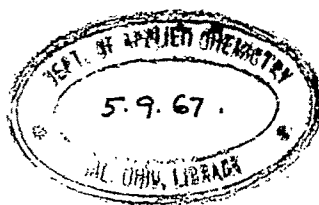


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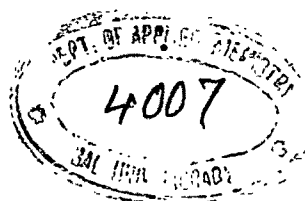


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## THE TECHNOLOGY GAP

A GREAT deal of well-intentioned nonsense is to be heard about the phenomenon called the technology gap, and there is a danger that it is infectious. The assertion is that Europe lags behind the United States in technological prowess, that the gap is steadily increasing, and that some means should be found for making it melt away. The Italian Government first raised the banners of deprivation early in the autumn of 1966. Mr. Harold Wilson, the British Prime Minister, echoed with a vision of how Britain could contribute more than most nations to the creation of a technological community in Western Europe. Towards the end of the year, both the President of the United States and the secretariat of the OECD embarked on special studies, and just before Christmas the issue came up in the Council of the North Atlantic Treaty Organization. Evidently the months ahead will be fully occupied with committee meetings of all kinds. Unhappily there is nothing like the same assurance that there will be agreement on whether there is a phenomenon which can be described as a technology gap, whether if it exists it is to be deplored or counted a blessing, and how, if at all, it might be removed or at least diminished.

The first need is to know what problem should be tackled. Although there is no reason to think that Europe is intrinsically incapable of some of the technological feats now being carried out in the United States, the plain fact is that advanced technology is much less obvious in Europe. But there is a similar disparity between Europe and North America in the general level of industrial production, in personal incomes (which determine the sizes of markets for goods of all kinds), and even in the amounts of food consumed. And if Europe sometimes looks a little woe-begone in comparison with the United States, it nevertheless seems unattainably sophisticated in comparison with the developing nations and those which are hardly developing at all.

In all these comparisons, the problem is to decide which comes first—the prosperity or the technology. By now there is plenty of evidence that technological developments alone do not bring prosperity. The collaboration of European nations in the development of the European space launcher under the umbrella of ELDO is unlikely to add much to the industrial competitiveness of the member countries. Certainly there would have been a better return from the investment of £100 million or so in more pedestrian development work—computers or even diesel engines, for example. Equally, the decision to commit £500 million to the Anglo-French development of a supersonic aircraft is unlikely to yield that amount of

benefit to the economies of the countries concerned. And even in the United States, it may well turn out that the Apollo programme to put a man on the Moon will prove to be more a drag on the economy than an incentive. What is called spin-off is grossly overvalued. The moral in all these cautionary tales is that lasting technological success depends on the existence of a healthy demand for its products. From this point of view, it is encouraging that the British Government seems to conceive of its European technological community in the context of the Common Market.

What, then, is likely to be done in Europe? Much more should be known about the British contribution after the Prime Minister and the Foreign Secretary have trudged around the capitals of the members of the European Economic Community in the next few weeks. No doubt they will make as much as they can of the contribution that British technology could make to the pace of development in Europe as a whole, and that will be entirely proper. But even if their enquiries result in a cheerful forecast about British and Scandinavian membership of the European Community, there will be a great deal of solid drudgery ahead. Working out common procedures for industrial specifications, testing procedures and patent applications will be a necessary preliminary—and progress on these matters within the Common Market has not been nearly as rapid as was at first expected. It would not be surprising if the amendment of national company legislation turned out to be high on the list of humdrum needs, together with arrangements for helping skilled people to move more easily from one place to another. At this stage, there is much less scope for the more ambitious schemes which are sometimes talked about—although there is a great deal which could be done immediately to put public laboratories more freely at the disposal of Europe as a whole. But none of these devices, and the more ambitious schemes which might follow, will work if the economic climate is not right. Mercifully, nobody seems to think that Europe can be turned into an engineers' Aladdin's Cave by investing money and effort in spectacular technology.

## ONE BASKET FOR EGGS

WITH the possible exception of a few men in Seattle, nobody will cry chicken because President Johnson and the United States Government have not followed the choice of the Boeing Aircraft Company as super-

sonic constructor to the administration with a decision that funds should be committed to the construction of a supersonic civil aircraft. For one thing, of course, this is a bad year for raising money, so that a decent show of diffidence in asking Congress for large sums of money is prudent as well as seemly. And in any case—a more practical consideration—there is \$200 million left in the kitty from last year, which should keep the design teams at Boeing employed for a month or two yet. Certainly there are enough problems to keep them busy, for all the consequences of the substantial modification of the design halfway through 1966 have not yet been fully worked out. As much as a year may be needed before all the details are tidied up. The President may have picked his gladiator, but there is no need to give him marching orders for a month or two.

A breathing space, however short, may help partially to resolve some of the problems which still haunt the supersonic projects now going forward in Europe and the United States. The strictly technical problems are probably the least worrying, which is not to imply that they are anything but formidable. The Boeing version of the supersonic transport is particularly ambitious. At take-off, lift enough for 300 tons or so of titanium and aviation fuel, with human appendages, will have to be transmitted through two wing hinges which must nevertheless perform so accurately that they do not become lop-sided in movement and turn the aircraft upside down. But, if the engineering will be difficult, it will also be fun. On strictly technical grounds, it is better that the Boeing design should have been chosen in preference to the more conventional solution proposed by Lockheed.

But getting off the ground will be easier than winning the right to do so regularly. Supersonic flight could easily turn out to be an intolerable public nuisance, with the result that operations of certain kinds are entirely forbidden. Even if supersonic flights over land areas are allowed, however, they are bound to be hedged around with restrictions more severe than those which at present keep the operators of subsonic jet aircraft lying awake at nights. Sometimes it may be necessary to build new airfields for supersonic aircraft, which will also help to tip the economic balance against them. But all this implies that all the supersonic projects, European and American, are gambles of a kind.

The simple commercial uncertainties of supersonic transport aircraft are even more daunting, and not merely because it is hard to see that the development programmes will pay for themselves. In Europe, indeed, the British and French Governments have apparently now abandoned hope that sales of Concorde aircraft will recover more than a nominal part of the £500 million to be spent on development. In the United States, no doubt, one of the issues to be hammered out in the next few months is that of how the administration and the Boeing company should share the risks and the putative profits of the programme that lies ahead. The most obvious difficulty on this score is that of knowing what the fare-paying demand for seats in supersonic aircraft will be. The Boeing

company may be right in its cheerful estimate that the demand for air transport will turn out to have doubled in the last half of the present decade, and there is every reason to expect that the rapid growth of air transport in the last two decades will continue for at least as long again, but that does not necessarily mean that people will pay the extra costs of travelling supersonically. Ironically, the Boeing company itself has chosen to invest an unprecedented amount of private capital in the construction of a line of jet aircraft capable of flying faster than any now in service and with twice as many passengers. As yet, it is too soon to know which of these will emerge as the more successful.

## NATURE BY AIR

BEGINNING with this issue of *Nature*, most subscribers to this journal in North America will be supplied each week with a copy which has been flown across the Atlantic by air freight and then distributed from New York by surface mail. The result will be that the journal should reach subscribers in the United States and Canada very soon after publication in London. Many subscribers in the United States will find that the journal reaches them on the date printed on the front cover—a circumstance which owes something to the near identity of the transatlantic time difference and the time taken for a jet aircraft to make the journey.

It is appropriate that there should be some kind of symmetry between the two sides of the Atlantic, for the numbers of copies distributed in the United States and the United Kingdom are approximately the same. (With Canada counted in, the centre of gravity lies somewhat to the West of mid-Atlantic.) But there are more cogent reasons why the delay in shipping the journal across the Atlantic by sea should have become intolerable. The increasing pace of change in science, and the urgency of a good many scientific communications, would be in themselves sufficient reason why copies of a weekly journal should not at the beginning of their useful life be incarcerated for two weeks or more in the hold of a ship. But periodicals of all kinds are more than mere providers of information. They serve also to give those who read them something which can properly be called a sense of community. (Readers know that at the least they have what they read in common.) But those who see their periodicals late tend to feel excluded from an experience which others have enjoyed. This, certainly, is why out of date newspapers are so hard to read. *Nature* seeks to provide the whole profession of science with a continuing and informed discussion of events as well as with a record of discovery, and therefore has a particular responsibility to be easily and quickly accessible. Sending it by air across the Atlantic is one of several steps which have been taken in the last century, and which will be taken in the next, to make it of greater service to those who read it.



## NEWS AND VIEWS

### What Future for Trieste?

THERE is continuing uncertainty about the means that will be found for financing the International Centre for Theoretical Physics at Trieste, at present sponsored jointly by the International Atomic Energy Agency at Vienna and the Italian Government. Although the centre has been widely acclaimed as a success, it appears that it may be hard for the IAEA to shoulder the chief cost of running the institution when the Italian Government withdraws its present support, originally intended to put the centre on its feet and not to support it indefinitely.

In the most recent annual report on the work of the International Agency's laboratories, the total budget of the centre at Trieste is given as \$414,447. Of this total, the contribution of the Italian Government amounted to \$278,000, while the International Agency contributed \$55,000. In addition to its contribution towards the running costs of the centre, the Italian Government has also provided the buildings in which the centre is housed. Because more than half the budget of the centre goes on salaries, there is obviously very little room for substantial economies without a drastic reduction of the scale of operations.

It is, however, unthinkable that the centre should founder for lack of support. The staff numbers close on a hundred, with Professor Abdus Salam as director. The centre has also pioneered a system of associate-ships under the terms of which physicists from countries described as "scientifically isolated" spend up to four months in the year at the centre. The work of the centre is organized into two divisions concerned with high-energy physics and plasma physics respectively, and an important part of the work of each of them is the organization of seminars lasting several weeks.

The central impediment to the continued financing of the centre by the International Agency is that there is no readily recognizable heading in its budget to accommodate what happens at Trieste. When the centre was formed two years ago, largely as a result of the enthusiasm of Professor Abdus Salam, it seemed that it would make an outstanding contribution to the training of physicists from developing countries, so that the agency was able to justify its modest support of activities at Trieste under the heading of aid. Experience has shown, however, that although the centre does include many people from developing countries on its staff and among its research fellows, its most valuable role is as an international meeting centre for physicists of all nationalities. In the circumstances, it is not surprising that Unesco has been suggested as the most natural source of continuing support. It is not yet clear whether that organization will choose to carry on the torch.

### Unfamiliar Modesty

MERELY a modest increase in expenditure on research and development in the United States is predicted by the Batelle Memorial Institute in its annual forecast

for the calendar year 1967. Total expenditure is expected to reach \$23.8 billion, an increase of only 2.2 per cent over 1966. This is the smallest percentage increase in the thirteen years for which records are available, and the dollar increase of \$500 million is the smallest since 1955.

The Batelle Institute is not, however, quite as clairvoyant as it may seem. It has been known for some time that Federal spending, the dominating factor in research and development—providing nearly 70 per cent of the funds—was not going to maintain its dizzy upward climb in 1967. The slowdown in Federal spending means that for the first time in ten years, industry, universities and non-profit institutions make up the major part of the annual increase—some 80 per cent. Industry, Batelle predicts, will spend \$6.8 billion, universities \$480 million, and other non-profit institutions \$326 million.

The figures show a shift in emphasis of the Federal research budget. The only component likely to get more money is basic research, with estimates of \$1.7 billion in 1965, \$1.9 billion in 1966 and \$2.1 billion in 1967; reductions are forecast for investment in plant and expenditure on applied research and development. The big spenders have been pinched hardest—the Department of Defense (DOD), the National Aeronautics and Space Administration (NASA) and the Atomic Energy Commission (AEC). Beneficiaries have included the Department of Health, Education and Welfare, the National Science Foundation, and the Departments of Agriculture, Interior and Commerce, but their increases hardly reflect the kind of commitment to the problems of health, air and water pollution, urban transport and re-development which is necessary if the aims of the "Great Society" are to be realized. What is particularly unfortunate is that if it were not for budgetary pressures, the lull in spending by DOD and NASA might have made possible a real re-distribution of funds.

By the time the budget pressure is relaxed, and the war in Vietnam settled one way or the other, DOD and NASA may well be clamouring for money once again. NASA has most of the money it needs for the "Apollo" programme, and seems uncertain what it wants to do after that, but nobody doubts that whatever it does decide to do will be very expensive. The Department of Defense is facing the problem of whether to establish an anti-missile defence system, with a budget of frightening size; estimates run between \$30 billion and \$50 billion. Another appropriation being deferred month by month is that for development of the Boeing supersonic transport. To put this into service is likely to cost \$4 to \$5 billion, with a Federal contribution of at least 75 per cent. It does begin to look as if the budget is simply not going to stretch far enough. The loser, especially after the mid-term election results, seems likely to be the Great Society.

### New Year Re-shuffle

SIR WILLIAM PENNEY is to leave the U.K. Atomic Energy Authority in September to become the next Rector of the Imperial College of Science and Technology—a post left vacant by the death of Sir Patrick Linstead three months ago. Sir William thus returns to academic life and his old college after an interval of 23 years in nuclear research and development,

most of it in weapons research, first as British representative at Los Alamos and then as Director of Research at Aldermaston. His three years as chairman of the Atomic Energy Authority leave it well organized and successful. It is now likely to enter a period of contraction and change which will impose an even greater burden on the new chairman.

It is also known that Sir Harry Melville is retiring in September from his post as Chairman of the Science Research Council. He has been chairman of the council since it was established in April 1965, was previously Secretary of the Department of Scientific and Industrial Research, and has been professor of chemistry at both Aberdeen and Birmingham. He too will probably wish to return to academic life. The name of Professor B. H. Flowers has been frequently mentioned in speculation about his successor at the Science Research Council.

## Nuclear Information

THE International Atomic Energy Agency is about to launch an ambitious scheme for collecting and processing technical information on atomic energy. At the end of December a working party drawn from sixteen of the member countries of the agency accepted in principle what is described as the concept of "an international nuclear information system", and which has already been blessed with the acronym INIS. The International Agency has for some time been concerned to sponsor developments in the handling of information in nuclear physics, and has been carrying out some work within its own organization. No doubt the new organization will work closely with others such as the Neutron Cross Section Centre in Paris which is operated by the European Nuclear Energy Agency.

## Aid Distribution

THE O.E.C.D. in Paris has now published statistics showing the way in which financial aid from advanced nations is distributed to developing countries. According to figures based on the membership of the Development Aid Committee of the O.E.C.D. (which includes Australia and Switzerland as well as the ordinary members of the organization), the total financial contribution to the less developed countries between 1960 and 1964 amounted to \$42.3 billion. Of this total, governments contributed \$25.9 billion, or close on two-thirds. The contribution of multilateral agencies such as the various institutions of the United Nations amounted to \$2.3 billion, and the rest of the aid represented the investment of private companies in the economies of developing nations.

It appears that the members of O.E.C.D. have put Asia at the top of their list of recipient countries, for the flow of public money to Asian countries worked out at an average of \$2,315 million a year in the first five years of this decade. Africa comes next on the list with an average of \$1,523 million a year from government sources. In terms of the populations of the two regions, of course, Africa is the more generously treated in that its population of 280 million people is merely a third of that of the recipient nations in Asia. Aid to Latin America and to undeveloped countries in Europe took \$674 and \$438 million a year respectively in the first five years of this decade. Except

in Asia, aid giving appears to be more or less the same from one year to the next, although the present contribution to the economy of undeveloped countries on the American continents is much greater than it was in the fifties.

## Publishing in Physics

THE Institute of Physics and the Physical Society, which has a long-standing interest in publishing of all kinds, is now planning to publish on its own account records of its own conferences and symposia. Three volumes, relating to conferences held in September, 1966, have been or are about to be published at prices ranging from £4 4s. to £5. Evidently the institute is hoping that it will be able to publish symposium proceedings more quickly and more cheaply than commercial publishers. Another of the benefits of the arrangement will be that librarians and others will be able to trace the publishers of particular conferences more easily than at present. Other learned societies will no doubt follow the experiment with interest.

## Less Smoking in Britain

A SIGNIFICANT decline in the consumption of tobacco in the United Kingdom is recorded in the fourth edition of the survey on the subject carried out each year by the Tobacco Research Council. Between 1964 and 1965, the total consumption of tobacco fell by four per cent to 255.1 million pounds. Since 1961, when smoking was at its peak, there has been a decline of eight per cent, coupled with a substantial change in smoking habits in favour of cigars and against pipes and cigarettes.

The decline is more marked when expressed in terms of the consumption by individuals, and among adult male cigarette smokers consumption declined from an average of 4,010 cigarettes per head in 1961 to 3,580 per head in 1965. The rising trend of cigarette smoking among women since the mid-fifties has now apparently been halted, and has settled down at 1,860 cigarettes per head or roughly half as much as for men. The incidence of cigarette smoking among children between 10 and 15 years seems not to have changed significantly in the first half of the present decade, but the numbers of men smoking tobacco in all its forms have declined significantly between 1958 and 1965, particularly among the age groups above the mid-thirties. Thus the proportion of men between 35 and 49 not smoking tobacco at all increased by nearly a third between 1958 and 1965, and amounted to 30 per cent at the end of the period. Although the proportions of women smokers are consistently lower than those of men in the same age groups, there has been no corresponding decline in the incidence of smoking in the years covered by the survey.

## Britain Five Years Ago

THE spate of primary publications of the 1961 census of the British Isles may soon be coming to an end, to judge from the appearance at the end of 1966 of the Summary Tables for Great Britain, together with the Household Composition Tables for England and Wales (H.M.S.O., £1 19s. and £3 7s. respectively). The

tables are comprehensive, careful and rather out of date.

The Summary Tables include details of population, age and marital condition, birthplace and nationality, fertility, migration, occupation, household composition, and age of leaving school. The population of Great Britain in 1961 was 51.35 m, made up of 24.7 m males and 26.6 m females, which represents a doubling of the population since 1870. Some of the tables are based on a complete return, while others are based on a 10 per cent sample. For these, sampling errors are defined. Even a 100 per cent return is no guarantee of accuracy, however, as the tables show. In 1961 for the first time a post-enumeration survey was carried out to check the information collected at the census; this shows that in some cases the information given in the census returns was inaccurate. The reasons for the inaccuracies are not given, but presumably they are caused either by ambiguities in the census form, or by simple reluctance of the people filling in the form to admit the truth. The number of women in employment, for instance, was understated, as was the number of households without a water closet. 1.08 m households without a water closet seems a remarkably high figure, but is likely to be an underestimate; the post-enumeration survey showed that the figure might be nearer 1.5 m. Facts like these show the great interest of the census returns; they would be easier to read, though, if they had better indexes.

## Transports of Despair

THE British Ministry of Transport must be feeling baffled by the sharp rise in road casualties over the Christmas holiday. Just two days before the holiday began, the Ministry issued figures for September 1966 which show that for the fourth successive month fewer people were killed and injured on the roads. In September there were 31,911 casualties, a figure which hardly gives cause for dancing in the streets, but which is nevertheless a reduction of 7 per cent compared with September 1965 despite a 7 per cent increase in traffic density since then. By contrast, the three days of the Christmas holiday showed an increase in casualties from 2,853 to 3,335 (17.5 per cent), and of deaths from 55 to 80.

Both sets of figures are worth closer examination. Over the Christmas period the number of injuries was actually produced by fewer accidents than last year—indeed, the Ministry of Transport figures show 5 per cent fewer accidents for Christmas 1966. A number of inferences can be drawn from this, but the most obvious way in which the number of people involved in each accident is increased is by increasing the number in each car. In response to the appeal not to drink and drive, people may well have left their cars at home and taken lifts with friends, a course of action long recommended by the ministry and the motoring organizations. If this interpretation is correct, minor injuries should show a much steeper rise than major injuries, since the back seats of cars are much safer than the front. In fact, this is exactly what did happen; slight injuries increased by 22 per cent, serious injuries by only 2.5 per cent. Of course, it is risky to interpret the figures without more detailed information; interpretation is really the job of the ministry.

The ministry attributes the improvement in the

September figures to the decline of two wheeled vehicles. The mileage covered by bicycles has declined for some years, and since 1960 the mileage covered by mopeds, scooters and motor bicycles has also declined. For every mile travelled, users of two wheeled vehicles suffered ten times as many fatal and serious injuries as drivers of cars, so the switch to safer vehicles has brought about a reduction in casualties, although the casualty rates for individual classes of vehicle have in many cases increased. To be sure, this conclusion will confound no one but the scooter manufacturers, but people are entitled to ask the ministry one plaintive question. Why, if two wheeled vehicles are so dangerous, does the ministry encourage their sale by letting young people ride them for a year before they are considered old enough to drive a car?

## Supersonic Contract

THE complex problem of deciding which of the two claimants to the U.S. Supersonic Transport contract has produced the better design has been settled to the satisfaction of the Federal Aviation agency, President Johnson and the Boeing Aircraft Corporation. The agency has selected the Boeing swing-wing design in preference to the Lockheed double-delta, and engines by the General Electric Company rather than Pratt and Whitney. The decision came as no great surprise, for the airlines consulted by the agency were reported to prefer the Boeing design.

The supersonic transport battle now resolves into one between the Boeing design, with a speed of 1,800 m.p.h. and room for up to 350 passengers, and the Anglo-French Concord, which will carry 136 passengers at 1,450 m.p.h. In the background, competing for prestige if not economic gain, is the Russian Tu144, which has a specification remarkably similar to that of Concord, carrying 126 passengers at 1,500 m.p.h. The Boeing design, which will cost \$35 m against the Concord price of \$16, is clearly the most ambitious of the three, but is three years behind the other two; both the Russian and the Anglo-French designs are to make their first flights in 1968, while the Boeing design is still at the mock-up stage, and earlier this year underwent radical design alterations.

The striking feature of the Boeing design is the use of wings which swing from a position at right angles to the fuselage at take-off through an intermediate position while climbing at subsonic speed and finally link up with the tail to form a delta-wing for high altitude cruising. Boeing claims that this design concept is well tested and proved, although some airlines are reported to have expressed alarm about it. It undoubtedly improves efficiency, particularly in take-off and landing, but the engineering which it makes necessary adds weight and offsets some of this advantage. In fact the take-off and landing speeds and distances required by the two rival American designs are remarkably similar; where the Boeing design would presumably gain would be in using the engines at lower power when circling and landing, a reduction both in cost and in annoyance by noise.

The problem of noise may well turn out to be more significant than is usually realized. It is already being seriously suggested that certification of aircraft should include permitted noise levels, and while this is unlikely to happen next week, it could be a reality in five

years' time. By that time Concord will be operating, and is unlikely to be trapped by noise legislation. If the noise level established means that the Boeing aircraft has to fly more slowly than its design speed, economy of operation will suffer severely, and indeed may make the SST quite uneconomic over land. A study commissioned by the FAA and carried out by the North American Aviation Co., however, indicates that the penalty of flying SSTs at subsonic speeds over land would be "not intolerable". People's interpretation of this depends on whether they build aeroplanes or operate them.

Aircraft builders are, of course, notoriously optimistic. This optimism is not always shared by the airlines, or by the political paymasters of the aircraft industry. The sacking of General Puget, until last week head of Sud Aviation, the nationally owned French airframe company which is collaborating with the British Aircraft Corporation in the Concord project, will diminish by one the number of enthusiasts in service. General Puget's successor, M. Maurice Papon, is known as a gifted administrator.

## Swinging Wings

BRITISH aerodynamic engineers are at once envious and optimistic about the outcome of the development work to be carried out by the Boeing Aircraft Company before its supersonic aircraft can take the air. The problem of matching the two wings together in such a way as not to upset the lateral stability of the aircraft has been dealt with on the F111 fighter aircraft by linking the two halves of the wing together mechanically with a common jack. Because the Boeing design has separate hinges for each half wing, and because these lie athwart the passenger cabin, it will be necessary to link the two halves together with an electro-mechanical servo-system. Apparently even as much as a degree or so of mis-alignment between the two wings would not be beyond the capacity of the roll control system, partly because the outer surfaces of wings contribute only a small proportion of the total lift.

The problem of designing hinges which can move reliably and yet transmit lifting forces comparable with the weight of the aircraft appears also not to be a cause for worry. Prototypes of hinges with dry bearings and lubricated bearings have been carried out. There appear to be no problems in using materials such as PTFE as dry lubricants or in using ordinary petroleum lubricants. Problems arising from the high temperatures at which some supersonic designs will operate may be more serious, particularly because they make it necessary to introduce new materials for incidental purposes such as the operation of hydraulics and lubrication systems.

Aerodynamic engineers therefore remain preoccupied with problems closer to the mainstream of classical aerodynamics. Thus it is necessary to design supersonic aircraft such as the Boeing SST so that they can if necessary land with the wings fully swept back if, for some reason, the wing moving mechanism should fail. In this kind of mishap, supersonic aircraft would necessarily have to seek out airfields with long runways and use up spare fuel before attempting to land at what would inevitably be a hair-raising landing speed. Another problem which excites lively interest

is that of striking a continuing balance between the centre of aerodynamic lift and the centre of gravity of a supersonic aircraft with swept wings. On the face of things, it would appear that moving back the wings would leave the front of an aircraft unsupported. British aerodynamicists claim that a decade ago Dr. Barnes Wallis showed that a clever distribution of the weight of an aircraft could go a long way to reduce the severity of this problem.

## Venus Observed

THE first observations of water vapour in the atmosphere of Venus from ground based observatories are described in two notes published in the current issue of the *Astrophysical Journal*. Hitherto, the identification of water in the atmosphere of Venus has been based on observations from balloon flights over the United States in the early sixties. Both the groups now reporting have worked with water vapour absorption lines in the near infra-red (8189 Å and 8193 Å) and have been able to recognize Doppler shifts due to the motion of Venus at different parts of its orbit about the Sun. The detection of water vapour in planetary atmospheres is necessarily complicated by the presence of water in the atmosphere of the Earth, but Doppler shifts make it possible to separate absorption due to the two sources.

M. J. S. Belton and D. M. Hunten worked with spectra of Venus formed by the 40 ft. spectrograph of the solar telescope at the Kitt Peak National Observatory (*Astrophysical Journal*, 146, 307; 1966). The second note in the same issue of the journal (*ibid.*, 146, 328; 1966) is by Hyron Spinrad and Stephen J. Shawl of the University of California at Berkeley, who have examined coude spectrograms of Venus taken at the Lick Observatory. The Doppler shift of the spectra due to the movement of Venus in its orbit amounted to between 0.2 and 0.3 Å, and the successful identification of water vapour from the Earth has thus been made possible only by the extremely high dispersion of the spectrographic instruments used. Both groups of astronomers have been able to estimate the amount of water in the atmosphere of Venus above the clouds. The first group has set an extreme upper limit of 125 microns to the amount of precipitable water, and the second has estimated the amount of water at 60 microns, emphasizing that this estimate is necessarily somewhat crude.

## Luna 13

LUNA 13, the Russian spacecraft which made a soft landing on the Moon on December 24, 1966, has now completed its mission. Pictures taken by Luna 13 from the landing site in the Ocean of Storms, 250 miles from the site where Luna 9 landed, show that there are no formations larger than 3.5 km in diameter within 62 km of the landing site, and no big formations rising above the ground. The spacecraft, inclined at 16° to the local vertical, completed a panoramic sweep and was able to distinguish objects 1.5 to 2 mm in size at a distance of 2 m. The spacecraft contained electronic programme devices, chemical batteries, and a temperature regulating system, and had four petal and four rod antennae, a television camera, and two devices for measuring soil depth and density.

The results seem to have confirmed those obtained by Luna 9. The level of radioactivity on the Moon's surface is low, and measurement of the cosmic ray reflectance of the surface shows that the Moon reflects 25 per cent of the particles which fall on to it from space. Photography showed once again that there is no layer of dust on the Moon's surface, which is covered with small stones a few centimetres in diameter. The Russian results suggest that these stones had fallen quite slowly onto the lunar surface and were certainly not meteorites, which would have given cratering.

In addition, Luna 13 carried out measurements of the physical properties of the surface. Rods were driven into the ground by an explosive device to a depth of 20-30 cm, and the density was measured by a beam of gamma radiation distributed along the surface. These measurements show that the surface of the Moon has mechanical properties similar to the properties of terrestrial soil of medium density. The density of the surface is much less than that of the Earth, and much less than the average density of the Moon.

## Economic Geochemistry

GEOCHEMISTRY is still struggling for a place in the hierarchy of recognized sciences. After the Second World War the subject promised to be an important tool for economic geologists, but this promise has not been fulfilled and the techniques of geochemistry have so far been principally used for such tasks as tracing the movement of underground water and prospecting for uranium. The investigation using geochemical methods of the Pequop Mountains area in Elko County, Nevada, by the U.S. Geological Survey is therefore welcome (*U.S. Geol. Survey Bull.*, 1198-E, 1966). The area had no previous history of metal production but possessed a host rock type (dolomite) and a structure favourable to the formation of ores. Conventional sampling and analysis of stream sediments failed to reveal anomalously high concentrations of metals, but analysis of river cobbles and pebbles showed contents of more than 1 per cent of lead and zinc, with lesser amounts of mercury, antimony and arsenic. The metals were concentrated in iron rich fracture fillings or gossan pods (with or without barite), both of which were associated with silicified limestone. Only detailed mapping will reveal whether these metals are present in commercially exploitable amounts.

## Crater in the Lake

A SWEDISH geologist has suggested that a submerged circular depression in Lake Hummeln, 150 miles south-west of Stockholm, was caused by the impact of a meteorite some 600 million years ago (Svensson, N. B., *Sver. Geol. Unders. Afh.*, 60, part 3, 1966). The crater, which is excavated in Pre-Cambrian granite, was probably exhumed by ice during the Ice Age. Evidence for a meteoritic origin is the presence of an unconsolidated breccia composed of pieces of the granitic basement and the shape of the crater, which is flat bottomed with a pronounced rim. It is thought that shortly after the formation of the crater the area was covered with Pre-Cambrian sandstone, thus preserving a feature which, if it had been exposed to the elements, would have rapidly become unrecognizable. A vol-

canic origin for the crater is improbable as no contemporary volcanic activity is known in the area, while a tectonic origin is ruled out on the grounds that the last demonstrable tectonism in the area was some 500 million years earlier than the formation of the crater. If the depression in the floor of Lake Hummeln is indeed an impact crater, then it is one of the earliest examples in the geological record.

## Sound of the Walrus

THE sounds made underwater by Olaf, a ten year old Atlantic walrus (*Odobenus rosmarus*), have been recorded at the New York Aquarium. The sounds can be heard on a gramophone record which is included in the latest edition of *Zoologica*, published by the New York Zoological Society.

The underwater sounds are distinct from the bellow, grunt and mellow whistle made by the walrus in the air. Underwater, the walrus produces rasps, clicks and bell-like sounds, with mouth closed and head submerged. The rasps and clicks are of short duration, 0.1-0.2 sec and 0.015-0.020 sec respectively. The clicks are produced in rapid sequences and resemble quiet machine gun fire. The rasp is the type of noise which is produced by blowing through closed lips. Olaf, with a damaged eye, had no difficulty in swimming, even with eyes closed. The rasps and clicks which he emitted in the process could have been for echo-location; but, as the report points out, the walrus was in familiar surroundings and may have been relying on memory for his orientation.

The bell like sound lasts longer (1-1.5 sec) than the rasps or clicks and is hollow and metallic, like the sound of distant cymbals. It is associated with the development of the pharyngeal pouches, which can inflate to balloon like proportions on each side of the head of this animal and which help in floating. The walrus floats when it is sexually active, and it was on such occasions that the bell like sound was heard. Confirmation that the pharyngeal pouches act as resonators for this sound was also obtained at the death of a walrus with a pouch inflated, after a hunt by Eskimos; when the pouch was stripped of skin and fat and struck with the flat of a knife blade, a bell like sound almost identical with that recorded was heard.

## Dorcas Gazelles

L. I. Ghobrial and J. L. Cloudsley-Thompson write from the Department of Zoology at the University of Khartoum about their recent note on the effect of water deprivation on the Dorcas gazelle (*Nature*, 212, 306; 1966). "We are disturbed to learn that some readers thought that the death from drought of two experimental animals was caused deliberately. Humanitarian reasons apart, the loss of these valuable gazelles was a severe blow to our programme of research. We never expected that death would follow a percentage loss of body weight so small compared with what the camel and the sheep can tolerate. As soon as our animals became weak and a big increase in blood urea was noted, they were given water and salt. Despite this, they died after about 12 h. We are sorry if our purely factual statement has misled your readers."



## University News:

## Liverpool

DR. M. R. SAMPFORD, at present senior principal scientific officer in the Agricultural Research Council Unit of Statistics in the University of Edinburgh, has been appointed to the newly established chair of mathematical statistics.

## London

PROFESSOR G. L. HOWE, professor of oral surgery in the University of Newcastle upon Tyne, has been appointed to the chair of oral surgery at the Royal Dental Hospital of London School of Dental Surgery from April 1, and Dr. M. Ginsburg, reader in pharmacology at Chelsea College of Science and Technology, has been appointed to the chair of pharmacology, tenable at that college.

## Southampton

DR. J. H. BIRD, reader in geography in University College, London, has been appointed to the chair of geography in succession to Professor F. J. Monkhouse; and Dr. M. Fleischmann, reader in physical chemistry in the University of Newcastle upon Tyne, has been appointed to the Electricity Council Faraday chair of electrochemistry.

## Appointments

DR. V. SUNDARAM, formerly senior scientific officer of the Cotton Technological Research Laboratory, Matunga, has been appointed director of the Cotton Technological Research Laboratory, Bombay.

## Announcements

DR. BRYAN THWAITES has been elected president of the Institute of Mathematics and its Applications for 1967, in succession to Professor M. J. Lighthill. Dr. Thwaites is principal of Westfield College in the University of London and is well known for his concern with the improvement of mathematics teaching in schools and for his founding of the Schools Mathematics Project. Professor Lighthill, Royal Society Research professor at Imperial College, London, and formerly director of the Royal Aircraft Establishment, Farnborough, played a major part in founding the institute, and has been its president since its incorporation in 1964. Dr. E. T. Goodwin, superintendent of the Mathematics Division of the National Physical Engineering Laboratory, and Dr. P. G. Wakely, manager of the Mechanical Engineering Laboratories of the English Electric Co., Ltd., at Whetstone, Leicester, have been appointed vice-presidents of the institute in succession to Professor A. Geary and Professor K. Stewartson. Professor D. C. Pack will continue to serve as honorary treasurer and Mr. F. W. Kellaway and Mr. W. G. Sherman as honorary secretaries.

At the election for the first elected council of the British Acoustical Society, which took place in November, Professor R. E. D. Bishop, Kennedy professor of mechanical engineering in University College London, was elected president. The other elected officers are: *Vice-presidents*, Professor E. J. Richards (head of Institute of Sound and Vibration Research, Southampton); Professor E. C. Cherry (Department of Electrical Engineering, Imperial College of Science and Technology, London); Mr. W. A. Allen (consulting architect).

DR. ELI BROOKNER, of the Raytheon Company, is the first recipient of the Journal of the Franklin Institute's \$1,000 award, which is to be made annually to the author of the outstanding paper appearing in the journal during the preceding year. Dr. Brookner received the award for his paper entitled "Effect of Ionosphere on Radar Waveforms", which deals with the problem of determining the effects of the ionosphere on the propagation of microwaves.

A MONTHLY abstract journal entitled "Abstracts of Mycology" is being published by BioSciences Information Service from January this year. There will be three trial issues, circulated to individual scientists with known interests in this specialized field of study. Further information can be obtained from Abstracts of Mycology, Professional Services and Education Department, BioSciences Information Service of Biological Abstracts, 2100 Arch Street, Philadelphia, Pennsylvania, 19103.

**ERRATUM.** In the legend accompanying Table 1 of an article "Induction of Thymine Dimers in Synchronized Population of Chinese Hamster Cells" by D. L. Steward and R. M. Humphrey (212, 298; 1966), the word "uridine" replaced the word "uracil" at two points, the words "cystine or cysteine" replaced the word "cytosine" at one point and the legend should read: "The two dimensional chromatographic analysis (ref. 8) does not separate uracil-thymine dimers from thymine dimers and the activity of both photo-products is referred to as thymine dimer activity in this report. The uracil-thymine dimer arises from the cytosine-thymine dimer deamination during acid hydrolysis of the DNA (ref. 17)".

**ERRATUM.** In the article "Flame Ionization and Magneto-hydrodynamics" by Jean Debiesse and Siegfried Klein in *Nature*, 212, 1405 (1966), figure 4 was printed upside down.

**ERRATUM.** In the first sentence of the third paragraph in the obituary of Dr. Arthur Patterson in *Nature*, 212, 1414 (1966), "increases" should read "decreases".

## CORRESPONDENCE

## What Kinds of Cities?

SIR,—Your article thus headed seems to me an odd compound of scientific scepticism and unscientific assumption. It rebukes the Greater London Council for neglect of research into what city people like, what they will put up with, and what they might like if they knew about it. Then in the belief, or hope, or hunch (which is it?) that closer packing in flats of 6 to 49 stories would be socially and culturally better, it accuses the G.L.C. of neglecting to consider that form of housing.

But this matter of high versus low housing happens to be one on which there has been the most exhaustive research; innumerable surveys and opinion polls have shown that 85 to 95 per cent of British people dislike flats and want houses with private gardens. The G.L.C. itself admits that London should have built 75 per cent of houses instead of 75 per cent of flats. Even you, without research, sense that London is too big; and I, as a proponent of a policy of decongestion and dispersal and an experimenter in its practicability, snarled at the L.C.C. for 40 years for not pursuing that obviously sensible policy. Even now that the G.L.C. is trying to do so I have to urge it to be speedier and more logical about the job, in co-operation with the Government and other regions. I know it wants to, and I realize the enormous political and technical difficulties.

London is the first great city in the world to set about reducing its population, over-centralization and density. As one of its persistent critics, I resent on its behalf the suggestion that through lack of research it is ignorant of the major social considerations.

Yours faithfully,

FREDERIC J. OSBORN

[Sir Frederic Osborn is Chairman of Council of the Town and Country Planning Association and Vice-President of the International Federation for Housing and Planning.]

# Lunar Air Tide

by

B. HAURWITZ\*

S. CHAPMAN†

National Center for Atmospheric Research,  
Boulder, Colorado

Lunar tides in the oceans are familiar and easy to observe. Lunar tides in the atmosphere also exist, although their recognition is more difficult

THE atmosphere, like the oceans and the solid Earth, is subject to the tidal forces of the Sun and Moon. We can observe and measure the tides of the oceans and of the solid Earth at their upper boundary, where we live. The atmospheric tides have to be determined mainly at the bottom of the atmosphere. One way in which this is done is by recording the changes of barometric pressure  $p$ , which indicates the total overlying mass per  $\text{cm}^2$  column. The analogous method of measuring the ocean tides would be by a pressure gauge on the ocean bed. In this case, in mid-ocean, the fractional tidal variation of pressure would be of order  $10^{-4}$ . This is because the tidal amplitude there is of order 0.5 metre (although at many places on coasts it is much greater), whereas the mean oceanic depth is of order 4,000 m. In the atmosphere near the equator, where the lunar air tide is greatest, its pressure amplitude is about 1/12 mbar, which is likewise about  $10^{-4}$  of the pressure at sea level (approximately 1,000 mbar). The lunar air tide, like the sea tides, also involves tidal currents and can therefore be studied by determining the lunar daily variations of the wind components.

The Moon's tidal force on the Earth is 2.4 times that of the Sun, and in the oceans the lunar tidal currents and changes of level bear approximately this ratio to those caused by the Sun. Thus the Moon dominates the ocean tides (and also the Earth tides). But in the atmosphere the Sun produces daily changes of temperature, and the associated pressure changes much exceed those caused by the tidal action of the Sun and Moon. Thus the Sun dominates the dynamics of the atmosphere. In the tropics the changes of temperature and pressure are more regular than in higher latitudes, and the barometer shows a regular solar semidiurnal pressure variation with an amplitude of about 1 mbar; the maxima occur at about 10 a.m. and 10 p.m. local mean solar time. As we proceed to higher latitudes the regular daily pressure change decreases, and the irregular weather changes of pressure grow, so as to make the regular changes difficult to detect on barographs. But they can still be determined by averaging the records over many days, thus reducing the irregular changes.

The observations are generally made at definite solar hours. The determination of the lunar daily variation, proceeding according to lunar time, can be done in a number of ways. It is desirable to use a method which permits the concurrent determination of both the solar and lunar daily oscillations; such a method was developed by Chapman and Miller<sup>1</sup>.

## The Harmonic Dial

It is convenient to denote by  $S$  a daily periodic change caused by the Sun, and by  $L$  one caused by the Moon. Thus  $S(T)$ ,  $S(p)$ ,  $S(v)$  may denote the solar daily changes

of air temperature  $T$ , pressure  $p$ , and wind velocity  $v$ , and likewise for  $L$ . These changes can be analysed into their harmonic components, of the form

$$s_n \sin(n\tau + \sigma_n)$$

for  $S$ , and

$$l_n \sin(n\tau + \lambda_n)$$

for  $L$ . Here  $t$  denotes solar time expressed in angle at the rate  $15^\circ$  per mean solar hour, and  $\tau$  lunar time at the same angular rate per mean lunar hour. Any such harmonic component can be denoted by  $S_n$  or  $L_n$ .

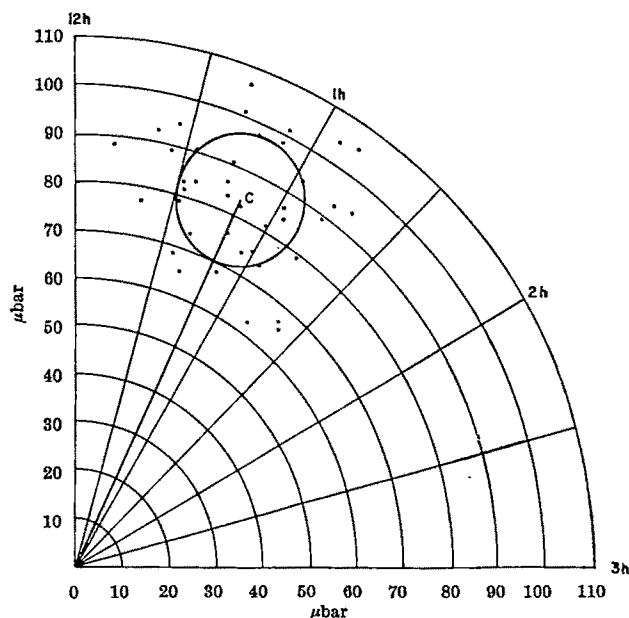


Fig. 1. Harmonic dial of the lunar barometric tide at Batavia (Djakarta). Each dot represents the determination for one year. The end point  $C$  of  $OC$  is the mean for 40 years. The circle of probable error for the individual yearly determinations is shown with its centre at  $C$ . (Bartels, 1927.)

Solar heating and cooling of the air generally produce one daily maximum of  $T$  and one daily minimum, but the variation is not simply sinusoidal; at night the fall of temperature is slower than the daytime rise to the early afternoon maximum. Thus  $S$  has components,  $S_2$ ,  $S_3$ ,  $S_4$ , . . . of period 12, 8, 6, . . . hours, as well as its main diurnal component  $S_1$ , of period 24 h.

There are associated components  $S_1(p)$ ,  $S_2(p)$ ,  $S_3(p)$ , . . . in the solar daily pressure variation, but their magnitudes

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are not simply proportionate to  $S_1(T)$ ,  $S_2(T)$ ,  $S_3(T)$ . . . . The semidiurnal variation  $S_2(p)$  is magnified by a small degree of resonance in the atmosphere, so that at most places it exceeds  $S_1(p)$ .

The lunar tidal potential has many periodic components; the semidiurnal term  $L_2$  is much the greatest. This is the term that has been most extensively studied in meteorological data. The varying distance of the Moon causes the lunar tidal force to vary, and its mean maximum is 1.37 times its mean minimum. The lunar air tide  $L_2(p)$  has been shown to increase from apogee to perigee in approximately this ratio<sup>3,4</sup>.

Thus  $S_2(p)$  and  $L_2(p)$  are the main solar daily and lunar daily variations of barometric pressure. At Batavia, now called Djakarta, Indonesia, their annual mean values are as follows (in mbars):

$$S_2 = 1.32 \sin(2t + 160^\circ), \quad L_2 = 0.082 \sin(2\tau + 68^\circ)$$

The maxima in these semidiurnal oscillations occur, respectively, at the solar and lunar hours (both a.m. and p.m.),

$$t_{\max} = \frac{90^\circ - \sigma_2}{30}, \quad \tau_{\max} = \frac{90^\circ - \lambda_2}{30}$$

Such oscillations can be conveniently indicated on a polar diagram called a harmonic dial<sup>4</sup>, because in its complete form it resembles a clock face. Fig. 1 is an example; it shows forty points ( $P$ ), each of which specifies the value of  $L_2(p)$  at Djakarta for one of the years 1866–1905. The distance  $OP$  from the dial centre  $O$  to any point  $P$  gives the value of  $L_2$  for that year;  $\lambda_2$  is the angle made by  $OP$  with the right-hand part of the horizontal axis. Regarded as a clock hour-hand  $OP$  also indicates the time  $\tau_m$  of maximum.

The point  $O$  is the centroid of the forty points  $P$ , and  $OC$  represents the 40-year mean value of  $L_2$ . The yearly points  $P$  differ from  $C$  because of the aperiodic barometric changes present even at the low latitude of Djakarta. Their scatter supplies a measure of the accuracy of the mean determination. Bartels<sup>5</sup> showed how to determine a probable error  $e$  for each yearly determination, when the distribution of the points  $P$  around  $C$  is reasonably symmetrical. The circle of "probable error" with radius  $e$  should contain half the points  $P$ , if the scatter is Gaussian. Nineteen of the forty points in Fig. 1 are within the circle of probable error there shown, and therefore the condition is well satisfied for  $L_2(p)$  at Djakarta. The probable error of the mean determination  $L_2$  is  $e \cdot n^{-1/2}$  where  $n$  is the number of individual determinations, in this case 40; thus for the 40 year determination it is 0.0021 mbars. Clearly  $L_2(p)$  at Djakarta is very well determined. No determination is regarded as satisfactory unless the amplitude is at least three times the probable error; for Djakarta the ratio is 39.

### Geographic and Time Variations

The first successful determination of the lunar air tide was made for St. Helena in 1842 by Lefroy<sup>6</sup>, then the director of the magnetic-meteorological observatory for the island, on the basis of 17 months' data (bi-hourly pressure values). For extratropical stations much longer series of data are required, because the tide, like the force which generates it, decreases polewards, and the irregular barometric changes due to the weather become very much larger. The first successful determination of  $L_2$  at an extratropical station, namely Greenwich, was made in 1918 by Chapman<sup>7</sup> from 64 years' data. Only about a third of these data were used for the computation; the most disturbed days were omitted—that is, those with barometric ranges larger than 0.1 in. mercury. The resulting mean lunar daily variation of barometric pressure based on 6,457 days of data with small diurnal range is

shown in Fig. 2, which contains also the harmonic dial representation.

Altogether there are now about eighty-five determinations of the lunar atmospheric tide<sup>8,9</sup>, although not all of them have yet been published. Unfortunately these stations are very unevenly distributed over the globe, as can be seen from Fig. 3. This figure shows the amplitude distribution of the lunar tide by isopleths (or isolines). Each dot indicates a station where the lunar tide has been determined.

The amplitudes are given in microbars (1  $\mu\text{bar}$  = 1 dyne/cm<sup>2</sup> = 0.001 mbar). They decrease from the equator towards both poles, as is to be expected because the force which generates the tide decreases with increasing latitude. But although the lunar tidal force is evenly distributed over the Earth, irregularities are found in the distribution of the lunar tide. Along the Pacific coast of North America the amplitudes are unusually low, presumably because the North American Cordilleras impede the tidal motions<sup>10</sup>. The low amplitude at Buenos Aires calls for an explanation. Over the Indian Ocean there appears to be a region of abnormally high amplitudes, although for lack of data it is not certain that this region of high amplitude really extends from East Africa to the

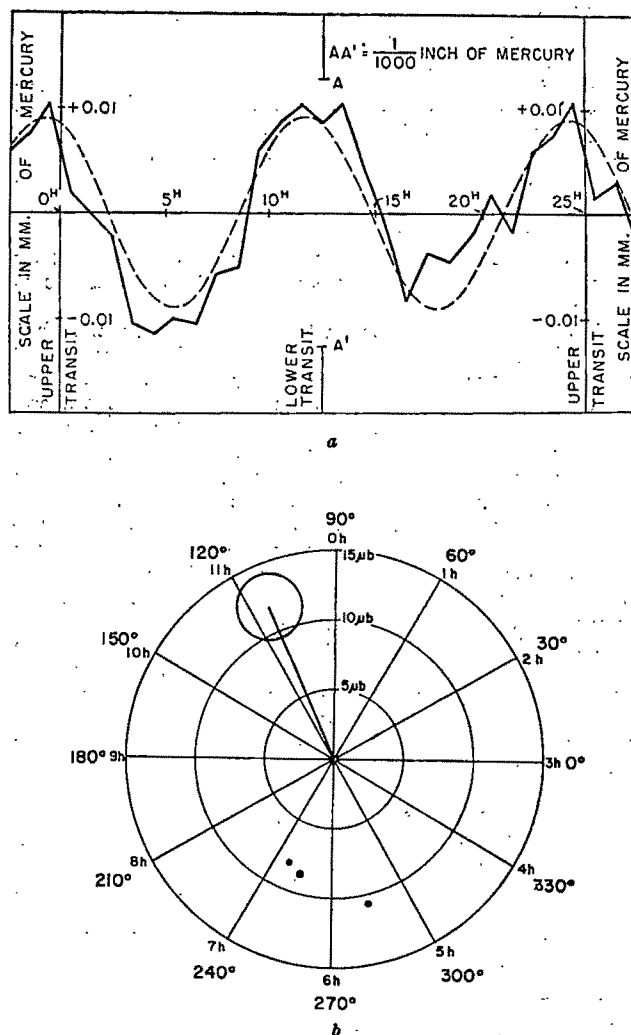


Fig. 2. The lunar barometric tide at Greenwich. *a*, Full curve: average lunar daily variation in mm mercury (1 mm mercury = 1,333  $\mu\text{bar}$ ), computed from 6,457 days' hourly data, 1854–1917; broken curve: lunar semidiurnal component of the variation. (Chapman, 1918.)  
*b*, Harmonic dial representation.

Western part of the Indonesian Archipelago. Some East African stations show high amplitudes, but others do not.

The phase angle of  $L_2$  is on the average about  $75^\circ$ , so that high tide (maximum barometric pressure) occurs about 30 min after the Moon crosses the local meridian. Individual stations, however, show deviations from this average; the barometric maximum at some stations, notably Greenwich, occurs nearly an hour before lunar transit. At the great majority of stations, however, the maximum occurs after lunar transit; the latest maximum, nearly 2 h later, is found at Haifa-Jerusalem. These phase

that this variation throughout the year, contrary to most annual meteorological variations, has the same phase in both hemispheres; that is, the minimum amplitude and the greatest delay of the barometric maximum after lunar transit occur simultaneously in the northern and southern hemispheres. A few stations do not follow the characteristic pattern shown in Fig. 4, but in most of these the difference between the summer and winter amplitudes and phases is within the limits of the probable errors. There does not seem to be any preponderance of deviating stations in either hemisphere. Thus this annual atmospheric variation has the same phase in both hemispheres.

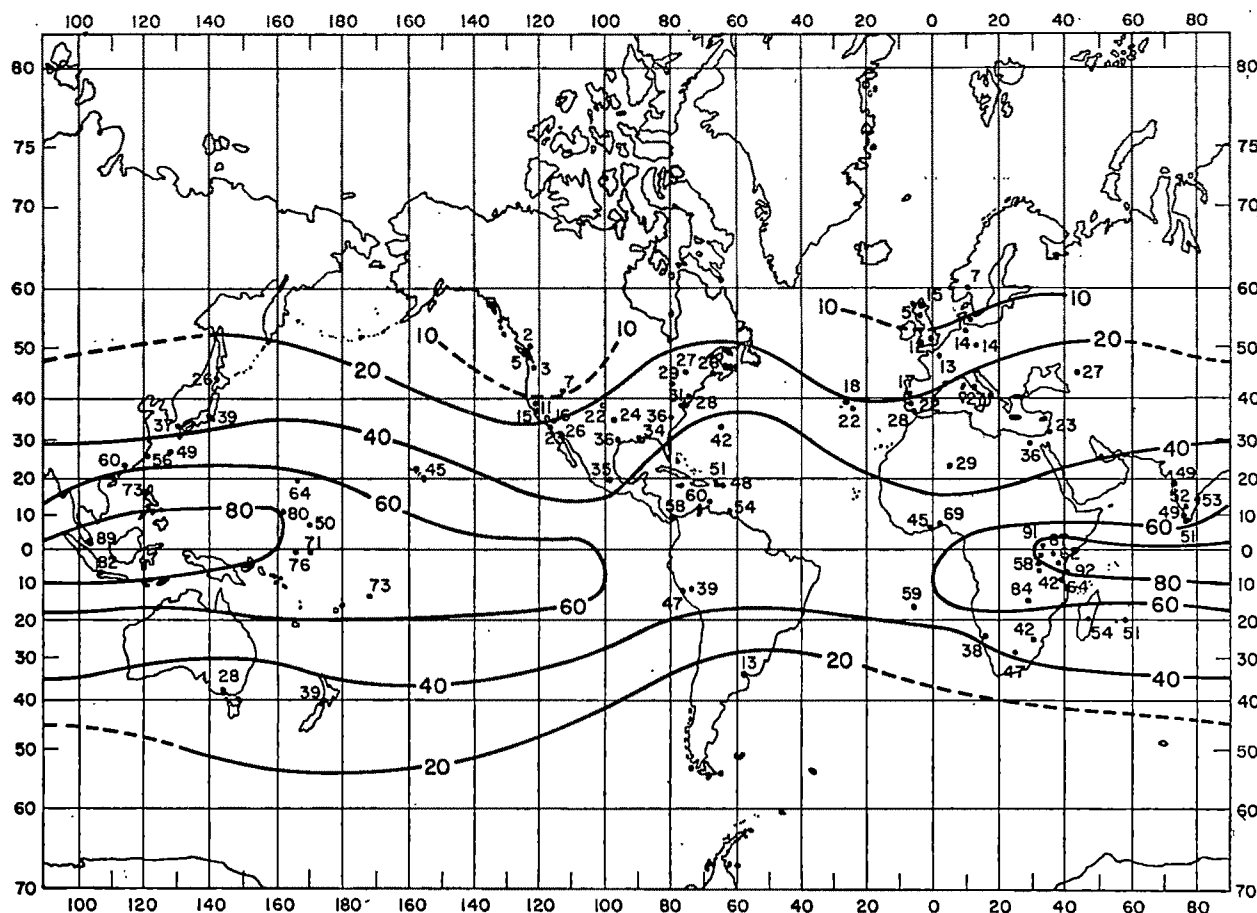


Fig. 3. Global amplitude distribution of the lunar semidiurnal tide (unit  $1 \mu\text{bar} = 10^{-3} \text{ mbar}$ ). The number at each station dot shows the local amplitude of  $L_2(p)$ .

differences may be partly due to errors in the determinations, because of insufficient data. But the larger part of these differences must be real, and their explanation must be sought in the irregular response of the Earth's atmosphere to the tidal force. The reduction of the length of the lunar day by about 10 min from perigee to apogee can clearly not account for these phase differences.

The lunar tide shows a pronounced annual variation, illustrated in Fig. 4, where two Puerto Rican stations are combined<sup>11</sup> to give a more reliable determination. The Roman numerals denote the 12 months; circles of probable error are shown for the odd months. The outstanding characteristics of the annual variation of  $L_2$  are the smaller amplitude and the later maximum during the northern winter months. It is particularly remarkable

### Temperature and Wind

The heating of the Earth's atmosphere by the solar radiation reflected and scattered by the Moon is quite negligible. But the lunar tidal pressure wave produces a lunar semidiurnal temperature wave. Depending on whether the changes of state produced by the pressure wave are isothermal or adiabatic, or follow an intermediate polytropic curve, the magnitude of the resulting changes of temperature and density will differ, as in the case of the Newtonian (isothermal) and Laplacian (adiabatic) calculations of the speed of sound. With the propagation of sound the changes of state are adiabatic, because they are so rapid that the temperature excess due to compression cannot be conducted away. It might be

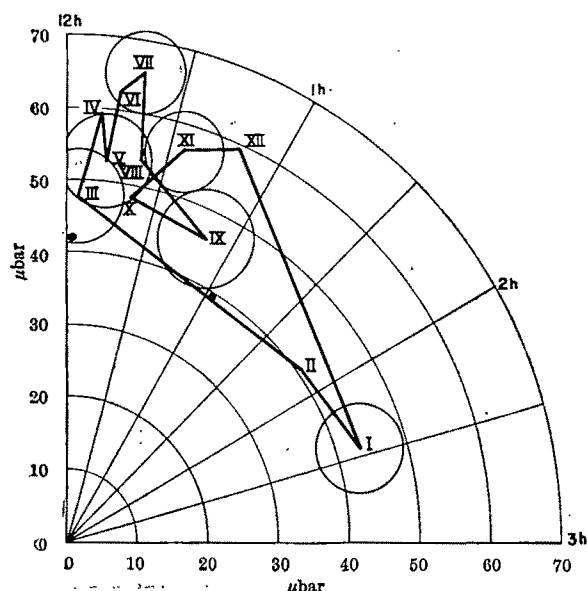


Fig. 4. Harmonic diurnal of the monthly means of the semidiurnal lunar barometric tide, Puerto Rico. Only every second circle of probable error is plotted. (Haurwitz and Cowley, 1966.)

expected that the tidal oscillations are slow enough to be isothermal. But the distances between regions of high and of low pressure are so great, in most latitudes, that no substantial heat flow can occur in the available time between the locations of greatest expansion and compression. Thus the motion should be adiabatic. This inference was confirmed<sup>12</sup> for Djakarta with the aid of 63 years of bi-hourly temperature data. The lunar temperature wave was found to have an amplitude of  $0.0086^\circ\text{C}$ , with a probable error of  $0.0030^\circ\text{C}$ , while the theoretical amplitude, assuming adiabatic changes, should be  $0.0072^\circ\text{C}$ . The phase constant is within  $2^\circ$  of that expected. It is possible that the atmosphere in contact with the ocean can conduct heat from or to it more readily than is possible at a land station like Djakarta, so that on a small oceanic island the tidal motions may not be adiabatic. A check of this surmise would require analysis of a long series of temperature data on the windward side of a small flat island. It is likely that thermal conduction in the high atmosphere, well above 100 km, is sufficient to make the lunar tidal motions there more nearly isothermal. But no data are yet available to check this.

The lunar air tide, like its oceanic counterpart, has associated tidal currents, and thus should also be apparent in the wind. Like the tidal barometric oscillation, the lunar tidal air motion is very small, with an amplitude of the order of 1 cm/sec. Because of the great variability of the wind, with much larger irregular variations than the lunar tidal amplitudes, scarcely any observational determinations of the lunar tidal wind have yet been made. The only published determination of the lunar tide is for Mauritius<sup>13</sup> ( $20.1^\circ\text{S}$ ,  $57.5^\circ\text{E}$ ) in the Indian Ocean, based on 16 years of data. The expressions found for the southward ( $u$ ) and eastward ( $v$ ) components are

$$u = 1.2 \text{ cm/sec } \sin(30\tau + 176^\circ) \pm 0.6 \text{ cm/sec}$$

$$v = 1.0 \text{ cm/sec } \sin(30\tau + 220^\circ) \pm 0.6 \text{ cm/sec}$$

This determination cannot be considered satisfactory; its probable error is too great. A more recent lunar wind determination for Balboa, Panama ( $9.0^\circ\text{N}$ ,  $79.5^\circ\text{W}$ ), based on somewhat more than 20 years of data, gave the following results:

$$u = 1.2 \text{ cm/sec } \sin(30\tau + 209^\circ) \pm 0.6 \text{ cm/sec}$$

$$v = 0.6 \text{ cm/sec } \sin(30\tau + 195^\circ) \pm 0.8 \text{ cm/sec}$$

This result is still less satisfactory. To reduce the probable error to acceptable proportions more data would be needed—the error decreases as the square root of the number of data. In this connexion it is of interest to note that a pilot study for Balboa based on one year of data (rather than 20 years as here) gave probable errors  $\sqrt{20}$  times larger, as is to be expected from the theory of errors.

#### • High Altitudes

Tidal theory shows that the wind speed due to the tidal oscillation must increase upward nearly in inverse proportion to the square root of the density. Under average conditions the density at 70 km is about one ten-thousandth of its surface value. Thus the speed of the lunar tidal wind should there be about 100 times larger than at the ground. The solar semidiurnal wind oscillations at those levels in fact have amplitudes of some tens of m/sec, compared with surface values of some tens of cm/sec. These solar semidiurnal wind oscillations at high levels have been determined by radio from the drift of meteor trains. These observations have permitted the determination of solar daily wind variations, but they are not numerous enough, as yet, to give the smaller lunar tidal oscillations. Greenhow and Neufeld<sup>14</sup> estimated from their radio meteor observations at Jodrell Bank that the amplitude of the lunar tidal oscillation is no larger than 2 m/sec at levels of about 80 km.

At these altitudes and higher in the ionosphere, additional indication of the lunar tide is obtained from ground based observations of geomagnetic and ionospheric parameters; indeed some of the first lunar daily magnetic variations were discovered only about 10 years after the first successful determination of the lunar barometric tide. The generally accepted explanation of these lunar daily geomagnetic variations, and of the corresponding solar daily geomagnetic variations, is given by the "dynamo theory". This theory attributes them to electric currents flowing in the ionosphere; which are induced by the mainly horizontal oscillatory motions, tidal and thermal, of the ionospheric air. The process resembles that in a dynamo: the moving air corresponds to the armature, the conducting ionospheric layers to the armature winding, and the geomagnetic field to the magnetic field of the dynamo electromagnet. From the current the type of the inducing air motion at these levels can be inferred, but to determine the speed requires a knowledge of the electric conductivity of the layers in which the current flows. Matsushita<sup>15</sup> from recent data on the lunar daily geomagnetic variations gave the following estimates of the required wind system ( $\theta$  = colatitude):

$$u = 1.7 \text{ m/sec } \sin\theta \cos\theta \sin(30\tau + 60^\circ)$$

$$v = 1.7 \text{ m/sec } \sin\theta \sin(30\tau + 150^\circ)$$

These amplitudes are more than a hundred times larger than those observed (and expected) in lunar tidal winds at the surface. As already explained, such an increase in strength with elevation is to be expected for this type of wave motion. The phase constant of the southward wind component  $u$  should be  $90^\circ$  larger, that of the eastward component  $v$  should be  $180^\circ$  larger, than the phase constant for the pressure oscillation. The surface pressure oscillation has a phase constant of about  $75^\circ$ , and Matsushita's wind determination from geomagnetic data would require for the lunar pressure variation aloft a phase constant of  $330^\circ$ . On the other hand, J. H. Chapman<sup>16</sup> has determined lunar tidal drifts in the  $E$  layer with amplitudes about ten times larger than those obtained by Matsushita. The phase constant of his determination agrees well with the theoretically expected values for both components, if it is assumed that the phase constant for the pressure variation is the same at the ground and in the  $E$  layer. Despite this consistency, the disagreement between these results and those of Matsushita and



Greenhow and Neufeld indicates that our knowledge of the lunar tide in the higher atmosphere is still very incomplete. Numerous data are available on the variation of geomagnetic and ionospheric parameters with lunar time<sup>15</sup>, but in most cases the interpretation of these data in terms of atmospheric motions, both horizontal and vertical, is still obscure.

Reliable lunar tidal variations of cosmic rays<sup>17</sup> have been found, mainly in the number of mesons received at the ground. From these measurements it has been inferred that the lunar tide at an elevation of about 18 km, where the mesons are generated by primary rays, is about ten times greater compared with the surface tide. Lunar variations have also been looked for and reported in other atmospheric parameters, such as the intensity of the oxygen green line 5577Å of the airglow<sup>18,19</sup>, the twilight intensity<sup>20</sup>, the polarization of sky light<sup>21</sup>, the rainfall<sup>22</sup>, the content of ozone in the atmosphere<sup>23</sup>, and the rate of meteors entering the Earth's atmosphere<sup>24</sup>. But the statistical basis of these relations is weak, and the physical connexions, if they do exist, are not clear. At least some of these relations, if they do prove to be real, may be semimonthly or monthly, not semidiurnal.

### Origin of Tides

According to the "equilibrium theory" of the tides, which ignores inertial effects, the amplitude of  $L_2$  at the equator should be 34  $\mu$ bar. As Fig. 3 shows, the amplitude is actually about 2.5 times larger. This is because the atmosphere requires a finite time to adjust to the changing tide generating force of the Moon. The result is a slight resonance magnification of the computed equilibrium tide, as observed. In order to determine the lunar tides to be expected from theory, the hydrodynamic equations of motion and continuity have to be used, together with a physical equation relating the pressure and density changes of the air<sup>25</sup>. For this equation it is generally assumed that the relation is adiabatic, and this assumption appears to be confirmed by the lunar tidal variation of temperature at Djakarta. The calculation shows that the surface pressure variation due to the lunar tidal force agrees reasonably well with what is to be theoretically expected. The actual amplitude and phase depend on the assumed vertical structure of the atmosphere. This was in particular shown by Sawada<sup>26</sup>, who computed them for various atmospheric models with different temperature distributions above the troposphere.

While there is thus no difficulty in accounting for the general distribution and magnitude of the observed lunar tidal oscillation, the irregularities of its distribution over the Earth, and the pronounced seasonal variation, require explanation. The lunar tidal potential is distributed quite regularly over the Earth; thus the irregularities in the barometric lunar tide must be due to the response of the atmosphere to the exciting force. One possible disturbing cause may be the ocean tide. Atmospheric tidal theory assumes in general that the Earth's surface is both horizontal and rigid; this is clearly not correct for the approximately 70 per cent of the Earth covered by water, which is subjected to tidal influences. Because of the irregular distribution of land and water this effect must be quite complex. (There will also be a smaller effect because of the solid-earth tide.) Sawada<sup>27</sup> has studied the effect of the ocean tide on the atmospheric tide, but only for an ocean completely covering the Earth. He found that such an ocean can indeed very appreciably affect the atmospheric tide, especially its amplitude. But more work is required to determine the effect of tides in oceans of limited extent, and to see whether it is in the right direction to explain the observed anomalies. Wulf and Nicholson<sup>28</sup> had earlier attempted a qualitative explanation of the geographical irregularities of  $L_2$  and its remarkable annual variation, stressing specifically the much greater and more widespread surface irregularities over the northern than over the southern hemisphere.

Sawada<sup>29</sup> has studied whether the effect of a zonal motion of the atmosphere, heretofore neglected in the theory of the lunar tide, would explain this peculiar annual variation. He showed that appropriate seasonal changes in the zonal wind distribution, especially its vertical shear, would account for the annual variation of  $L_2$ . This effect depends very much on the zonal wind distribution in both hemispheres. Unfortunately the wind distribution in the southern hemisphere is not yet sufficiently well known to decide whether this explanation of the annual variation of  $L_2$  is satisfactory. In any event the meridional temperature gradient associated with the zonal wind system has less influence on the annual variation than the winds themselves. But an annual variation of the mean global vertical temperature distribution could also give rise to the observed annual variation of  $L_2$ . The solution of this problem and of many others concerning the lunar tide lies in the future.

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In *Nature* (101, 359; 1918) there appears the following abstract of the proceedings of the Royal Meteorological Society—"Dr. S. Chapman: The lunar atmospheric tide at Greenwich, 1854-1917. The tidal forces due to the moon affect the aerial as well as the fluid ocean, and the lunar atmospheric tide is manifested by the periodic variation in the height of the barometer having two maxima and two minima (high and low tide) in the course of a lunar day. This variation is much smaller than the solar semidiurnal barometric variation, which is not a simple solar tidal effect; the minute lunar variation, however, can be detected with ease in the records of tropical observatories, where the irregular fluctuations of pressure are small. Attempts to determine it in the records of European observatories have been made, but hitherto without success. By treating hourly observations of 'quiet' days only, on which the barometric range did not exceed 0.1 in., and by abstracting the solar variation, the lunar atmospheric tide at Greenwich has now been ascertained. Its total amplitude is less than 0.001 in., the harmonic formula being  $0.00036 \sin(2t + 114^\circ)$  in., where  $t$  represents lunar time measured, at the rate of 360° per lunar day, from the epoch of upper transit. A comparison with the variation at Batavia (lat. 6° S.), viz.  $0.00256 \sin(2t + 65^\circ)$  in., suggests that the amplitude varies as the fourth power of the cosine of latitude, and that the phase also varies with latitude."

## BOOK REVIEWS

### STRUCTURAL ANTHROPOLOGY

#### *The Savage Mind*

By Claude Lévi-Strauss. (The Nature of Human Society Series.) Pp. xii+290. (London: Weidenfeld and Nicolson, 1966.) 45s. net.

WHEN the speculative, theoretical and comparative approach of the evolutionist school of anthropology fell into disrepute, it yielded to studies of single societies based on intensive field research. During the past half century a body of essential basic knowledge of societies and their cultures has been built up in a series of monographs, but the theoretical and comparative use made of this material has been relatively slight. Now, once again, there is a return to the comparative approach, but it is a return with a difference. In involves the search for consistencies of structures, functions and meanings through analysis in depth, making use of the findings of field research and in turn inspiring more detailed field studies.

In the forefront of this return to comparative anthropology is its chief inspirer, Professor Lévi-Strauss, who is both an ethnologist and a philosopher. *The Savage Mind*, first published in French under the title *La Pensée Sauvage* (1962), is an important landmark in the development of modern anthropological thought and might be said to have inaugurated the new era.

Already this book has brought about a profound change in attitude towards the philosophical systems and classifications of peoples throughout the world. Only a few years ago the primitive, intellectual level of thought was assumed to be as low as the technological and economic level. Primitive people in general lacked words for abstracts and did not think in general categories, only in concrete, particularistic terms—so it was asserted. When the occasional field worker produced evidence to the contrary both the field material and the conclusions were frequently doubted. This book once and for all destroys these assumptions, revealing their essential naivety by reviewing the wealth of primitive systems of thought and belief in many cultures, the ways of classifying the natural world and integrating this world with society and culture. Analytical reasoning, Lévi-Strauss shows, occupies a considerable place in all societies, primitive and complex alike. After reading this book no one can ignore the possibility of finding in his field of study elaborate classifications of categories, elements, species and numbers, of encountering a wealth of knowledge relating to natural life with complex classifications used in order to conceptualize society and culture. There is now no excuse for failing to make a deliberate investigation into these possibilities and their social significance.

The two major systems which are used as examples are totemic classifications and caste. The approach Lévi-Strauss uses is, in both cases, essentially a structural one. On one hand, the diversity of species in nature and on the other the diversity of functions on a cultural plane have furnished man with models of social classification and structure. The function of each is succinctly stated; totemic groups exchange women, castes exchange goods and services. Both are a means of securing or displaying the interlocking of social groups with one another.

Totemism is only a particular case of a general problem of classification, one of the many examples of the role which specific terms often play in the working out of a social classification (page 62). Names are discussed as

another mode, the prohibition on using the names of the dead being expressed as a structural property of certain systems of naming. The couvade, games and myths are other important subjects which are covered. This structural approach, not only to ritual forms but also to their religious and magical content, is particularly interesting since beliefs had not hitherto seemed to lend themselves to structural analysis in the way that institutions and general social organization have done.

The steps by which Lévi-Strauss reaches his conclusions are frequently complex and open to argument. His style of expression, moreover, is often turgid. Nevertheless, in spite of the disagreements and disputes which will rage around many of the author's assertions—for his approach is always a challenging one—his postulates invariably possess the virtue of that inspiration which produces creative thought. In the structural school of anthropology, of which he is the leading theoretician and proponent, we may disagree with Lévi-Strauss; we cannot afford to ignore his work which, when pondered over, increasingly illuminates the interpretation of social systems.

AUDREY J. BUTT

### MEMORY

#### *Aspects of Learning and Memory*

Edited by Derek Richter. Pp. x+182. (London: William Heinemann Medical Books, Ltd., 1966.) 35s. net.

THIS book contains eight chapters, by various authors, and are largely independent of each other. They are all concerned, in one way or another, with experimental evidence bearing on the problem of memory and learning. These range from a discussion of human psychology (M. Metcalfe) and of the effects of human brain damage (W. R. Russell, F. Newcombe and J. H. Brierley) through a discussion of learning in animals (I. S. Russell) and its possible neurological basis (J. H. Gaddum and B. S. Meldrum), to biochemical aspects of the problem (D. Richter). As will be apparent from this list of topics, the coverage is wide and the book draws the reader's attention to most relevant lines of research. The references are extensive and well chosen and the book is, on the whole, well written.

On the rare occasions where anything of an arithmetical nature is discussed, however, it is usually wrong or misleading. J. H. Gaddum (page 54) discusses the total number of possible patterns of instantaneous activity for the nerve cells in the brain and obtains the number  $2^{100}$ , rather than  $2^{10^6}$ , which is very different. His use of the word "pattern" is muddled and inconsistent—a pattern cannot at the same time cover all nerve cells and be spreading (pages 67–68). D. Richter's calculation of  $10^{130}$  patterns of synaptic modification (page 94) is based on such a special assumption that it seems quite pointless. It is wrong, anyway, to be very impressed by the size of numbers like  $2^{100}$ ; the information content of a choice from one of  $2^{100}$  is only 100 bits, while that of a choice from  $2^{10^6}$  is  $10^6$ , which is probably comparable with the human memory capacity (see, for example, page 91). On a different topic, I. S. Russell (page 164) states that Wilson, Oscar and Bitterman found matching in probability learning in the monkey and that this was because the monkey adopted the strategy of avoiding the previously rewarded alternative. This strategy would lead to anti-matching; the actual behaviour was more complex, the data sparse and I incline to think the matching observed was partly fortuitous.

I. S. Russell has marred an otherwise interesting chapter by a lot of loose talk about "data processing", "encoding", etc. Many biologists nowadays love using words of this sort, but it seems to me usually both pointless and undesirable. To those who are familiar with the words in their normal contexts, there are overtones which are by

no means necessarily correct in the biological situation and therefore the words are probably misleading at least. An example of positive error is the assertion on page 153 that retrograde amnesia in man shows that a certain period of time is required for memory to be encoded. As a distinction is made (page 156) between "encoding" and "storage", that assertion is clearly untrue.

There is a centrally important matter about which definite statements are made, but for which I think there is no solid evidence one way or the other. J. H. Gaddum (page 61) states that "most neurones in the brain of an animal are continuously active while the animal is awake" and B. S. Meldrum (page 118) that "every time a stimulus is received and a learned response made, most of the cells of the brain are involved". These statements may well be true, but I do not think we can be sure. Also one might usefully note the apparent inconsistency of the specific proposal (I. S. Russell, page 159) of the relevance of the hippocampal-fornix system for memory with the very interesting observation (J. H. Brierley, pages 34, 37) that bilateral section of the fornix has no apparent effect on memory.

The extent of criticism in this review is much more a reflexion of my own interest in the subject than of wholesale defects in the book. I can recommend it as a clear and, on the whole, well written and accurate survey of a large part of the field. In particular it is a pleasure to see that none of the authors has that uncritical approval of the RNA hypothesis about memory which we have seen so much of in recent years.

J. S. GRIFFITH

## NEMATODES

### Nematodes

By H. D. Crofton. (Hutchinson University Library.) Pp. 160. (London: Hutchinson and Co. (Publishers), Ltd., 1966.) 32s. 6d. hardcase; 13s. 6d. paperback.

NEMATODES may be unpopular with many students because some teachers cling to the absurd belief that the study of parasites is not quite respectable. Nevertheless, research on free living nematodes as well as forms which parasitize plants, animals and man has created the formidable subject known as nematology. These round worms originated in the sea and more than half the existing nematode species are still marine, less than a sixth living in fresh waters and soils, less than a quarter living parasitic lives. In spite of notable structural uniformity over an immense range of sizes, nematodes follow various modes of life and display versatility in the patterns of their life histories. The turgid body is covered by a complex proteinaceous cuticle. The muscles of the body wall act in conjunction with a pseudocoelomic hydrostatic skeleton to control form and play some part in locomotion. This unique turgor-pressure system is related to modified bodily functions: a muscular pharynx is needed to force liquid food into a collapsed intestine; defecation requires special muscles and reproduction special mechanisms—to inject non-flagellate sperms under pressure at copulation and to transport oocytes and eggs along the ducts of the female system. Functional simplicity is often more apparent than real; metabolic processes change according to conditions which may vary in the same habitat, and the basic life cycle with its five stages of growth and development and four intervening moults may be modified to fit in with special problems of parasitic life.

In dealing with general organization, the structure of the body wall and somatic musculature, various organ systems, embryology and development, metabolism, patterns of life history and the characters and features of the free-living and parasitic types of Nematoda, Dr. Crofton has condensed and dispensed a large amount of information and many interesting ideas which indicate

the directions of future progress within a very small space. He has given also an outline scheme of classification and a list of references, which could be enlarged with benefit in later editions. Many students and teachers of zoology will be grateful for this concise account of a group of animals which has very great economic and medical importance in the modern world and a much greater interest for many of us than some teachers believe.

The paperback edition will be a "must" for all students with an eye for good value.

BEN DAWES

## THE COMPLEAT RUMEN

### The Rumen and Its Microbes

By Robert E. Hungate. Pp. x+533. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 158s.

ACKNOWLEDGED as one of the most distinguished investigators in the field of rumen biology, Professor Hungate has drawn together the results of his own considerable research and those of other workers into a comprehensive and scholarly account of rumen ecology. The result is an outstanding contribution to our knowledge of microbe-ruminant symbiosis.

The book falls naturally into two sections. The first is concerned with the fundamentals of rumen biology and includes descriptions of the microbial populations and their activities, both from qualitative and quantitative points of view. Consideration is given to autecological studies of the various micro-organisms, but the author emphasizes that the microbial populations are interrelated in too many ways to allow simple interpretations of their symbiotic relationship to the ruminant. The diversity of microbes and the complexity of metabolism in the rumen are related clearly to the chemical heterogeneity of the plant diet. In this context the concept of maximum biochemical work is developed: of the various possible carbohydrate fermentation pathways, certain combinations effect greater cell yields than do others. Consequently, maximum growth necessitates a complex microbial population possessing many more pathways for biochemical work than are present in a single species. The author argues that carbon dioxide, methane, acetate, propionate and butyrate are the major fermentation products in the rumen because the anaerobic routes leading to them provide the most efficient conversion of substrates into microbial cells. This concept goes a long way in rationalizing the diversity of the rumen microbiota.

One of the most interesting expositions in this book is devoted to the kinetics of ruminant digestion and its approximation to continuous fermentation systems, an approximation which becomes remarkably close when animals are fed at frequent regular intervals. Increasing turnover rates in the rumen produce greater host yields but, as in continuous culture systems, increases in rate beyond the point at which microbial reproduction can maintain a constant population result in washing out. This discussion is not a restricted academic one; the reader's attention is directed to the importance of rumen turnover and to resultant feeding practices that are significant in ruminant economy.

The second part of the book evaluates the applied aspects of rumen ecology and covers such areas as ruminant metabolism, rumen variations, practical applications and abnormal rumen functionings. Here, too, are considered such factors as feed additives, including enzymes and antibiotics, and rumen inoculation. Throughout the discourse is made complete by reference to the techniques of rumenology—cultivation of micro-organisms, methods of sampling, estimation of feed digestibility and nutritional status of diets, to mention but a few examples.

A measure of Professor Hungate's success is unmistakable in the authoritative way in which he deals with the

multifarious aspects of his subject, whether he is considering the neuromuscular control of stomach movements or the physiology of cellulolytic bacteria. The book is well illustrated and the proof reading has been assiduous. The only detraction would appear to be its very high price.

ALAN T. BULL

## MUTUAL BENEFIT

### Symbiosis

Vol. 1: Associations of Micro-organisms, Plants, and Marine Organisms. Edited by Henry S. Mark. Pp. xviii + 478. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 132s.

THE original definition proposed by De Bary in 1879 states that symbiosis is a constant and intimate association between dissimilar organisms. Such a definition covers all forms of parasitism as well as mutualism and commensalism. Many modern workers prefer to restrict to symbiosis only those associations in which mutual benefit is involved. In electing to review the subject of symbiosis in the wide sense of the word, leaving out only the less relevant aspects of parasitism, Mark has taken on a very difficult task. The result is, however, in Volume 1, an excellent comprehensive review of a very wide range of associations involving micro-organisms, plants and marine animals.

Chapter 1, which deals with symbioses involving only micro-organisms, is particularly valuable, because this aspect of the subject is not well documented in review form in the literature. The next four chapters deal respectively with lichens, bacterial symbioses with plants, mycorrhizas and endozoic algae. Chapters 6 and 7 deal with symbioses involving marine animals, and Chapter 8 is concerned with methods for the experimental analysis of behaviour in symbiosis.

This volume will prove very valuable in highlighting similarities between apparently very different associations. Perhaps more important, it will indicate similar methods for experimental investigation of these associations. The review will be welcomed by all biologists working on problems of symbiosis.

B. W. FERRY

## NEUROSCIENCE IN JAPAN

### Correlative Neurosciences

Part B: Clinical Studies. Edited by T. Tokizaire and J. P. Schadé. (Progress in Brain Research, Vol. 21B.) Pp. xi + 437. (Amsterdam, London and New York: Elsevier Publishing Company, 1966.) 145s.

THE books in this series are always beautifully produced and often contain work of major importance. This is the second part of a work dealing with clinical neurological studies and the relationship of neuro-anatomy, neuro-physiology and neuro-chemistry to the clinical sciences. This is work entirely from Japan and for this reason alone it is of considerable interest. Of the sixteen papers only eight have any direct relationship to clinical subjects. There is a detailed description of the effects of hemispherectomy in ten patients which is beautifully illustrated and makes an important contribution to neurological science. The conclusions about thalamic participation in sensation in these cases are of considerable interest and illustrate how such clinical investigations can enrich the basic sciences.

There is a paper on an extensive epidemiological and clinico-pathological study of cerebral haemorrhage and cerebral-arterial disease in a small Japanese town. It could well serve as a model for similar investigations in this country. Another well documented and interesting survey is on multiple sclerosis in Japan.

One of the most important papers in the volume has already been published elsewhere. This is "Sedative Stereencephalotomy", which marks one of the first incursions into the human hypothalamus. A number of the papers appear to have little connexion with any practical clinical work, but there are three interesting and important papers on sleep.

It would have been interesting to see an account of Forel *H*-tomy in the treatment of epilepsy or, indeed, any of the significant contributions made by Japanese workers in the surgical treatment of dysrhythmia. Instead there is a scholarly account of the human triangular tract of Helweg.

This volume is representative of the considerable neuro-scientific work proceeding in Japan, and makes available material normally inaccessible in Britain.

EDWARD HITCHCOCK

## DOWN'S ANOMALY

### Down's Anomaly

By L. S. Penrose and G. F. Smith. Pp. vii + 218. (London: J. and A. Churchill, Ltd., 1966.) 42s.

THIS book about Down's anomaly (mongolism) covers a field of wide importance and implications to human biology, which transcends what could be considered the rather narrow boundaries of the study of mental deficiency. It makes its timely appearance on the hundredth anniversary of Langdon Down's celebrated description of "mongolian idiocy" and seven years after the recognition of the autosomal trisomy that is the cause of the clinical syndrome. The authors are clearly at home with the subject, and the fact that the senior author has for years given a great deal of original and stimulating thought and research energy to the subject of this book is amply revealed in its outstanding excellence and clarity and thought-provoking quality. After a sweeping historical introduction, which outlines the stages of the characterization of the disorder, the authors review physical signs, devoting special attention to the facial characteristics, including those of ears and eyes, and to those of hands and feet. Various aspects of the intellectual development of the affected person are given prominence, while the limitations of the formal I.Q. studies are critically examined, and the more modern but as yet scanty studies done with methods devised to test specific neural faculties are stressed. Social maturity, which is advanced in these patients, and their personality traits and developmental patterns are given due consideration in the light of their importance to adaptation of the patient and his integration into the community. A chapter is devoted to the distinctive dermatoglyphics found in Down's syndrome and a section of this considers the effects of somatic chromosome mosaicism on the sensitive dermal patterns.

There is a chapter on the haematological changes, in which the increased incidence of acute childhood leukaemia is emphasized, and one on the biochemistry of Down's anomaly which reviews the older work and stresses the more recent descriptions of serum and cell-enzyme changes and of metabolic alterations and their relationship to the trisomic state. The clinical diagnosis of Down's syndrome, the discriminative value of single traits, both metrical and qualitative, and of combinations of characters are given prominence, also because these elements are essential to a proper interpretation of the chromosomal findings in the light of the phenotype, particularly in doubtful cases with mosaicism or with so-called partial trisomy. Thirty pages are devoted to the description of the cytological findings. Standard (or primary) trisomy and the identity of the specific autosome, usually defined as number 21, are considered. Next the centric-fusion type of interchange involving a number 21 and a member of the 13-15 or 21-22 groups, or long-arm isochromosome

formation, are discussed, together with their origins and transmission, in some instances by asymptomatic balanced carrier parents. Chromosome mosaicism and the occurrence of double trisomy, for example for number 21 and XXY, are considered in detail. There follow a chapter on vital statistics in which mean survival age (about ten years or so in some statistics), causes of death and secular changes are discussed, and one on the aetiology of the anomaly. Here the different possible causes of Down's syndrome independent of maternal age are detailed and ideas are put forward about possible factors in the origin of the maternal-age dependent trisomy. Treatment and prevention, for example, by limiting conceptions after a certain maternal age, are considered. The genetic prognosis for those families in which an affected child is born is discussed, and the importance of chromosome studies of the patient and, if necessary, of the parents is emphasized.

The authors of this admirable book express the hope that their way of presenting the material may not only show the advances made but also stimulate further work. They cannot fail to succeed, because they have already achieved their aim of getting at least one reader both interested and stimulated.

P. E. POLANI

## BIOLOGY AND STATISTICS

### Biostatistics

By Alvin E. Lewis. Pp. xi + 227. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1966.) 68s.

THIS is an elementary and essentially non-mathematical exposition of statistical ideas and methods, illustrated with examples mainly from biological sources; it is intended for "students in biology and medicine who have reached the stage where they are ready to judge data and to begin their own investigations and experiments". The standard topics of probability, random sampling, the normal distribution, the chi-squared distribution, tests of significance, and the analysis of variance are all presented, and there are brief chapters on quality control, sequential analysis and distribution-free methods. There is a summary and a good set of examples at the end of each chapter, and an excellent appendix of statistical tables.

It is extremely difficult to write a good statistical text with such a scope and aim, and Dr. Lewis only partially succeeds. Many of his approaches are good, such as the introduction of the *t*-test for paired observations before that for two separate samples. Others are less satisfactory, such as introducing the analysis of variance by way of the variance ratio test; this may mislead the beginner into regarding it as a method for studying heterogeneity of variances when the aim is to study heterogeneity of means. Some of the examples are a little strained ("spherical seeds") and at times the author gives the impression of not being quite at home either with biology or statistics.

IAN SUTHERLAND

## ORIENTATION. STATISTICS

### Structural Diagrams

By Andrew B. Vistelius. Translated by R. Baker. Translation edited by N. L. Johnson and F. C. Phillips. Pp. xi + 178. (London and New York: Pergamon Press, 1966.) 80s. net.

THIS book was originally published in 1958, and deals with the treatment of orientation data from structural geology and petro-fabrics. These data are usually too complex to be tested statistically by the methods used by palaeomagnetists; and although tests have been proposed,

none has been accepted for general use by structural geologists.

In such tests, including those of Vistelius, the results are plotted on a two-dimensional projection. Vistelius rejects the use of equal-angle and the equal-area projections in favour of an equal-interval projection. His arguments are not convincing and his choice increases the labour of testing. He warns against the use of polar instead of equatorial versions of projections, although they give identical plots of data. An introduction to probability theory and statistics is provided, but it is difficult to follow because too much statistical jargon is used in an attempt to be rigorous, and symbols change their meaning from equation to equation and even within paragraphs. Some of the tests proposed before 1958 are critically reviewed.

Vistelius then proposes four new tests which all involve determining the numbers of poles (data) falling in each of the cells of a net laid over the two-dimensional projection of the data. The  $\chi^2$  and normal distributions are used to test for significant differences between the frequency distributions representing diagrams under test, and frequency distributions representing model random diagrams or other real diagrams. The tests include various objectionable features. The nets of cells are tailored to fit the data, which makes the tests partially subjective. The first test uses a false model of random distribution. The cells are so large that they tend to smooth out or randomize the data, thus decreasing the chance of detecting weak preferred orientations; yet the detection of weak preferred orientations is the most important task these tests can perform. Nevertheless, the second and fourth tests given by Vistelius are novel and could be useful if improved.

The book is poorly translated and badly edited. Typographical errors include "n" in place of "m" in Table A14 and a mis-reference to an equation (page 45). Errors arising from a failure to understand the subject are more common and give rise to meaningless and even self-contradictory sentences. Such errors include the literal translation of obscure Russian sentences; unjustified changes in the original punctuation; the omission of "not" in one sentence; and inversion of a mathematical substitution on page 44. Mis-translations include "position" instead of "attitude", and "fissure" instead of "joint". "Exposure", "cell", "thin section", and "cut" in "cut effect" are all translated as "section", causing considerable confusion. Equations 2-13 and 2-20 have been changed from the original without appropriate changes being made in the text.

The book is concerned solely with the statistical testing of diagrams. There is no discussion of sampling methods and neither discussion nor interpretation of the patterns of preferred orientation is shown by structural diagrams.

DEREK FLINN

## OPTICAL ANALYSIS

### Treatise on Analytical Chemistry

Edited by I. M. Kolthoff and Philip J. Elving, with the assistance of Ernest B. Sandell. Part I: Theory and Practice, Vol. 6. Pp. xxiii + 3347-4246. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 175s.

THIS book deals with optical methods of analysis. The ten chapters cover a wide field, from emission spectroscopy to electron microscopy, and from flame photometry to neutron absorption.

A scientific critique of such a wide range of disciplines is clearly beyond the scope of a single reviewer; more is to be gained by taking the position of an interested reader seeking guidance. A random spot-checking within the various chapters for information on specific items gave me



a most favourable impression. The authors of individual sections have achieved a satisfactory balance between the encyclopaedic and the critical and they have together produced a creditable exposition of dispassionate scholarship. The "mainstream" analytical chemist will welcome particularly the chapters on microscopy and refractometry where a hitherto scattered literature has been collected into a unified whole with an analytical emphasis.

With several of the techniques discussed the problem is not complete when a result is read from the instrument; representation, storage and retrieval of information are becoming increasingly pressing problems, and the authors have rightly considered these in fair detail.

Weaknesses can inevitably be found in a work of such dimensions. For example, Chapter 1 on emission spectroscopy lists 185 selected literature references, yet line broadening is discussed without reference to the literature and hollow cathode sources are described without reference to design considerations. These are, however, minor shortcomings in a volume notable for clarity of layout and excellence of literary presentation.

The firm editorial hand is evident throughout in the maintenance of overall balance and adequate cross-referencing between this and other volumes in the series. This volume more than maintains the high standard set by earlier ones in the series and is encouraging testimony to the worthwhile nature of the daunting task which the editors have undertaken.

J. K. FOREMAN

## FRESH IDEAS IN SOLID STATE THEORY

### Many Body Theory

Edited by Ryogo Kubo. (1965 Tokyo Summer Lectures in Theoretical Physics, Part 1.) Pp. iv+160. (Tokyo: Syokabo; New York: W. A. Benjamin, Inc., 1966.) \$7.45.

THIS book contains ten of the more important contributions to the 1965 Tokyo Summer Lectures in Theoretical Physics. It is to the credit of Professor Kubo, the editor, that it escapes the scrappiness and lack of cohesion which can result when isolated papers are detached from the coherent background of cross-questioning and informal discussion against which they were originally presented.

The contributions form a well-balanced selection, though the authors do not always attack their subject on the same level: H. Mori's article on "Relaxation Phenomena near the Critical Points" requires careful study, while D. Pines's "Elementary Excitations in a Homogeneous Base Liquid" is more intuitive, although it ends with some interesting speculations on the occurrence of zero sound. Between these extremes lies a wide spectrum of different approaches, but they have this in common: the authors are concerned with physically relevant ideas, and for the most part include only enough mathematical formalism to clothe them respectably. Nevertheless, the mathematics makes no concessions to the unsophisticated, and heavy reliance is placed on diagrams.

The presentation is consistently clear, and the many misprints are not, on the whole, obtrusive. J. M. Luttinger's "A New Mechanism for Superconductivity" is, however, mathematically cryptic, and the elision of definite and indefinite articles, common in "Japanese" English but rare elsewhere in this book, is an unnecessary barrier to comprehension.

Apart from those already mentioned, the authors include K. A. Brueckner, W. Kohn, J. R. Schrieffer and P. G. de Gennes. To the solid state theorist these are names to conjure with, and they do not disappoint here: Brueckner's articles on "Liquid Helium-Three", "Nuclear Structure" and "Correlated Crystals" are lucid and com-

prehensive, while Schrieffer studies the quasi-particle approximation in normal and superconducting metals, using Migdal's simplification of the electron-phonon vertex function to obtain explicit expressions for the electron self-energy valid to all orders in the electron-phonon coupling strength. W. Kohn, summarizing his own work on the subject, formulates the problem of the inhomogeneous electron gas in terms of a functional  $F[n(r)]$  characterizing the ground state, while de Gennes discusses Landau-Ginsburg theory and Type II superconductors.

In short, ideas abound in this lively volume; many of them need the firm anchorage of further work, but this is not in the nature of a criticism. I recommend the book both to graduate students and to more senior workers who are seeking inspiration suitably leavened with information.

A. E. K. DOWSON

## NMR THEORY

### The Theory of Nuclear Magnetic Resonance

By Igor Vladimirovich Aleksandrov. Translated by Scripta Technica, Inc. Translation edited by Charles P. Poole, jun. Pp. x+197. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 70s.

THIS book is a translation of the original Russian version which was published in 1964. In view of its title and contents, it immediately invites comparison with the classic and authoritative book by Abragam which was published in 1961. Incidentally, it is surprising to find no reference to Abragam's book in this. The comparison is not favourable to Aleksandrov, for his book gives an impression of a laboured and pedestrian approach, in spite of some quite elegant mathematics, and one feels that the author experienced no joy in his work. The field is one capable of more exciting and yet sound presentation, as has been shown by Abragam, Slichter and Pople.

The book presents the basic theories of magnetic resonance relaxation, a few topics in the theory of line shapes in solids, the theory of the chemical shift and the indirect spin-spin coupling in molecules, and in a final chapter a hotchpotch of minor problems in nuclear magnetic resonance. This presentation is in quite a logical order but does not achieve the unity required of a book, and this is emphasized by the different notation used in reporting the theories of Kubo and Tomita, Bloch and Redfield. While this is helpful to persons familiar with their notations, and it is no doubt deliberate, it makes it very difficult to compare the several theories. No real and searching comparison of the differences and relative advantages of the theories is presented, which is unfortunate because many experts in the field would be glad to see this discussed.

The discussion of the difficult and complex matter of the chemical shifts and  $J$  couplings is thorough, but I suspect that it may by now be rather out of date in view of the great interest in this field. It is remarkable that here, as indeed throughout the book, virtually no experimental results are quoted. They are unusually lacking even for an avowedly theoretical treatment and they are certainly necessary in the molecular theory which is notorious in its disagreement with experiment.

The translation is not convincing and the editing is not thorough. There are words not to be found in English or improperly used (kinetical, nontrivial, nonzero, etc.), a number of misleading mathematical errors ( $t-t_0-i\hbar\lambda$  for  $t_0-t-i\hbar\lambda$ , errors of sign, omission of symbols, etc.), and a large number of printer's errors (Poole for Pople, both  $T_r$  and  $S_p$  used, etc.). The price is on the high side. A useful reference book but no more.

J. G. POWLES

## USES OF ISOTOPES

**Les Radioéléments, Facteurs de Progrès Economique**  
Par Albert Nils. Pp. xii+214. (Paris: Dunod, 1966.)  
32 francs.

As an introduction the reader gets eight pages of very elementary explanations about the nucleus, the atom, artificial radioactivity and the production of artificially produced radioisotopes, written for a man with some basic knowledge of chemistry but not necessarily of physics.

The second chapter "Nuclear Energy and Radioisotopes" deals in a very succinct way with the present demand and resources of energy. The figures given in this chapter might be slightly out of date. The main part of the book is a summary of other publications on the advantages—mainly the financial ones—of using radioisotopes in industry, but also in medicine and agriculture.

It must be admitted that it is very difficult to undertake such a study in detail and therefore there are bound to be some shortcomings in such a book. Perhaps too great a simplification is applied to the economic treatment of isotopes in medicine and agriculture where, for example, entomological considerations such as the sterile male technique are treated in a few lines without emphasizing the enormous economic advantages of this technique. On the other hand, food preservation or extension of the shelf-life of food has been treated in far more detail. The main emphasis, however, is on the industrial uses of isotopes and here some summaries and the main figures have been taken from the *I.A.E.A. International Survey on the Use of Isotopes in Industry*.

While the subject matter is surely of great interest to agriculturalists as well as to industrialists, the book is not written for them, or for the scientists or technicians. For the scientist it is perhaps too technical and for the technician it is not technical enough. It also has a bias to Belgian conditions.

I think that an industrialist, by quickly glancing through this book, might get quite a lot of first-hand information which might be useful to him although by no means comprehensive. A complete survey would be an extremely difficult, if not impossible, task.

Generally, it can be said that this book does not contribute much more, or in better form, than some of the publications referred to in its bibliography, but does contain some detailed information giving an idea of the field.

HENRY SELIGMAN

## Introduction to Atomic and Nuclear Physics

By Rogers D. Rusk. Pp. xiv+470+12 plates. (London: Iliffe Books, Ltd.; New York: Appleton-Century-Crofts, 1965.) 63s. net.

THIS book sets out to describe a large area of what is frequently called modern physics, aiming at providing an introduction for undergraduates. It treats a broad range of topics: quantum mechanics, relativity, atomic and molecular spectroscopy, nuclei, and glances at solid state physics and elementary particles. I think it is a failure, because the author is too frequently inaccurate, or expresses himself in a misleading way; in many cases these two possibilities are difficult to distinguish. As a reasonably typical example I quote from p. 323: "the suggestion by Heisenberg that the forces binding a proton in the nucleus to a neutron in the nucleus might arise from exchange of charge such that the proton momentarily becomes a neutron and the neutron momentarily becomes a proton and the two may scarcely be said to lead independent lives". Knowing something about this subject one can see some relation to the truth in these remarks but one cannot agree that they are correct; it is difficult to decide how much is actually wrong and how

much is misleading presentation. Whichever it is, an undergraduate would surely be confused. There is a high density of such confusing statements, in places at least one to each page, so that it seems impossible to recommend the book. I also remark on another fault, which also occurs in other books. Each chapter is followed by a short list of books for "Recommended Reading". These range from popular accounts to advanced textbooks, many covering a much wider area than the particular chapter. The chief result of such general recommendations, without any guidance about what can be recommended in each book, must surely be to produce severe indigestion. I think this practice should be deplored.

A. B. CLEGG

## Introduction to the Theory of Flow Mechanics

By Albert Betz. Translated by D. G. Randall. Pp. xvi+281. (London and New York: Pergamon Press, 1966.) 75s. net.

THIS book is an edited translation of the German work of Betz, first published in 1959. The difficulty with textbooks on turbo-machine theory (and there are not many) is that they are either too elementary or they present a collection of mathematical solutions of various problems in the field. Lack of space usually prohibits a thorough description of these solutions so that much of the material is available only in the original papers. This book suffers slightly from this fault—although it can be read with immediate profit. A good collection of references is included, many of them to original work of many years standing and to much of the German work.

The contents are roughly as follows: Some twenty pages are devoted to general principles, a fairly good knowledge of fluid mechanics being assumed. The next fifty pages briefly discuss flow in ducts, including topics such as diffusers and boundary layers in curved ducts. This is followed by some seventy pages on cascade theory in which approximate treatments of ideal fluid problems are interleaved with discussions of the effects of losses, compressibility and end effects. The remainder of the book, apart from the appendixes, contains a discussion of almost every type of flow machine (including very short descriptions of thermodynamic machines, such as ram-jets and pulse jets); the treatments are in many cases rather too brief. The appendixes refer mainly to the sections on cascade theory and include Betz's vortex influence charts and his cascade velocity and state diagrams. Perhaps the best part of the book is the section on cascade theory, although it is a pity that the translator could not have included an appendix on at least one of the theories outlined by Betz (in particular, the work of Isay). The translator is to be congratulated on achieving a most readable text.

J. W. RAILLY

## Problems of Atmospheric Circulation

Edited by R. V. Garcia and T. F. Malone. (A Session of the Sixth International Space Science Symposium, Mar del Plata, Argentina, May 11–19, 1965.) Pp. vi+186. (Washington, D.C.: Spartan Books; London: Macmillan and Co., Ltd., 1966.) 58s. net.

At a price which most research workers can afford for their private shelves, this book provides in one volume several review articles of the highest quality as well as much other material of great interest to all concerned with the broad problems of atmospheric physics.

There is a discussion on radiation and dynamics in the upper atmosphere from the vigorous laboratory at Leningrad under Professor K. Ya. Kondratiev. Professor R. E. Newell (Massachusetts Institute of Technology) contributes a brilliant survey of the energy and momentum budget of the atmosphere above the tropopause. W. L. Webb combines and discusses the results of the

American Meteorological Rocket Network up to January 1, 1965. There are details of the *EOLLE* drifting balloon experiment (P. Morel) and of the even more elaborate *GHOST* scheme (V. E. Lally). Professor K. P. Feoktistov and others give a fascinating account of various observations in atmospheric optics made by the Soviet astronauts aboard the *Voskhod*.

These and five other papers (I overlook two useless abstracts) rather more than fulfil the editors' claim to "offer an up-to-date account of the progress made in the use of rockets and satellites for meteorological research and of the promises for the near future".

The book is very well produced.

C. D. WALSHAW

### Bio-organic Mechanisms

Vol. 2. By C. Thomas Bruice and Stephen J. Benkovic. (Frontiers in Chemistry Series.) Pp. viii+419. (New York and Amsterdam: W. A. Benjamin, Inc., 1966.) \$25.

THIS book is the second volume in Drs. Bruice and Benkovic's survey of bio-organic mechanisms, which they define as "those mechanistic studies likely to be of importance in the understanding of enzymic reactions or chemical transformation of biochemically important compounds such as the co-factors". This book is intended as a reference work for research workers and advanced graduate students and contains chapters on phosphate esters, phosphoro- and phosphonohalides, polyphosphates, thiamine pyrophosphate and pyridoxal-5-phosphate, nicotinamide, nucleotides, folic acid and biotin. It achieves its objective as a reference work on biorganic mechanisms from the standpoint of a physical organic chemist, and for this reason alone would make a valuable addition to an enzymologist's bookshelf. There is, however, a marked bias towards the study of model systems as opposed to studies on compounds of known biological significance and enzyme reaction mechanisms. For example, three chapters on phosphate derivatives without an adequate discussion of enzyme phosphate transfer mechanisms, the mode of action of phosphorohalides and the properties of nucleotides and polynucleotides cannot be described as "reasonably complete coverage". Nevertheless, the objectives of the book are fully realized in the succeeding chapters on enzyme co-factors. It is unfortunate that a chapter on flavins could not be included in this volume.

D. E. GRIFFITHS

### Genetics of the Dog

The Basis of Successful Breeding. By Marca Burns and Margaret N. Fraser. Pp. viii+230+18 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1966.) 45s.

THE first edition of this book, by Dr. Marca Burns, a livestock breeding research worker and a breeder of cocker spaniels, appeared in 1952. The present edition, to which Miss Margaret Fraser, of the Commonwealth Bureau of Animal Breeding and Genetics, has contributed chapters on behaviour, is much enlarged, and the whole brought up to date in the light of much recent work on dog genetics. Genetic abnormalities in dogs, some of them distressing to the dog and unattractive to its owner, are common: some would say far too common, although the authors point out that they occur in mongrels perhaps as frequently as in show breeds. There is, however, little doubt that favouring certain extreme characters on the show bench can bring suffering to the dog and little credit to breeder or judge.

This balanced and well documented account of the genetics of the dog is written in language that any intelligent breeder can understand. It ought to lead to more sense and humanity in pedigree breeding than have been seen in certain breeds—bulldogs, dachshunds and German shepherds, to mention only three. There are eighteen plates (one in colour), 559 references, an index of breeds and a subject index. The book is well produced.

W. LANE-PETTER

## OBITUARIES

### Professor H. H. Swinnerton

EMERITUS PROFESSOR HENRY HURD SWINNERTON died on November 6, aged 91. He was a palaeontologist of world-wide repute, an outstanding teacher of the natural sciences and a man regarded with deep affection by many students and colleagues. Swinnerton belonged to a generation of scientists with many interests which it is difficult to match today. He took his degrees in London as a zoologist, and moved to the department of natural sciences at Nottingham to teach zoology, geology and botany, and developed the department of geography there; but he made his main scientific impact as a geologist, with archaeology and the study of modern land forms as sidelines.

He was awarded his D.Sc. at the Imperial College of Science and Technology, for investigation of the skeletons of the primitive lizard *Sphenodon* and modern fishes. There, influenced by Professor A. Morley Davies, he developed the interest in palaeontology which became his main scientific concern. In all his writings, and particularly in his classic textbook *Outlines of Palaeontology*, he stressed the dynamic aspects of evolution and classification, and in his presidential addresses to the Geological Society and to the British Association he dealt particularly with the mechanics of evolution. His other researches included work on the Triassic rocks of the Midlands and their fish faunas, the stratigraphy and fauna of the Cretaceous rocks of Lincolnshire, the glacial and post-glacial deposits of the East Midlands and the Lincolnshire coast and the physiographical development of eastern England. He also wrote a series of popular books on geology.

Apart from science he had a particular interest in young people and their intellectual problems—problems which had worried him while he resolved the conflict between religion and science—and he gave a great deal of his time to this in the Church and in the schools with which he was connected. In retirement Swinnerton continued his work on the Jurassic oyster *Gryphea*, a highly variable genus of which specimens were available at many horizons in numbers sufficient for statistical analysis; his final paper was published a year before his death.

P. E. KENT

### Michael Stewart Pease

MICHAEL PEASE died on July 27, aged 76. He had retired as director of the Poultry Research Station in 1957.

Pease took his natural sciences tripos and diploma in agriculture at Trinity College, Cambridge, in 1909–13. He went to Germany to do postgraduate work in genetics, but at the beginning of the First World War he was interned in Ruhleben camp. He returned to England in 1919, and joined Professor R. C. Punnett at the Small Animal Breeding Station, where they worked on the development of autosexing breeds of poultry—animals which show differential sex characters when they hatch.

In 1934 he went to the Animal Nutrition Institute, and from 1940 he was also in charge of the Small Animal Breeding Station. In 1951 he took up his last post at the Poultry Genetics Station and became a senior principal scientific officer. Until his retirement Pease worked mainly on the effects of inbreeding and subsequent crossing on poultry. Some of the lines of chickens which he developed are still used by immunologists, as the only highly inbred chickens available. Pease never held a university post, but he was a regular lecturer in animal genetics at the School of Agriculture in Cambridge.

Pease, who came of a Quaker family, was socially and politically very active. He was a parish councillor; a member of his rural district council for thirty-eight years, and of Cambridgeshire County Council for eight years. He received an O.B.E. for political and public service.

# High Energy Neutrons from the Sun

by

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P. J. LAVAKARE  
R. SUNDERRAJAN

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A balloon flight made from India on April 15, 1966, has provided new evidence that energetic neutrons can be produced directly in solar flares

THE emission of energetic neutrons from the Sun and their detection near the Earth could reveal the presence of fast charged particles in the coronal regions of the Sun. It would also help to provide information about conditions on the Sun at times of intense solar activity which would be free from the bias associated with the detection of fast charged solar particles near the Earth arising from the existence or otherwise of favourable conditions near the Sun and in interplanetary space. If the neutron production spectrum at the Sun is of the type deduced by Lingenfelter *et al.*<sup>1</sup>, then, because of the neutron half-life of about 12 min, the differential energy spectrum of the neutrons when they reach the Earth's orbit will have a maximum roughly in the region of 50–100 MeV if the proton spectrum responsible for the production of neutrons is assumed to be similar to that in solar proton events. The neutrons arriving in the vicinity of the Earth would reach the top of the atmosphere with equal intensity at all latitudes, thus implying that experiments made from equatorial latitudes will have an advantage in that the general background of neutrons produced by charged particles of galactic and solar origin will be a minimum. The first possible evidence for the production of solar neutrons was reported from this laboratory<sup>2</sup> from an experiment made with freshly coated nuclear emulsions flown in a balloon flight made on March 23, 1962, from Hyderabad (17°6' N., 78°5' E.). The purpose of the present report is to present the first positive evidence for a solar

neutron event using a scintillator-spark chamber detector assembly flown on April 15, 1966, also from Hyderabad.

The detector system shown in Fig. 1, consisting of the producer, the scintillators  $S_1$  and  $S_2$  and the spark chamber, was flanked on five sides by five plastic scintillators 1 cm thick each directly viewed by a 2 in. photomultiplier. The sixth side was left open for photographing the spark chamber. The discrimination levels for the pulses from  $S_1$  and  $S_2$  which trigger the spark chamber were separately set at a value corresponding to  $2.5 \times I_0$  where  $I_0$  is the ionization of a relativistic singly charged particle; this setting corresponds to an energy release of  $\geq 2$  MeV in each scintillator. The spark chamber was triggered, and an event recorded, when there was a coincidence pulse in  $S_1$  and  $S_2$  with no pulse in any of the anticoincidence scintillators; the event was recorded by photographing the spark chamber, together with a timer watch, by a stereoscopic camera. The recorded events were classified under the following six types: *Blank* is an event in which none of the spark gaps has fired,  $G_1$  represents an event in which only the top gap has fired,  $G_{1-2}$  is an event in which the two top gaps have fired and similarly  $G_{1-3}$ ,  $G_{1-4}$  and  $G_{1-5}$  are events in which the top three, four and five gaps respectively have fired.

The different kinds of events to which the present instrument could respond are the following: (i) Nuclear disintegrations produced within the anticoincidence shell by neutrons of energy  $\geq 50$  MeV. (ii) Favourable neutron-

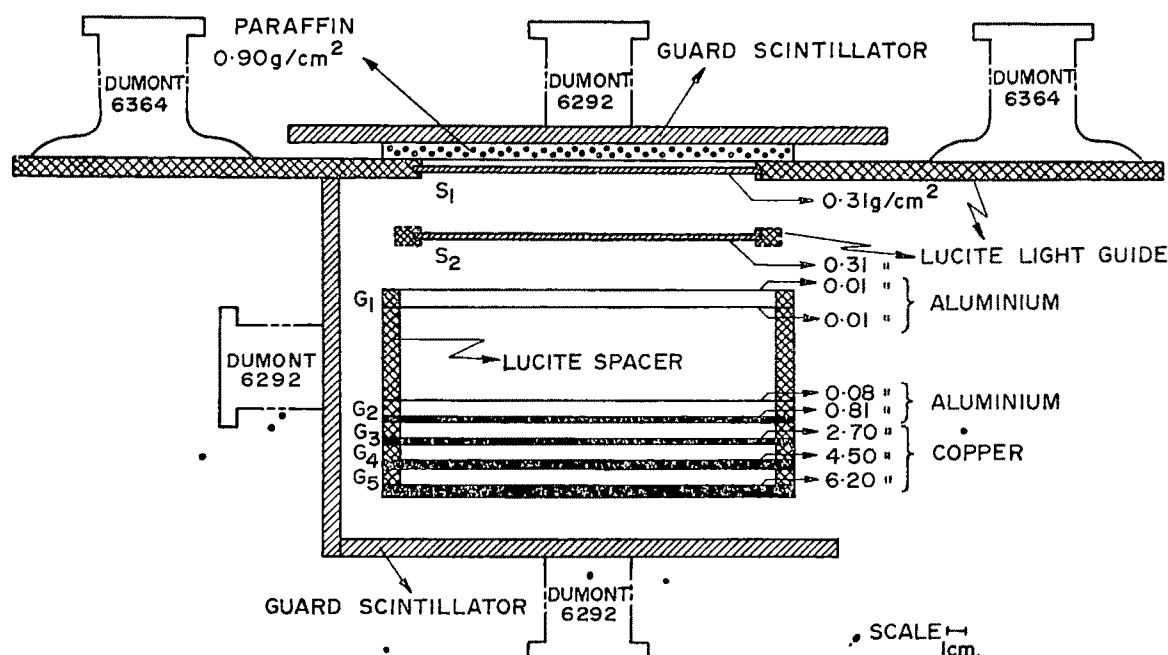


Fig. 1. The high energy neutron detector.  $S_1$  is viewed by two photomultipliers through a light guide at right angles to that of  $S_2$ . The guard scintillators constitute the anticoincidence shield on five sides.

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proton elastic collisions occurring in the paraffin producer or the scintillators  $S_1$  and  $S_2$ ; for energies  $> 50$  MeV, events due to this process will be much less than 10 per cent of those due to (i); and for energies  $< 50$  MeV, it would be the most important process giving rise to events predominantly of types *Blank* and  $G_1$ . (iii)  $\gamma$ -Rays materializing within the anticoincidence shell; the efficiency of the detector for such events is very low because of the triggering system which demands an energy release  $\geq 2$  MeV in each of the two scintillators  $S_1$  and  $S_2$ . (iv) Slow secondary protons having the right geometry and energy, leaking into the detector through the camera window. Because of the geometry of the detector system such events also would contribute predominantly to *Blank* and  $G_1$  events only. (v) Fast protons entering through the camera window can produce nuclear disintegrations in any constituent part of the detector within the anticoincidence shell and, if the resulting secondary particles satisfy the triggering conditions, an event can be recorded. A simple estimate of this contribution from known fluxes of fast protons at the flight altitude, and the characteristics of interactions they produce, clearly shows that this kind of event will be  $< 10$  per cent of the events due to atmospheric neutrons at 10 mbar. It is clear from the foregoing observations that events of the type  $G_{1-2}$ ,  $G_{1-3}$ ,  $G_{1-4}$  and  $G_{1-5}$  arise mainly from nuclear disintegrations produced by high energy neutrons (process i).

The detector assembly was launched in a balloon flight made from Hyderabad, India, at 0717 IST on April 15, 1966. The balloon reached a ceiling altitude of 11 mbar at 0930 h and floated there at constant altitude (within about 1 mbar) till the instrument was cut down at 1345 h. The detector system was switched on when the balloon reached an altitude of 210 mbar. Owing to the high counting rate at the ceiling altitude, we could get records only up to 1100 h when the film was exhausted. The stereophotographs obtained during the flight were scanned and the counting rates determined for the different kinds of events for 10 min intervals. From an analysis of the spark

chamber photographs it was found that the efficiency of the top four gaps remained constant within statistical errors and had mean values of  $77 \pm 4$  per cent,  $83 \pm 4$  per cent,  $62 \pm 7$  per cent and  $80 \pm 10$  per cent for gaps  $G_1$ ,  $G_2$ ,  $G_3$  and  $G_4$  respectively.

A careful examination of the different counting rates summarized in Fig. 2 reveals three striking features which provide positive evidence for the emission of solar neutrons:

(i) There is a broad maximum in the total counting rate at  $\sim 100$  mbar (corresponding to the Pfotzer maximum) followed by a decrease which continues up to about 25 mbar where the counting rate is  $34.6 \pm 1.8$ ; it then starts increasing rapidly (beginning at about 0900 h) as though there was a sudden increase in the influx of particles responsible for the events.

(ii) The total counting rate has a value of  $49.6 \pm 2.2$  when the balloon reached the ceiling altitude and it continued to increase up to a maximum value of  $77.0 \pm 2.8$  during the time the balloon was at constant ceiling altitude; the general level of counting rate at the ceiling altitude is higher than that expected from secondary atmospheric particles by a factor of about three.

(iii) The variations in the counting rates at the constant ceiling altitude are far too large to be accounted for by statistical fluctuations.

That the detector system worked normally in the altitude region  $> 25$  mbar is seen from the fact that the absorption mean free path obtained from the present investigation between 1,000 mbar (ground observations) and 200 mbar is  $162 \pm 7$  g/cm<sup>2</sup>; this may be compared with the value of 150 g/cm<sup>2</sup> obtained by Hess *et al.*<sup>3</sup> for neutrons of energy  $> 50$  MeV.

If the particles activating the detector were all of secondary origin in the atmosphere, the counting rate would have continued to decrease between 25 and 11 mbar, and would then have remained constant at the ceiling altitude. The expected counting rate at the ceiling altitude obtained by extrapolating the straight line fitted for the

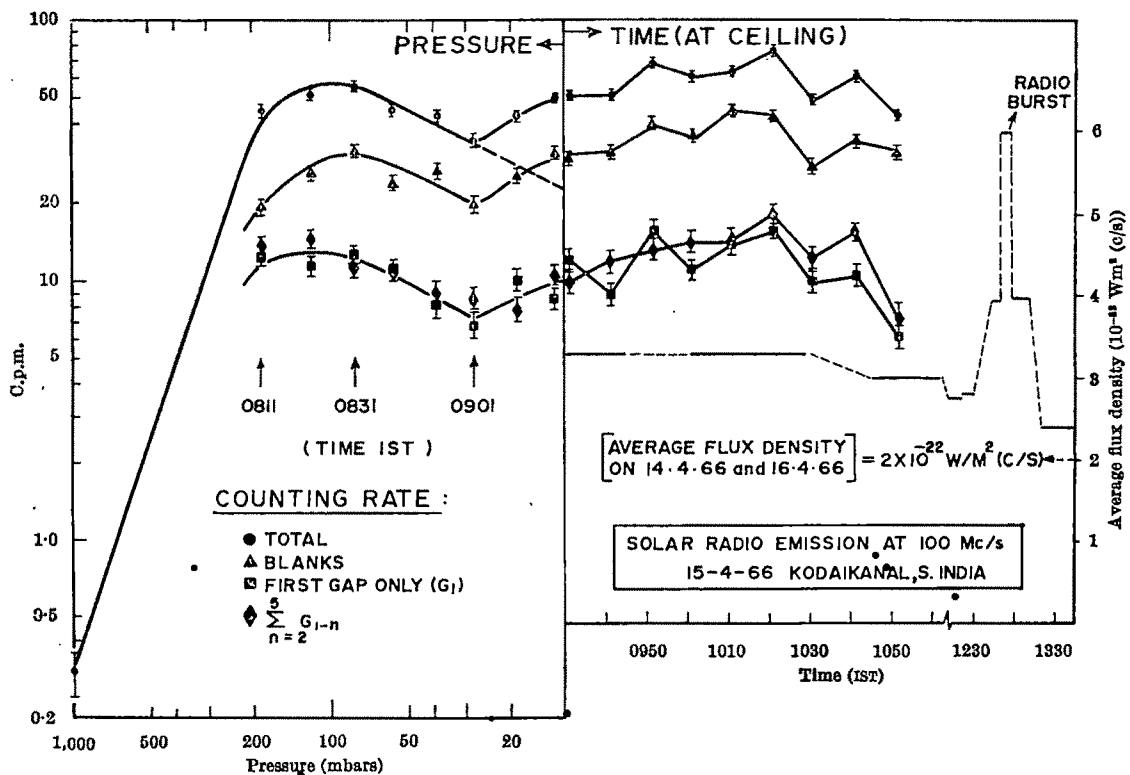


Fig. 2. Plot of the various counting rates as a function of pressure up to the point at which the balloon has just reached the ceiling altitude, and thereafter as a function of time. The solar radio emission at 100 Mc/s is also shown in the figure.



total counting rates recorded at the four levels of 80, 58, 39 and 28 mbar is only 23 per min. We have also been able to calculate the expected total counting rate at the ceiling altitude by making use of the flux of atmospheric neutrons of energy between 20 and 160 MeV measured by Apparao *et al.*<sup>2</sup> under almost identical flight conditions. The energy spectrum of the neutrons was then constructed using these data and the production spectrum for protons as given by Powell *et al.*<sup>4</sup>. We then calculated the counting rate by taking into account all possible contributions from nuclear disintegrations and elastically scattered protons due to neutrons of energy between 20 and 500 MeV, as well as from atmospheric protons leaking in through the camera window of the detector system. The calculated value thus obtained for the total counting rate is 16 per min with an accuracy better than 50 per cent. Considering this uncertainty in the calculations, this value seems to be consistent with the extrapolated value of 23 while the observed level of counting at the ceiling altitude is about three times this value.

It might be argued that the abnormally high counting rate and the large fluctuations observed at the ceiling altitude may be due to some erratic behaviour of the instrument which set in after about 0900 h. Such an erratic behaviour may arise from either (a) an erratic variation in the efficiency of the anticoincidence shield or (b) a similar behaviour of the triggering system unaccompanied by any charged particle traversing  $S_1$  and  $S_2$ . We have carefully considered these two possibilities and find that, if such erratic behaviour existed, the variations in counting rates should be restricted only to *Blank* and  $G_1$  types of events in (a) and to *Blank* events only in

(b). It is clear from Fig. 2, however, that events  $\sum_{n=2}^5 G_{1-n}$

do show an unambiguous correlation with the total counting rate. From these arguments we conclude that the large variations in the counting rates for different types of events observed beyond 25 mbar pressure level in Fig. 2 are due to real variations in the influx of neutrons responsible for the events.

The observed variation in the intensity of energetic neutrons can either be due to very large variations in the flux of charged primary cosmic rays (of solar or galactic origin) or to the arrival of neutrons which could only be of solar origin. In the same balloon flight as the present one, the Physical Research Laboratory, Ahmedabad, had a simple Geiger counter detector system, and this did not show any variation in the counting rate beyond statistical

fluctuations during the entire time when it was at the ceiling altitude. We therefore conclude that the large variations of counting rates we observed, when the equipment ascended beyond 25 mbar and was at ceiling altitude, were due to solar neutrons.

On April 15, when the present experiment was conducted, the Sun was under optical and radio observations at the Astrophysical Observatory, Kodaikanal. Judged from the brightening in the optical region (in H $\alpha$ ) and the enhancement of radio emission (at 100 Mc/s) the solar activity could be classified as a sub-flare. It seems very likely that this solar event and our observations on solar neutrons have a close relation.

The task of estimating the flux of solar neutrons in the present experiment was made very difficult because of the difficulty of separating the contribution of events due to neutron-proton elastic collisions at lower energies. If, however, all events other than *Blanks* and  $G_1$  are attributed to stars produced by energetic neutrons—that this is reasonable has been shown early in this article—one can obtain a rough estimate of the average flux of solar neutrons for the 1.5 h of observations as  $\sim 1,000 \text{ m}^{-2} \text{ sec}^{-1}$  in the energy interval of 50–500 MeV.

To summarize: (i) we have obtained, for the first time, positive evidence for the emission of high energy neutrons from the Sun; (ii) the observations indicate that the neutron emission set in at about 0900 h IST on April 15, 1966, and continued for at least about 90 min and during this time the neutron intensity seems to pulsate with periods  $\leq 10$  min; and (iii) there seems to be a plausible association between this neutron event and a sub-solar flare.

We are grateful to the Balloon Flight Group of this Institute for the successful flight operations, to Mr. George Abraham for the excellent technical help, to Dr. G. S. Gokhale for help and suggestions in different stages of this experiment and to Professor M. G. K. Menon for useful suggestions regarding the preparation of this paper. Our thanks are also due to Dr. Vainu Bappu, Director of the Kodaikanal Observatory, and Dr. N. W. Nerurkar, Physical Research Laboratory, Ahmedabad, for permitting us to quote their data here.

<sup>1</sup> Lingenfelter, R. E., Flamm, E. J., Canfield, E. H., and Kellman, S., *J. Geophys. Res.*, **70**, 4087 (1965).

<sup>2</sup> Apparao, M. V. K., Daniel, R. R., Vijayalakshmi, B., and Bhatt, V. L., *J. Geophys. Res.*, **71**, 1781 (1966).

<sup>3</sup> Hess, W. N., Patterson, H. W., Wallace, R., and Chupp, E. L., *Phys. Rev.*, **116**, 445 (1959).

<sup>4</sup> Powell, C. F., Fowler, P. H., and Perkins, D. H., *The Study of Elementary Particles by the Photographic Method*, 423 (Pergamon Press, London, 1959).

## Supersonic Liquid Jets

by

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C. N. SCULLY

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Liquid jets have been produced by ballistic extrusion with velocities of 4.58 km/sec

This article describes the development of methods for the production of very high velocity liquid jets. These can be used to study problems associated with the impact of very high velocity liquid droplets and jets with solids and liquids, including luminous radiation from droplet collisions and the use of high velocity liquid streams for the acceleration of spheres and irregularly shaped objects using the liquid as sabot. Previous workers<sup>1,2</sup> have used an impact acceleration technique for obtaining velocities up to 1.1 km/sec (3,600 ft./sec). By using liquid columns, usually water, accelerated in the smooth

bore of a smokeless powder gun and extruding the liquid column through a converging tapered section fixed to the end of the gun barrel, we have achieved water jet velocities of up to 4.58 km/sec (15,000 ft./sec).

A smooth-bore smokeless powder gun of bore diameter 1.145 cm was used to fire water columns vertically through convergent conical tapered sections into an evacuated (0.2 torr) 'Lucite' chamber. Before firing, each water column was supported in the gun barrel by a tight fitting Lexan piston. A 'Mylar' diaphragm 1 mil thick between the evacuated chamber and the water column prevented

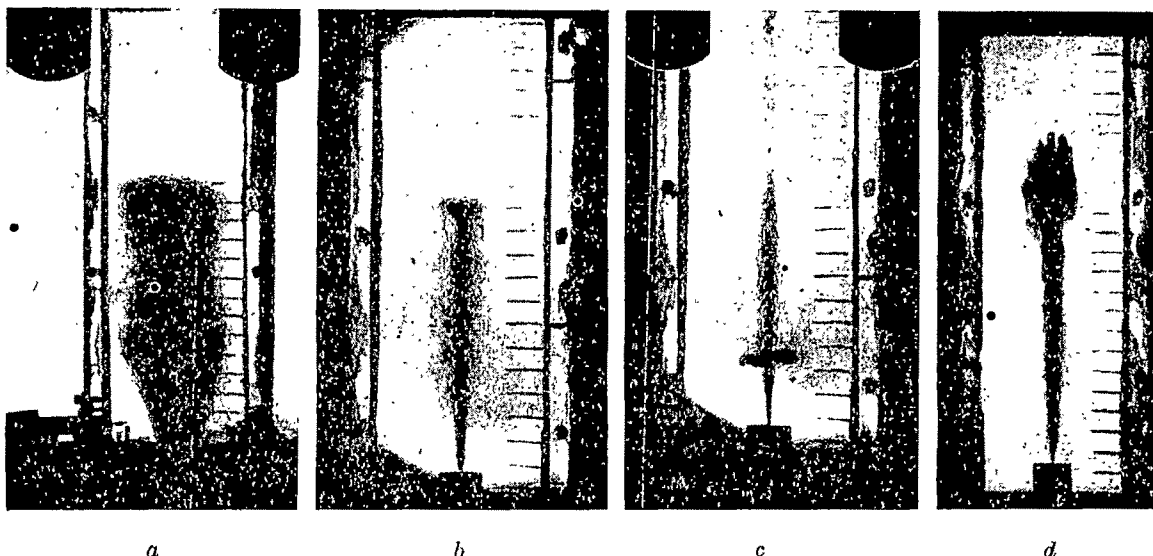


Fig. 1. Water jet effluxing from nozzle for various conditions. *a*, Input velocity 1.77 km/sec (5,800 ft./sec), 12/1 area ratio, 8 cm length, efflux velocity 4.58 km/sec (15,000 ft./sec). Typical high velocity jet. *b*, Input velocity 0.66 km/sec (2,180 ft./sec), 12/1 area ratio, 8 cm water, efflux velocity 1.91 km/sec (6,250 ft./sec). Typical low velocity jet. *c*, Radially expanding pulse resulting from the formation of a shock front. *d*, Jet expanding into air at one atmosphere pressure with input velocity of 1.22 km/sec (4,000 ft./sec), 12/1 area ratio, 8 cm water column and efflux velocity of 2.88 km/sec (9,400 ft./sec).

evaporation, and water columns 4, 6, 8, 12 and 16 cm long were fired. The gun was calibrated for the various projectile weights and fired the piston water column at velocities from 0.714 to 1.90 km/sec into a steel conical tapered section screwed directly to the end of the gun barrel. The tapers all had half-angles of  $1.5^\circ$  and large diameters matching the gun bore, but were of different lengths and, therefore, of different area ratios (ratio of area of gun bore cross section to area of exit end of taper). The velocities of the leading edges of the water jets were derived from time and position data obtained from 'Dynafax' camera records taken at 25,000 frames per second.

The motion of the water column in the converging taper section can be adequately described by the one dimensional, unsteady equations of compressible fluid flow with a slowly varying cross-sectional area ratio in Eulerian co-ordinates<sup>3</sup>. These are

$$\begin{aligned}\frac{\partial \rho}{\partial t} + \rho \frac{\partial u}{\partial x} + u \frac{\partial \rho}{\partial x} + \frac{\rho u}{A} \frac{\partial A}{\partial x} &= 0 \\ \frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + \frac{1}{\rho} \frac{\partial p}{\partial x} &= 0 \\ \frac{dp}{dt} - c^2 \frac{d\rho}{dt} &= 0\end{aligned}$$

From this system may be obtained the physical characteristic equations

$$\frac{dx}{dt} = u \pm c$$

and the corresponding state characteristic equations

$$du = \mp \frac{1}{\rho c} \left( \frac{\partial p}{\partial c} \right)_s dc \mp \frac{1}{A} \frac{dA}{dx} dx$$

where  $\rho$ ,  $p$ ,  $u$ ,  $c$  are the density, pressure, velocity and sonic velocity of the fluid and  $A$  is the cross-sectional area. The left bounding characteristic is given by the equation of the piston path and the pressure condition behind it by

$$\begin{aligned}\frac{dx}{dt} &= u \\ p &= p_b - \frac{m_p}{A} \frac{du}{dt}\end{aligned}$$

where  $m_p$  is the mass of the piston and  $p_b$  is the gas pressure behind the piston. The right bounding characteristic is given by the equation of the free surface and its pressure condition

$$\begin{aligned}\frac{dx}{dt} &= u \\ p_f &= 0\end{aligned}$$

where  $p_f$  is the free surface pressure.

The equation of state of water was given by Tait<sup>4</sup>

$$p = 2.94 \left\{ \left( \frac{\rho}{\rho_0} \right)^{7.47} - 1 \right\}$$

The boundary conditions corresponding to the experimental configuration were  $p_b = 0.216$  kbar; a constant piston sectional density of 1 g/cm<sup>2</sup> at  $t=0$ , a uniform pressure, density and velocity in the columns and one atmosphere of pressure on the free surface of the liquid, and the free surface positioned at the beginning of the converging section.

The above equations with appropriate boundary conditions were numerically integrated on an IBM 7094 computer using an iterative technique similar to that given in Shapiro<sup>5</sup>.

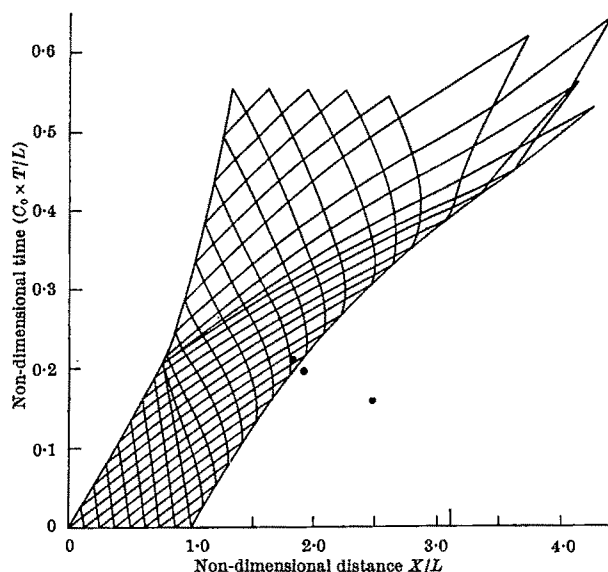


Fig. 2. Characteristic mesh for following conditions, input velocity 1.22 km/sec (4,000 ft./sec), 12/1 area ratio, piston mass = 0, converging section terminates at 3.12.

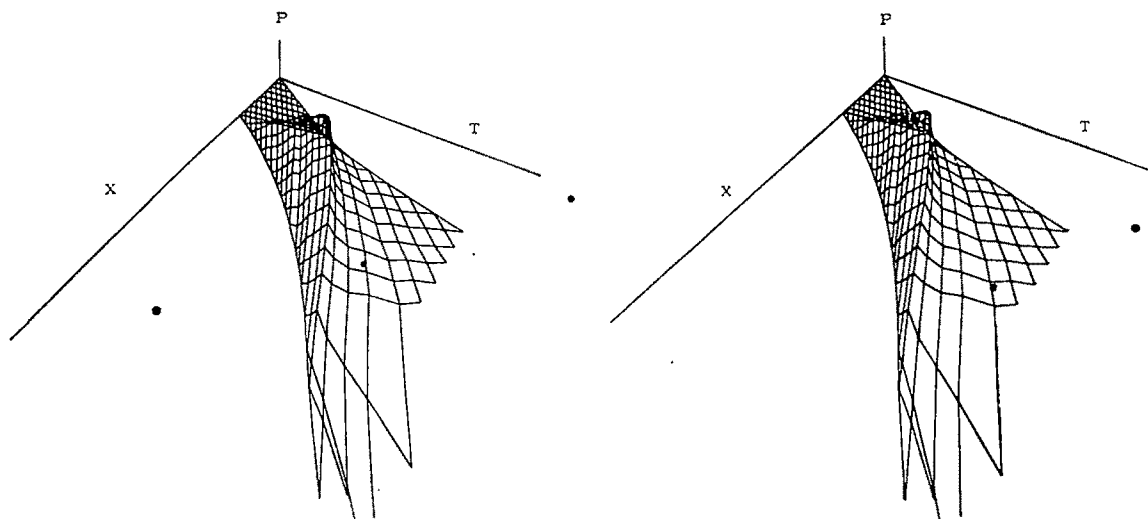


Fig. 3. Stereoscopic pair of pressure-time history for conditions in Fig. 2.

The dynamics of the process are illustrated in the characteristic plots (Figs. 2 and 3). The pressure pulses produced as the water column enters the converging section propagate into the liquid at velocities  $u \pm c$  where  $u$  is the local fluid velocity and  $c$  is the local sonic velocity. The curvature of the characteristics is a function of the strength of the pressure pulses. If the right or left running characteristics intersect, a right or left running shock will be formed at that time. In Fig. 2 for the conditions given, there is no shock formed because the pulses have partially transmitted their pressure and momentum to the boundaries of the column. The case where a water column enters the converging section supersonically is similar, but when a rear running shock is formed it will be stationary near the entrance of the converging section.

The calculated jet velocities did not include the effect of the vaporization, with the result that the velocities were under-estimated. To confirm the analysis, a jet was extruded into an ambient atmosphere at 1 bar (Fig. 1d). The calculated and the experimental values agreed within 3 per cent.

Figs. 4a and 4b illustrate the effect of input velocity and area ratio on jet velocity. In the velocity range considered, the jet velocity does not reflect processes typical of an incompressible fluid.

The effect of initial column length on jet velocity is shown in Fig. 5a. The leading edge velocity increases linearly up to some critical length, depending on the piston

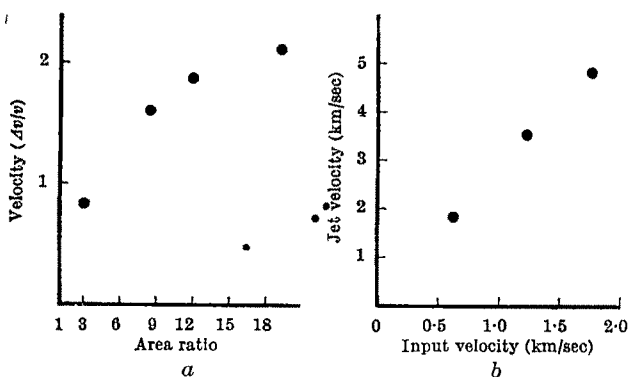


Fig. 4a. Jet velocity versus input velocity for constant 12/1 area ratio, 8 cm water column.

Fig. 4b. Jet velocity versus area ratio for constant input velocity of 1.22 km/sec (4,000 ft./sec), 8 cm water column.

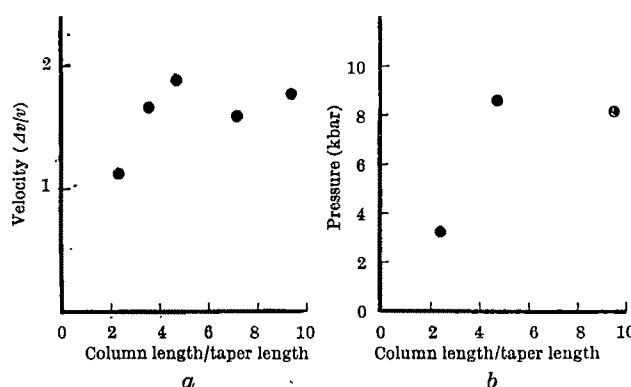


Fig. 5a. Jet velocity versus column length divided by taper length for constant input velocity 1.22 km/sec (4,000 ft./sec), 8 cm water column.

Fig. 5b. Calculated peak taper pressure versus column length divided by taper length for constant input velocity 1.22 km/sec (4,000 ft./sec) and 12/1 area ratio.

mass and the taper angle. The analysis is carried out for zero piston mass, which for the conditions in Fig. 2 gives the maximum initial column length that can be used before a shock would be formed.

The pressure-time history of the column is shown in the stereoscopic pair<sup>6</sup> in Fig. 3. As can be seen, the pressure gradients increase rapidly in time, causing the leading edge of the column to accelerate supersonically. In Fig. 5b the calculated peak pressures for various column lengths are plotted, and in the cases where a shock is formed the pressure at the beginning of formation is given. When the entrance velocity was supersonic, pressures of 11 kbar were produced, and were great enough to cause a radial expansion of the hardened steel taper sections.

Two piston densities were used, 1 g/cm<sup>3</sup> and 8.5 g/cm<sup>3</sup>, for the conditions given in Fig. 2 and for the 12 to 1 taper ratio. The denser piston increased the jet velocity from 3.51 km/sec (11,500 ft./sec) to 4.13 km/sec (13,500 ft./sec).

The yield strength of the steel taper sections appears to be the factor which limits the maximum jet velocities. Small changes in efflux velocity cause large changes in taper pressure.

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<sup>3</sup> Stanyukovich, K. P., *Unsteady Motion of Continuous Media*, 48 (Pergamon Press, 1960).

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# Radioactive Ruthenium and Manganese Isotopes in the Atmosphere

by

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The movement of air masses between the stratosphere and the troposphere can be traced by studying the concentration and ratios of radioactive ruthenium and manganese injected into the atmosphere by nuclear bursts on the ground and in the air

RADIOACTIVE debris injected into the atmosphere by ground and air nuclear bursts is well suited for tracing the movements of air masses between the stratosphere and the troposphere. This air transport is studied by determining the concentrations of the products of fission and neutron activation in the air samples collected by filtering large volumes of air at various altitudes and latitudes in the atmosphere. Filter samples, collected between July 1962 and October 1963 in the northern hemisphere up to an altitude of 68,000 ft. in connexion with the Star Dust Program, were received in the autumn of 1963 by the University of Arkansas. The results of analyses of these samples for antimony-125 and antimony-124 have been published by Kauranen<sup>1</sup>. In this report we present the results of analyses for ruthenium-106 (rhodium-106) and manganese-54.

The filter samples were decomposed and brought into solution according to the methods described by Friend *et al.*<sup>2</sup>. From a large portion of this solution, the sulphides of antimony, tin, ruthenium and manganese were precipitated and antimony was separated from them as described by Kauranen<sup>1</sup>. The remaining solution was processed for ruthenium as described by Rao and Shahani<sup>3</sup> and manganese was subsequently precipitated as dioxide. The chemical yields were usually about 60 per cent. The introduction of any oxidizing agents was carefully avoided while the filters were being decomposed. The ruthenium and manganese samples were counted with a 3 in.  $\times$  3 in. sodium iodide (thallium doped) crystal in a 512 channel pulse-height analyser and the  $\gamma$ -ray spectra showed that both the ruthenium and manganese samples were pure. In order to calculate the respective concentrations, 0.513 MeV  $\gamma$ -ray peak for ruthenium-106 (rhodium-106) and 0.84 MeV  $\gamma$ -ray peak for manganese-54 were used.

The concentrations of ruthenium-106 (rhodium-106) and manganese-54 and the ratios of ruthenium-106 : manganese-54 in the northern hemisphere at different seasons in 1962 and 1963, as a function of latitude and altitude, are summarized in Fig. 1. The data are calculated in disintegrations per minute (d.p.m.) per standard cubic foot (scf) at the time of collection and they are corrected for decay to January 1, 1963. The overall error in these values is assumed to be about 20 per cent. The air volumes and the tropopause heights were provided for us by Dr. Feely of Isotope, Inc., Westwood, New Jersey.

Despite several tests carried out by the United States and the Soviet Union in 1961 and early 1962, the concentrations of ruthenium-106 shown in Fig. 1A are quite low (1–3 d.p.m./scf) in the northern stratosphere by July and September 1962. But the corresponding concentrations of manganese-54, also shown in Fig. 1A, are rather high (12 d.p.m./scf) in July and September 1962.

In the autumn of 1962, a series of large-scale nuclear tests were carried out by the U.S.S.R. at Novaya Zemlya (76° N.) and some high altitude tests by the United States at Johnston Island (17° N.). As a result of these tests, there was a considerable increase in the concentrations of ruthenium-106 throughout the northern stratosphere by January 1963. The concentrations of ruthenium-106 at polar and mid-latitudes between 60,000 and 70,000 ft. are about 2–3 times higher than the ruthenium-106 activity in tropical latitudes, which indicates that the contribution of fission-product debris into the lower stratosphere by Soviet tests was much higher than that of American tests. But the concentrations of manganese-54 did not show such an increase at this time at polar and tropical latitudes. According to Holland<sup>4</sup>, manganese-54 appears to have been produced largely in the 55–60 megaton Soviet arctic thermonuclear detonation in October 1961. All nuclear explosions involving fission-type bombs contribute, however, to the production of ruthenium-106.

A study of the concentrations of ruthenium-106 and manganese-54 and their ratios in Fig. 1A–D shows that a semi-horizontal mixing is taking place in the lower stratosphere. Fig. 1A shows that a ruthenium-106 activity of 1.5 d.p.m./scf was located at 65° N. and 62,000 ft. by September 1962, and Fig. 1B shows that this activity had increased enormously by January 1963 as a result of Soviet tests, giving rise to a maximum ruthenium-106 activity of 122 d.p.m./scf at the same latitude and altitude as already mentioned. A part of this huge ruthenium-106 activity, introduced into the polar stratosphere by Soviet tests in the autumn of 1962, appears to have migrated southward by January 1963 to 45° N. and 60,000 ft. where the air samples showed a ruthenium-106 activity of 103 d.p.m./scf, as shown in Fig. 1B. Furthermore, the ratios of ruthenium-106 : manganese-54 in January 1963 in these two regions seem to be the same, as indicated by the ratio-isoline of 18 in Fig. 1B. A similar trend is also observed in the case of manganese-54. As shown in Fig. 1A, by September 1962, a manganese-54 activity of 10 d.p.m./scf occurred at 63° N. and 63,000 ft. But by January 1963, as shown in Fig. 1B, this centre of manganese-54 activity had moved southward to 43° N. and 61,000 ft. These observations seem to indicate that in winter 1962 and early spring 1963, the ruthenium-106 and manganese-54 activities moved southwards from the North Pole to mid-latitudes at about 70,000 ft.

An evidence of northward and downward movement of the lower stratospheric air (at about 50,000 ft.) from tropics to mid-latitudes and probably into lower polar regions could be found in winter and spring from a study of Figs. 1A–D. The ruthenium-106/manganese-54 ratio-isolines of 5 and 9 in Fig. 1B show a tendency to slope northward and downward, and the concentration isolines in this figure also show the same trend. By April 1963,

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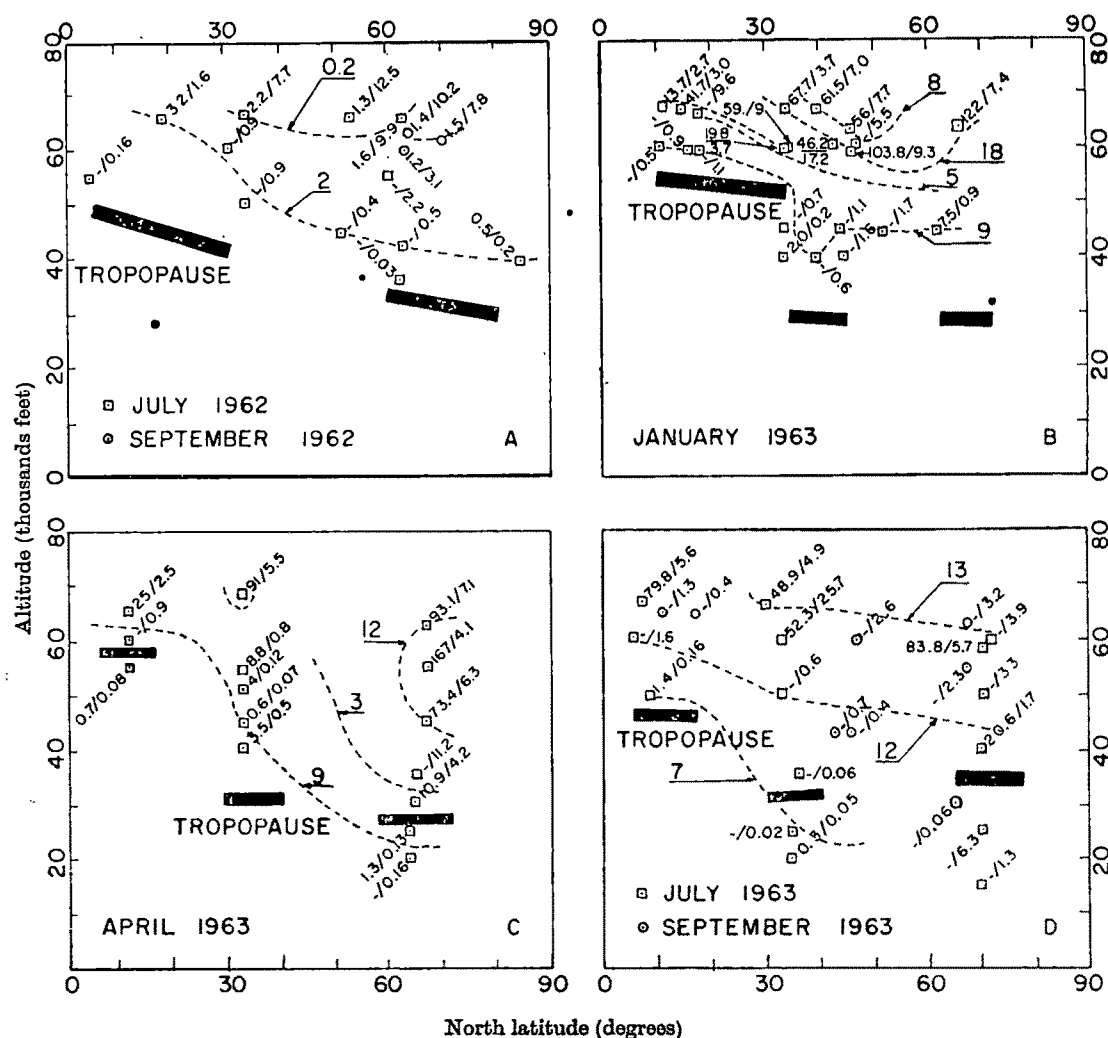


Fig. 1. Concentrations and ratios of ruthenium-106 and manganese-54 in the northern hemisphere during July 1962–September 1963. The values are given in d.p.m./sof. Near the squares in the figure values for ruthenium-106 and manganese-54 are given as, for example, 50/5.1, where the value of the numerator represents the concentration of ruthenium-106 in d.p.m./sof and the value of the denominator represents the manganese-54 concentration in d.p.m./sof. In cases where the values for a particular nuclide were not determined, they are indicated as —. The dashed lines represent the ratio-isolines of ruthenium-106 : manganese-54 and the corresponding values are indicated by the arrows.

however, as shown in Fig. 1C, the ruthenium-106/manganese-54 ratio-isoline of 9 shows a steep downward and northward slope from tropics to pole. Another example of northward and downward movement of lower stratospheric air could be found in the air mass at 45° N. and 60,000 ft., with a ratio of ruthenium-106 : manganese-54 of 3 (as shown in Fig. 1B) from winter 1963, which has moved to 65° N. and 45,000 ft. by spring 1963 (as shown in Fig. 1C). The concentrations of ruthenium-106 and manganese-54 in this air mass also show the same trend. This type of air transport seems to contribute much to the increased concentrations of these nuclides, detected in the lower polar stratosphere by spring 1963. In addition, the vertical mixing as a result of the formation of polar cyclonic vortex in winter in the lower stratosphere contributes significantly to the "Spring-Peak" resulting in the increased concentrations of the fission products in rainfall by spring time<sup>5</sup>.

Our data in Figs. 1A–D show that air can pass between the stratosphere and the troposphere in the vicinity of the jet stream and the tropopause discontinuity, as proposed by Staley<sup>6</sup> and Newell<sup>7</sup>. In Fig. 1C, the ruthenium-106/manganese-54 ratio-isoline of 9 in the lower stratosphere follows the path of the tropopause indicating higher con-

centrations above the tropopause surfaces and protrudes into the troposphere near the jet stream region in the mid-latitudes. In Fig. 1B the ruthenium-106/manganese-54 ratio-isolines of 9, and in Fig. 1D the ratio-isoline of 7, also show a similar tendency. Further attempts are in progress in this laboratory to study the movement of air masses in the stratosphere with the help of strontium-90 and cerium-144 distributions and comparing them with these results.

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# Dielectric Absorption of Microwaves in Human Tissues \*

by

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Calculations show that differences in absorption of microwaves by the human body may be a means of localizing tumours and some body structures

MEASUREMENTS of the electron spin resonance from "surviving" animal tissues at 3 cm wavelength have shown a difference in the amount of resonance absorption at room temperature at  $g=2.004$ , between malignant and normal tissue<sup>1-4</sup>. The signal for normal rat liver is some twenty times greater than that for rat liver tumour, while at  $g=2.016$  a broad absorption signal from rat hepatoma and metastases has been observed which is absent in normal rat liver tissue<sup>4</sup>.

It is more difficult to obtain suitable samples of human tumours and normal tissues for comparison, but preliminary measurements indicate that a differential exists between some tumours and the corresponding normal tissue. Using a reliable spectrometer of high sensitivity and reproducibility, it is therefore possible to distinguish between normal and malignant tissue samples, but this would be a cumbersome and expensive way of discriminating between them when the examination of a section under the microscope would give the same information and more.

One wonders whether a machine using electron spin resonance could be constructed which could measure or display *in vivo* the differential between the normal tissue and the tumour embedded within it: is it possible that a machine which detects and measures the transmitted intensity of microwaves through the body could scan the region of interest to display and localize a tumour? The first question to answer in the design of such a machine is how much of a beam of microwaves incident on the body would emerge after transmission through the body, or after reflexion from the interface between tumour and normal tissue within the body.

First, the considerable dielectric absorption of the microwave beam by the body tissues must be calculated and it is here that some interesting observations can be made. Experimental information on the dielectric constant of human tissues is sparse. England and Sharples<sup>5</sup> and England<sup>6</sup> have at 3 cm reported values of the power absorption coefficient ranging from  $\alpha=2.65$  to 3.2 (average value 2.84) for six samples of breast carcinoma (scirrhous) and a value of  $\alpha=0.49$  (leg fat  $\alpha=0.44$ ) for breast fat. Thus there is a difference in dielectric constants for a particular tumour and what might be regarded as its surrounding tissue. The tumour value reported was also

slightly different from that of other soft tissue (for example, skeletal muscle  $\alpha=2.60$ ), but Buchanan (personal communication) has pointed out the great difficulty of such measurements and the uncertainties which are attached to smaller differences. It would appear that a more extensive series of dielectric measurements on tissue samples is needed to establish values more accurately from tissue to tissue and to investigate the reality of any differences between tumours and normal tissues. For the calculation of tissue absorption reported in this preliminary study, the values of England and Sharples<sup>5</sup> have been used throughout.

The calculations have been carried out for the model shown in Fig. 1. A slab of breast fat 10 cm thick has a tumour of 1 cm thickness embedded within it, the position of the tumour being defined by  $l_2$ . The waves propagated in the tissues are attenuated, reflected and changed in phase: diffraction will also occur, but this was not taken into account in this simple model.

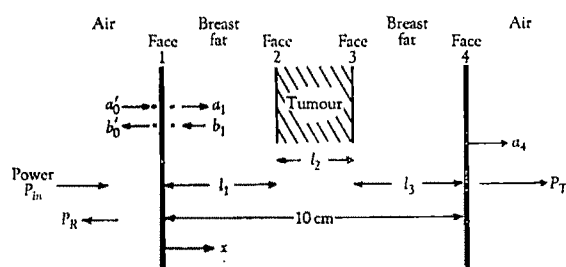


Fig. 1. Diagram of the model used for the calculations. A breast tumour ( $\alpha=2.84$ ) of thickness 1 cm is embedded in breast fat ( $\alpha=0.44$ ) and is irradiated with 3 cm microwaves. The transmitted and reflected intensities ( $P_R$  and  $P_T$ ) are calculated both with and without the tumour (see Fig. 2).

If  $P(x)$  is the power at  $x$ , then  $P(x) = P(0) e^{-\alpha x}$  where  $\alpha$  is the linear power absorption coefficient.

Let  $a$  and  $b$  (see Fig. 1) be the wave amplitudes and let  $R$  and  $T$  be the reflexion and transmission coefficients, then at an interface (for example, face 1)

$$1 + R_1 = T_{0,1}$$

For the model in Fig. 1

$$\begin{bmatrix} a_1 \\ b_1 \end{bmatrix} = \frac{1}{T_{1,2} \cdot T_{2,3} \cdot T_{3,4}} \cdot [A] \begin{bmatrix} a_4 \\ 0 \end{bmatrix}$$

\* This material is part of that presented at the First International Conference of Medical Physics, Harrogate, 1965, and the Eleventh International Congress of Radiology, Rome, 1965.

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$$\text{where } [A] = \prod_{j=1}^3 B^j \text{ and } B^j = \begin{bmatrix} 0 & 1 \\ e^{-i\varphi_j} & R_{j+1} \end{bmatrix} \begin{bmatrix} 1 & R_{j+1} \\ R_{j+1} & 1 \end{bmatrix}$$

and the complex propagation factor  $\varphi_j = \left(k_j - \frac{i\alpha_j}{2}\right)l_j$  and

( $j=1, 3$  for fat;  $j=2$  for tumour)

with  $\lambda_j$  the wavelength,  $k_j = 2\pi/\lambda_j$ ,  $\alpha_j$  = absorption coefficient in the medium  $j$  and  $l_j$  = length of medium.

This matrix expresses the relation between the transmitted amplitude  $a_4$  and the amplitudes  $a_1$  and  $b_1$  which result from multiple reflexions, phase changes and absorption.

Defining  $T = a_4/a_1$

and  $R = b_1/a_1$ ,

$$\text{then } T = \frac{T_{1,2} \cdot T_{2,3} \cdot T_{3,4}}{A_{11}} \text{ and } R = \frac{A_{21}}{A_{11}}$$

Referring to Fig. 1,

$$P_T = P_{in} \cdot |T|^2 \cdot |T_{0,1}|^2 \cdot 1/(1 + RR_1)^2$$

and

$$P_R = P_{in}(R + R_1)^2/(1 + RR_1)^2$$

The transmission coefficients and  $R_1$  are found from the dielectric constants of the media, and it only remains to calculate  $A_{11}$  and  $A_{21}$  in order to determine  $R$  and  $T$ .

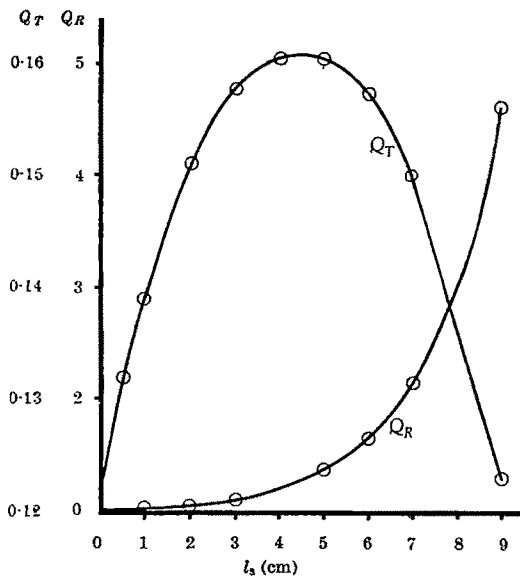


Fig. 2. The results of the calculations plotted against  $l_3$ , the position of the 1 cm thick tumour, for the model of Fig. 1.

$$Q_T = \frac{\text{Transmitted power with tumour}}{\text{Transmitted power without tumour (fat only)}}$$

$$Q_R = \frac{\text{Reflected power with tumour}}{\text{Reflected power without tumour (fat only)}}$$

The matrix elements give  $|A_{11}|^2$  and  $|A_{21}|^2$  as functions of  $\alpha_1$ ,  $\alpha_2$ ,  $l_3$ ,  $R_1$ ,  $k_2$  and  $k_3$  which are calculated for various values of  $l_3$  and also for the case when no tumour is present.

The following ratios are obtained.

$$Q_T = \frac{P_T \text{ with tumour}}{P_T \text{ fat only}}$$

and

$$Q_R = \frac{P_R \text{ with tumour}}{P_R \text{ fat only}}$$

which are plotted in Fig. 2. The values of  $Q_T$  and  $Q_R$  are related to the input power  $P_{in}$  by the ratio of  $P_T/P_{in}$  in the absence of tumour. For 10 cm of fat without tumour

$$P_T/P_{in} = 1.8 \times 10^{-3}$$

$$\text{and } P_R/P_{in} = 0.12$$

The values of  $Q_T$  in Fig. 2 show that the transmitted power through the tumour and fat is less than one-fifth of that through fat alone and that the transmitted power is greatest through the model when the tumour is centrally placed in the fat. The values of  $Q_R$  in Fig. 2 show that, as the tumour is moved from the back face to the incident face, the reflected power increases from 0.12 to greater than 0.5.

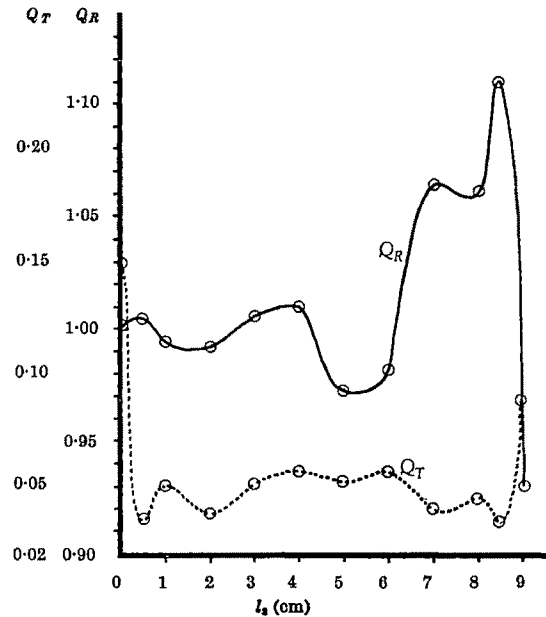


Fig. 3. The results of the calculations plotted in the same way as Fig. 2, when a layer of skin 1 mm thick ( $\alpha = 2.60$ ) is added to Faces 1 and 4 of the model shown in Fig. 1.

It would seem possible, therefore, that the presence of breast tumour tissue in this model could be demonstrated either by reflexion or transmission. Because of heating effects it is recommended that the continuous incident power at the surface of the body must not exceed 10 mW/cm<sup>2</sup>, over a frequency range from 30 Mc/s to 30 Gc/s, for a human being<sup>7</sup>. As a result the transmitted power levels would be of the order of 10<sup>-5</sup> W/cm<sup>2</sup> for the model considered here. It is possible that a more intense short pulse could be used in order to increase the power to be detected but only with great care.

For this model, the differential of intensity beneath the tumour to that of the surrounding regions is greater than would be achieved in *in vivo* diagnostic radiography. The model taken, however, has been a favourable one because of the considerable difference of dielectric constant reported by England and Sharples<sup>8</sup> for breast fat and breast tumour. The calculations have been performed also on the much less favourable case of the breast tumour

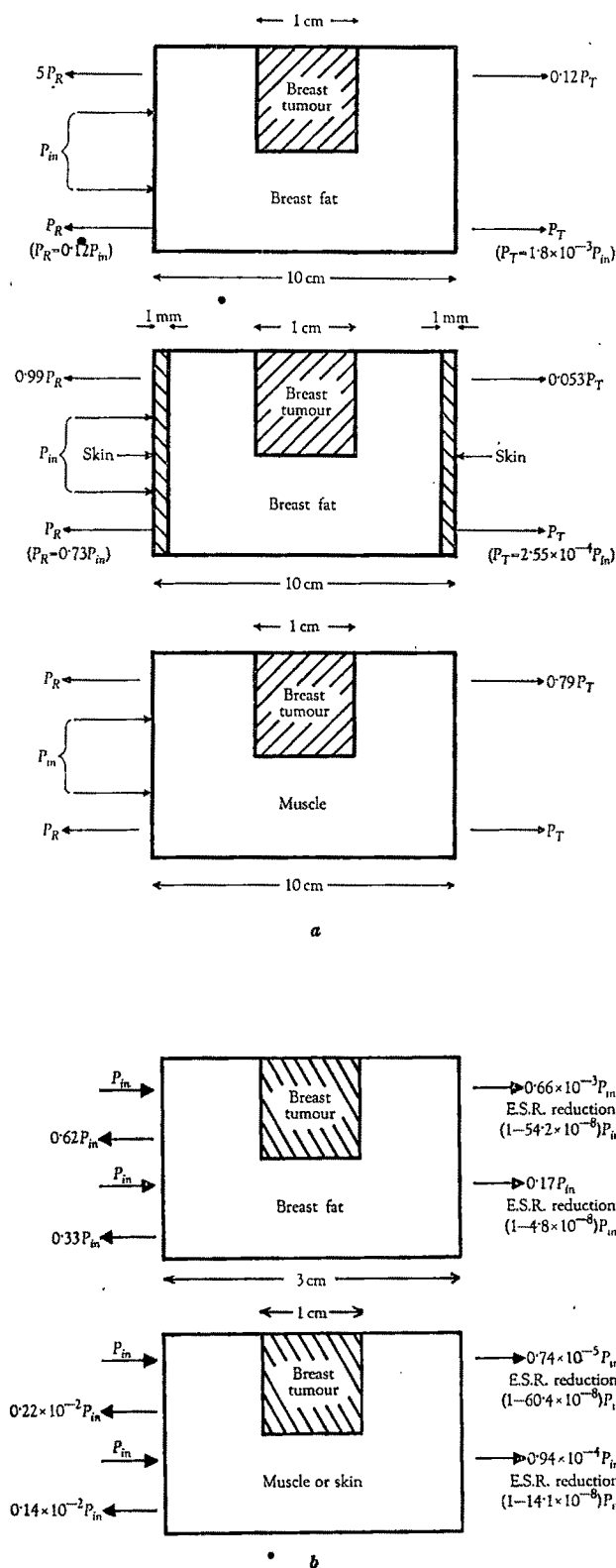


Fig. 4. *a*, Summary of calculated results for the model shown in Fig. 1, 10 cm thick. The central figure shows the results with a 1 mm thick skin layer added as shown, whilst the lowest figure has the breast fat replaced with muscle. There is a much greater difference in transmitted intensity with and without tumour when skin is added, and a much smaller difference when fat is replaced with muscle. *b*, Summary of the calculated results for a model of a 1 cm breast tumour embedded in 3 cm of breast fat (upper figure) and muscle (lower figure). In addition, the extra absorption caused by electron spin resonance (E.S.R.) processes is also shown. E.S.R. calculations assume tumour has  $\approx 10^{16}$  spins/g and normal tissue has  $\approx 10^{15}$  spins/g.

( $\alpha = 2.84$ ) embedded in muscle ( $\alpha = 2.60$ ). In this case, for a centrally placed tumour,

$$Q_T = 0.79 \text{ and } Q_R = 1$$

If this 20 per cent change in transmitted intensity does occur *in vivo*, then it should be possible to detect it.

Further calculations were carried out for the model of Fig. 1 with the addition on Faces 1 and 4 of a layer of skin 1 mm thick ( $\alpha = 2.60$ ). The matrix elements are more complex and lead to the result of Fig. 3. The transmission case is approximately symmetrical and the large effect with the tumour near the edges is caused by the importance of the phase angle when there is a small distance between two tissues of similar dielectric constant. The ratio of the transmitted power, with and without tumour, is even greater than before and the output power levels are measurable. In these circumstances  $P_T/P_{in} = 2.55 \times 10^{-4}$  and  $P_R/P_{in} = 0.74$ .

Very useful reductions in unwanted reflexions can be made by matching the impedances of materials at interfaces, that is, by blooming the surfaces. For example, the two skin-air interfaces can be matched by coating them with a material, the dielectric constant ( $K$ ) of which is related to those on either side by  $K = (K_{air} \cdot K_{skin})^{1/2}$ . Such a coating will approximately match impedance, not only for the incident waves which travel inwards but also for the emergent waves which travel outwards; it would also increase still further the ratio between tumour and non-tumour intensities.

Fig. 4(a) summarizes the results on the 10 cm thick model with and without skin, while Fig. 4(b) presents the results on a model which is only 3 cm thick. For the case of this smaller model, the extra absorption of microwaves due to electron spin resonance processes, with the model placed in a magnetic field of 3,400 G, has been calculated. It has been assumed in these calculations that the tumour has a concentration of approximately  $10^{16}$  spins/g, and normal tissue approximately  $10^{15}$  spins/g, which are the values determined for rat liver and rat liver tumour<sup>4</sup>. It is seen that the electron spin resonance absorption of microwaves is very small (about  $10^{-4}$ ) compared with the dielectric absorption. As a result, the detection of the difference in electron spin resonance absorption between tumour and normal tissue, by a machine based on the rationale discussed here, would seem to be a more formidable task than the detection of the difference arising from dielectric properties.

It seems likely that by measuring the transmission of microwaves through the body, it should be possible to demonstrate differences in dielectric absorption from point to point in the body and perhaps outline and localize—by photographic or scanning methods—tissues or body cavities, or abnormal regions of organs which have different dielectric constants. It seems possible that this might have some value for tumour detection and localization. Perhaps the technique of holography may prove to be an aid in this procedure. The reflected beam should enable interfaces (particularly mobile interfaces) to be detected in a similar manner to ultrasonic localization methods. It is realized that the practical difficulties are considerable.

We thank Mr. M. Kent, with whom this work has evolved, for his help and comments, and Dr. T. J. Buchanan for reading and checking the calculations. We also thank the Science Research Council for support.

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# Killing of Staphylococci by Penicillins

by

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What happens after the inhibition of cell wall synthesis by penicillins?  
Effects of antibiotics suggest that mucopeptidase plays an important  
part by destroying mucopeptide

THE penicillins inhibit the formation of mucopeptide in bacterial cell walls<sup>1,2</sup> and when staphylococci are tested under suitable conditions this can be shown to occur without lag and with minimal growth inhibitory concentrations of the antibiotics<sup>3,4</sup>. The particular step in the biosynthesis of the complex polymer which is inhibited is likely to be a very late one<sup>5,6</sup>. Inhibition of growth and mucopeptide synthesis<sup>7</sup> is reversible and is not followed by death of the bacteria unless they have grown for a sufficient time in the antibiotic (succeeding article). Death of the cells is thus not related by an obvious mechanism to the primary inhibition of the formation of cell walls, although fairly rapid permeability changes have been shown in *Escherichia coli*, *Erwinia* and *Staphylococcus aureus* as a result of growing the bacteria in media containing penicillin.

The present work is intended to define the steps that follow the inhibition of cell wall synthesis. Two considerations form the basis of this work. The first is derived from the observation that the penicillins in high concentrations do not directly inhibit protein formation<sup>3,4</sup>. It is, therefore, presumed that at low concentrations they have no effect on the formation of wall lytic enzymes (mucopeptidases) by the cell although, as described later, it eventually became necessary to suppose that at concentrations considerably higher than those that just inhibit growth they could specifically depress the formation of mucopeptidases. The second is that all bacteria produce autolytic enzymes, called here mucopeptidases, that can attack mucopeptide. A number have already been described<sup>8,9</sup> which attack the structure at a number of points. The possible roles of autolytic enzymes in the remodelling of the wall of bacteria have been discussed<sup>10,11</sup>. Some enzyme which hydrolyses mucopeptide probably would be essential for normal wall extension and division of a micro-organism.

It is known that the bactericidal effects of benzylpenicillin on streptococci and *E. coli* can be antagonized by the simultaneous presence of other antibiotics such as chloramphenicol<sup>12,13</sup>. Fig. 1 and Table 1 show that both lysis and death of staphylococci caused by the addition of methicillin to cultures growing in a dilute casein hydrolysate medium can be prevented by chloramphenicol, provided the latter is added not later than 15 min after the penicillin. This argues that, provided protein synthesis is switched off within a time that corresponded to about a fifth of a generation time (70 min) in the medium used, irreversible damage to the cell is not caused by the inhibition of mucopeptide synthesis. The correlation of the lytic effect on the cells with the antagonism of the bactericidal effects suggested that the continued formation of a mucopeptidase might be a factor leading to the death of staphylococci. The effects of excess chloramphenicol and the growth-inhibitory concentration of methicillin on the amounts of mucopeptide formed by staphylococci incubated in a casein hydrolysate growth medium are

Table 1. NUMBER OF LIVING BACTERIA IN CULTURES INCUBATED WITH METHICILLIN AND WITH CHLORAMPHENICOL ADDED AFTER VARIOUS TIMES

Time of growth* (min)	Additions: None	Living counts $\times 10^{-6}$			
		Methicillin (2 $\mu$ g/ml.)	Methicillin (2 $\mu$ g/ml.) + chloramphenicol (50 $\mu$ g/ml.) after:		
			15 min	30 min	60 min
50	2.1	2.0	2.4	—	2.0
110	4.6	2.5	3.0	2.6	2.6
320	15.1	0.08	3.0	1.0	0.09

*Staphylococcus aureus* strain 524SC was grown at 35° C, with shaking, in a 0.1 per cent casein hydrolysate medium supplemented with thiamine, nicotinic acid, 0.5 per cent glucose and 0.002 molar magnesium sulphate until the extinction indicated a dry weight of 0.08 mg/ml. Methicillin, sufficient to give 2  $\mu$ g/ml., was added to four of the five flasks. At the times indicated after this, sufficient chloramphenicol was added to give a concentration of 50  $\mu$ g/ml. Samples were taken after 50, 110 and 320 min, diluted in saline and plated on Hedy-Wright agar. The number of colonies on appropriate plates was counted after 18 h of incubation at 35° C.

\* Time of total growth of culture after inoculation. Methicillin was added immediately after the 50 min sample had been taken and chloramphenicol 15, 30 and 60 min after this time.

shown in Fig. 2. When chloramphenicol is present from the beginning, together with growth-inhibitory concentrations of methicillin, there is a slow increase in the amount of mucopeptide. In the absence of chloramphenicol and the presence of penicillin there is an increase for a period of 90 min, but this is followed by a rapid decrease. This phenomenon can be explained when it is remembered that, in the absence of chloramphenicol, mucopeptidases would continue to be formed at the normal rate, whereas the activity, but probably not the formation, of one of the enzymes that biosynthesizes mucopeptide is inhibited considerably. The ratio of the amount of hydrolytic enzyme to the amount of mucopeptide in the walls therefore increases as the cells grow. Consequently the proportion of the total mucopeptide that is removed in unit time

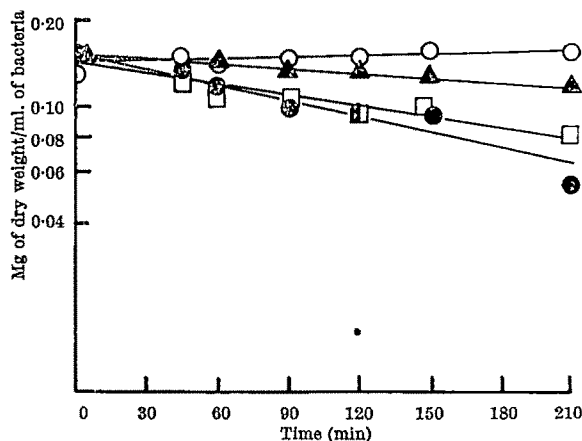


Fig. 1. The effect of time of addition of chloramphenicol after methicillin on lysis of *Staphylococcus aureus* strain 524SC. The casein hydrolysate medium of the organisms was as given in the legend to Table 1. The dry weight of bacteria was estimated from the extinction measured with an absorptiometer and the use of a calibration curve. The time given on the abscissa is the time in minutes after lysis began (that is, 2 h after the addition of methicillin). The concentration of methicillin was 2  $\mu$ g/ml., and of chloramphenicol 50  $\mu$ g/ml. ●, No chloramphenicol; □, 60 min; ▲, 30 min; ○, 15 min.

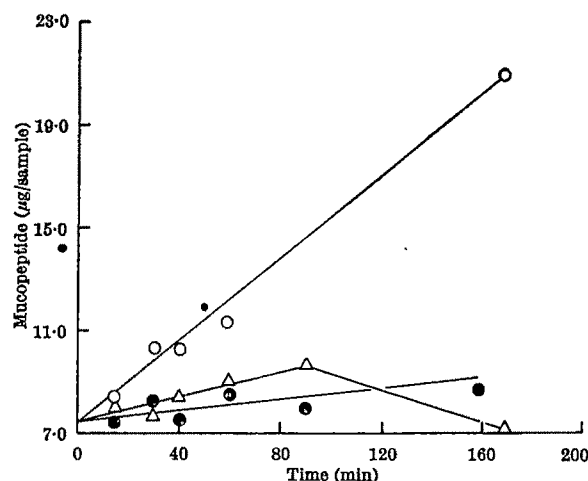


Fig. 2. The effect of methicillin on the synthesis of mucopeptide in the presence and absence of chloramphenicol. The conditions for growth of the organisms were as given in the legends to Table 1. Methicillin (2  $\mu\text{g}/\text{ml.}$ ) and chloramphenicol (50  $\mu\text{g}/\text{ml.}$ ) were added to the appropriate flasks when the bacteria had grown to a density equivalent to 0.06 mg/ml. Mucopeptide was estimated chemically by the method described in the legend to Table 2.  $\circ$ , Chloramphenicol only;  $\Delta$ , methicillin only;  $\bullet$ , methicillin and chloramphenicol.

increases as the culture is incubated. The situation obtaining when no further mucopeptidase can be formed in the presence of methicillin is described in Table 2, which shows the effect of increasing concentrations of methicillin on mucopeptide formation by organisms incubated continuously in the presence of chloramphenicol. In this experiment mucopeptide formation was measured both chemically, which gives the net increase or decrease in the total amount of material, and by incorporation of  $1\text{-}^{14}\text{C}$ -glycine, which gives an indication of the total synthesized. It will be seen that 5  $\mu\text{g}/\text{ml.}$  of methicillin is just sufficient to prevent an increase in the amount of mucopeptide (measured chemically). The incorporation of radioactivity, however, shows that about 15.4  $\mu\text{g}$  of mucopeptide/10 ml. of culture were still formed. This would represent an increase of about 50 per cent over the original amount present, but an approximately equal amount has been removed by the mucopeptidase with the result that the total amount remains constant. This calculation, of course, assumes, without evidence, that both new and pre-existing mucopeptides are equally susceptible to the mucopeptidases. Chemical examination of the mucopeptide formed in the presence and absence of methicillin showed no analytical differences in the proportions of the amino-acids. In other words, the mucopeptide in the walls is "turning-over" in these conditions.

Table 2. INHIBITION OF MUCOPEPTIDE SYNTHESIS BY METHICILLIN ACTING IN THE PRESENCE OF CHLORAMPHENICOL

Concentration of methicillin ( $\mu\text{g}/\text{ml.}$ )	Mucopeptide synthesis chemical measurement ( $\mu\text{g}/10\text{ ml.}$ of culture)	percentage inhibition	Radioactive measurements in mucopeptide preparations Total counts $\times 10^{-3}$ in 10 ml. of culture	Mucopeptide synthesized (calculated) ( $\mu\text{g}/10\text{ ml.}$ of culture)
0	7.4	—	6.55	—
5	— 3	100	1.35	15.4
10	— 11	(100)	0.52	5.9
20	— 14	(100)	0.03	0.5
50	— 17.5	(100)	0.01	0.5

*Staphylococcus aureus* strain 524SC was grown at  $35^\circ\text{C}$ , with shaking, in a 1 per cent casein hydrolysate medium supplemented by thiamine, nicotinic acid, 0.5 per cent glucose and 0.002 molar magnesium sulphate until the extinction of the culture corresponded to 0.06 mg/ml. At this point sufficient sterile solutions (1 mg/ml.) of chloramphenicol and methicillin were added to give respectively 50  $\mu\text{g}/\text{ml.}$  and the concentrations shown in the first column above. Sufficient labelled glycine was added to give 0.01  $\mu\text{g}/\text{ml.}$  Samples of 10 ml. were removed immediately into 0.5 ml. of 50 per cent trichloroacetic acid, and again after 2 h incubation with shaking. Mucopeptide was prepared (ref. 18), and one sample of the final preparation was counted in a scintillation counter by suspending the preparation directly in the scintillation fluid. Another sample was hydrolysed in a sealed ampoule overnight with 6 normal hydrochloric acid at  $105^\circ$ . After removal of the hydrochloric acid and solution of the residue in water, the concentration of total ninhydrin-reacting material was measured (ref. 19). The amount of mucopeptide present in a 10 ml. sample at the time of addition of the antibiotic (that is zero time in the culture) was 39  $\mu\text{g}$ .

As the concentration of methicillin is increased, the synthesis side of the balance is gradually decreased until, at a concentration of 20  $\mu\text{g}/\text{ml.}$ , no significant incorporation of carbon-14 from glycine occurs, and in this experiment the full decrease of about 15–17  $\mu\text{g}/\text{sample}$  in the amount of mucopeptide is expressed. Despite removal of mucopeptide, neither death nor lysis of the bacteria occurs in an incubation period of 9 h. A chaser type of experiment in the presence of methicillin, in which a pulse of labelled glycine was followed by an excess of unlabelled glycine, showed that the radioactivity could be removed again from the walls of bacteria incubated in the presence of 2  $\mu\text{g}/\text{ml.}$  of methicillin, as would be expected if "turn-over" were taking place.

It must be presumed that penicillin does not, as part of its primary effect, prevent the synthesis of the enzymes which biosynthesize mucopeptide as distinct from inhibiting their action. The concentration of a penicillin which is just sufficient to kill a micro-organism might then be expected to be that which will inhibit mucopeptide synthesis in a growing culture for long enough to allow the increasing proportion of the polymer removed by the mucopeptidase in a given time to cause irreversible damage to the wall and hence to the membrane underneath. The quicker the organisms grow, the steeper will be the increase in the proportion of hydrolytic enzyme (mucopeptidase) to its substrate (mucopeptide) in the walls, as long as the action of the biosynthetic enzyme remains inhibited. The rate of death of the bacteria, therefore, will be related to the rate of protein biosynthesis in the culture which, in turn, controls the rate of formation of the hydrolytic enzymes.

This hypothesis suggests that cells would die the more rapidly the higher the concentration of the penicillin and the more complete the inhibition of mucopeptide synthesis, as long as the rate of protein biosynthesis remained constant. Methicillin, however, like benzylpenicillin, acting on some strains<sup>14,15</sup> but not others (O'Grady and Pennington—personal communication), has an optimal concentration (1–2  $\mu\text{g}/\text{ml.}$ ) for the rate of killing of *Staphylococcus aureus* strain 524SC, although again some other strains of *Staph. aureus* do not show this phenomenon<sup>16</sup>. The rate of lysis of strain 524SC of staphylococcus is also optimal at about 2–5  $\mu\text{g}/\text{ml.}$  (see Fig. 3). Nevertheless the experiment shown in Table 2 and a number of other results obtained suggest that concentrations of methicillin much greater than 2  $\mu\text{g}/\text{ml.}$  do not inhibit the action of the mucopeptidase.

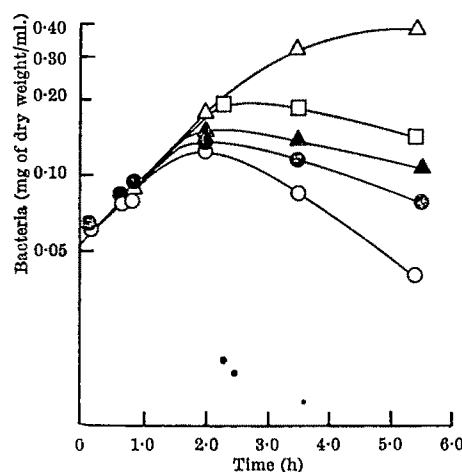


Fig. 3. The effect of various concentrations of methicillin on the growth and lysis of *S. aureus* strain 524SC. The organisms were grown at  $35^\circ\text{C}$ , with shaking, in the supplemented casein hydrolysate medium (see legend to Table 1). When growth reached a density equivalent to 0.06 mg/ml. the various concentrations of methicillin were added and samples removed during incubation at  $35^\circ\text{C}$  with shaking. The extinction was measured in an absorptiometer and the dry weight was calculated from a calibration curve.  $\Delta$ , 6  $\mu\text{g}/\text{ml.}$ ;  $\square$ , 1  $\mu\text{g}/\text{ml.}$ ;  $\blacktriangle$ , 20  $\mu\text{g}/\text{ml.}$ ;  $\bullet$ , 10  $\mu\text{g}/\text{ml.}$ ;  $\circ$ , 2  $\mu\text{g}/\text{ml.}$

The presence of chloramphenicol throughout the incubation of the organisms with methicillin prevents, or almost prevents, lysis of growing bacteria in the presence of 2  $\mu\text{g/ml}$ . of the penicillin but, from the results in Table 2, allows some action of a mucopeptidase up to a concentration of 50  $\mu\text{g/ml}$ .

Permeability changes in the outer layers of growing staphylococci, like the rate of death and lysis, are at a maximum when the organisms are growing in 2  $\mu\text{g/ml}$ . of methicillin in the absence of chloramphenicol (see Fig. 4). The results recorded in Fig. 4 were obtained 2 h after the addition of methicillin, at about the time that measurable lysis starts in the medium containing 2  $\mu\text{g/ml}$ . of methicillin (see Fig. 3). With this concentration of antibiotic, changes in permeability were just perceptible 15 min after its addition, were marked after 30 min, and the bacteria were completely permeable after about 2.5 h. The optical density of the culture was still increasing after 30 min. The increase in permeability with time occurred exponentially for the first 2 h, which is consistent with the continued exponential production of a mucopeptidase.

The higher concentrations of methicillin do not inhibit the activity of the autolytic enzyme normally present in the wall (see Table 2), and so some other reason for the inhibition of lysis must be sought. Three explanations seem possible. (1) The higher concentrations of methicillin themselves or the intermediates of mucopeptide synthesis which accumulate to a higher concentration as a result of the more complete inhibition of cell wall synthesis may specifically inhibit the formation of mucopeptidase. (2) A concentration of 2  $\mu\text{g/ml}$ . of methicillin is not sufficient to stop mucopeptide synthesis, so that disorganized expansion of the cell wall can occur during turnover. Both the deposition of new mucopeptide and the action of the lytic enzyme during this process may be localized. More damage may be caused than with higher concentrations of methicillin where the synthesis is completely stopped. (3) Higher concentrations of methicillin have some unrelated effect in preventing access of the mucopeptidase to its substrate. There is insufficient evidence to allow us to distinguish between

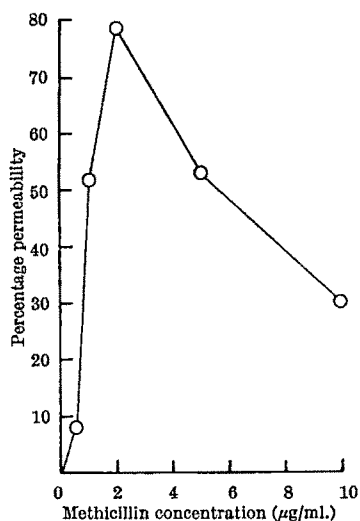


Fig. 4. The effect of methicillin concentration on the permeability of staphylococci to 8-anilino-1-naphthalene sulphonic acid (ANS). *Staphylococcus aureus* strain 524SC was grown to a density equivalent to 0.06 mg/ml. in the supplemented casein hydrolysate medium and the various concentrations of methicillin added. Incubation with shaking was then continued for 2 h. At the end of this time samples (10 ml.) were taken, the organisms centrifuged out rapidly and resuspended in 1 per cent buffered casein hydrolysate warmed to 35° C. An absorptiometer reading was taken for each sample and 0.5 ml. of 1.0 molar ANS in water added. The solutions were then read on a fluorimeter at 470 m $\mu$ , using light of 400 m $\mu$  to activate the fluorescence. The readings obtained were corrected for scatter from a calibration curve prepared from suspensions of untreated organisms of known extinction. Finally the reading after correction was compared with a similarly corrected reading for a sample of cells of the same density grown in the absence of penicillin and kept at 100° C for 5 min before the addition of ANS solution. Such cells are taken as 100 per cent permeable. This technique was adapted from the one used by Strange and Postgate (ref. 20).

the first two hypotheses. The third seems unlikely in view of the removal of mucopeptide which occurs in the presence of chloramphenicol and higher concentrations of methicillin up to 50  $\mu\text{g/ml}$ . (see Table 2). In considering specific inhibition of mucopeptidase formation, it is possible that the muramyl-peptide part of the nucleotide precursors could be end-product type repressors of mucopeptidase formation. Lytic enzymes, for example, might be expected to produce fragments related to these precursors in cells inhibited by penicillin. The second hypothesis also becomes attractive when the very localized formation of mucopeptide in cultures of multiplying cells<sup>17</sup> is compared with what appears to be the more general formation over the whole surface of the cell which takes place in cultures in which protein biosynthesis is inhibited<sup>10,11</sup>. If the lytic enzyme is also formed locally in multiplying cells, a little enzyme may be able to do much more local damage to the freshly synthesized mucopeptide than the same amount spread over the whole surface of the cell, as it may be when mucopeptide synthesis is completely stopped. The fact that considerable amounts of mucopeptide can be removed from the walls of the staphylococci incubated in the presence of chloramphenicol and high concentrations of methicillin (Table 2), without death or lysis of the micro-organisms, emphasizes the probability of a local removal in the growing cells.

The above experiments were all made with the single strain of *Staphylococcus aureus* 524SC plus methicillin, but similar although less dramatic results were also obtained with strain Oxford using both methicillin and benzylpenicillin, and some experiments were repeated with strain P 20. It is not necessary, of course, that the secondary reasons for death from penicillins of all species and genera of micro-organisms should be the same. Nevertheless the presence of mucopeptidases is widespread in bacteria, and may indeed be necessary for the growth of their walls<sup>10,11</sup>. The absence of lysis of an organism in the presence of penicillin does not, of course, exclude the involvement of mucopeptidases, especially if this action is localized. Relatively slight damage to the existing wall when synthesis is reduced by the antibiotic may be sufficient to cause great changes in the permeability of the cytoplasmic membrane without lysis occurring, as is shown by comparison of the results in Figs. 3 and 4. It should, in conclusion, perhaps be pointed out that the conditions used in the above experiments are quite different from those used in earlier investigations of mucopeptide synthesis<sup>1-4</sup>. In the earlier experiments conditions were such that little or no protein synthesis would occur even in the absence of chloramphenicol.

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# Reversal of the Action of Penicillin

by

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Growth curves of *Staphylococcus aureus* suggest that cells remain viable for some time after cell wall synthesis has been inhibited by penicillin

DURING an investigation of the mechanism of production of "persisters"<sup>1</sup> by following the changes in turbidity in a culture of *Staph. aureus* to which penicillin had been added, we found that the staphylococci had to be exposed to the action of the penicillin for a considerable period before lysis of the cells occurred, and for much of this time the action of penicillin was readily reversible.

Previous workers using viable counts as the index of the action of penicillin have indicated that viability is rapidly lost within minutes of exposure to the antibiotic in adequate quantities<sup>2-4</sup>. Many of the metabolic functions of the cells are, however, known to proceed normally for some considerable time after exposure and there is reason to believe that interruption of cell wall synthesis and death and lysis of the cells are separate events. Cell wall synthesis has been shown to continue when growth is inhibited by chloramphenicol, and while addition of penicillin to such cultures inhibited mucopeptide synthesis, it did not bring about death or lysis of the cells (preceding article).

It was found, again using viable counting techniques<sup>3,4</sup>, that staphylococci exposed for as little as 15 min to penicillin underwent a long quiescent phase sometimes lasting 3-8 h before growth was resumed. Yet cell wall synthesis, it seems, may be resumed within 15 min of the termination of a short pulse of penicillin<sup>5</sup>. Our observations show that cell wall synthesis may be interrupted and restored after a much longer period of exposure without affecting the viability of the cells.

A strain of *Staphylococcus aureus* which was sensitive to penicillin (M.I.C. 0.06 U/ml.) originally isolated from a wound swab was used throughout these experiments. This organism was chosen because of its sensitivity to penicillin and because the bactericidal action of the penicillin was readily manifested by lysis of the cells<sup>6,7</sup>.

A pear shaped flask suspended between the photo cells of a photometer contained 35 ml. of nutrient broth and 0.5 ml. of an overnight broth culture giving an approximate concentration of  $1 \times 10^6$  organisms/ml. Arrangements were made to maintain the temperature at 37° C and the culture was continuously stirred.

Solutions of benzyl penicillin were used throughout and were added to the culture chamber in quantities of 0.1 ml. The action of the penicillin was terminated by the addition of Wellcome penicillinase. At the concentrations used, even the largest amount of penicillin used was destroyed in less than 10 min.

The addition of penicillin to the culture chamber before or at the time of inoculation did not completely suppress an increase of turbidity. The staphylococci were able to "grow" for some time in the presence of what, as judged by conventional determination, was an adequate dose of penicillin. The effect was more marked when the penicillin was added in the mid-log phase (Fig. 1). Considerable concentrations of penicillin were required at this time to produce an immediate change in the growth curve.

If the action of the penicillin was terminated by the addition of penicillinase a series of curves was obtained as shown in Fig. 2. The activity of the penicillin had to be maintained for almost 100 min before appreciable lysis occurred. Withdrawal before the end of this critical period resulted in the rapid resumption of growth and the curve quickly resumed its former slope (Fig. 3).

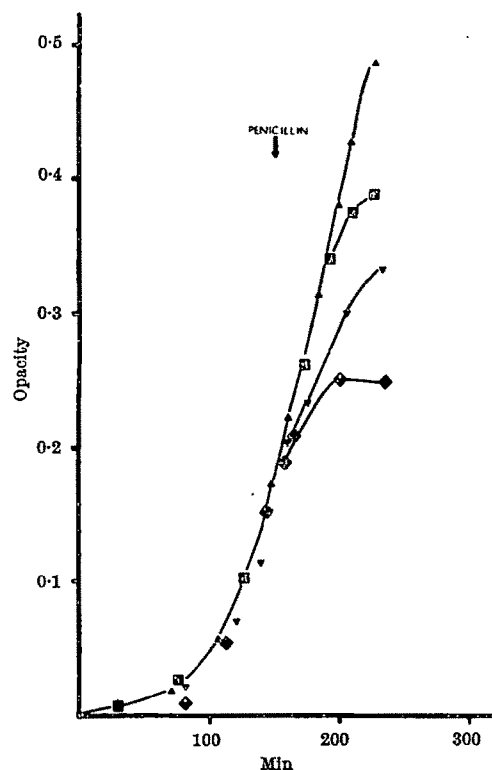


Fig. 1. The effect of penicillin on the growth curve when added in the mid-log phase. ( $\Delta$ ), Control; ( $\square$ ), 3 U/ml.; ( $\nabla$ ), 12 U/ml.; ( $\diamond$ ), 3,000 U/ml.

Turbidimetric measurements have been criticized on the ground that they are influenced by the bloated and apparently inviable spheres which penicillin has been shown to induce.

Cooper<sup>8</sup> has shown that the dry weight of a culture increased for approximately 60 min after exposure to penicillin, but only after this time was a rapid increase in size apparent. It is unlikely that the swelling of the cells giving a false appearance of growth could account for the very regular nature of the growth curve for some time after the addition of penicillin, nor would it be expected that the early addition of penicillinase would so restore

growth as to produce a curve indistinguishable from the normal. Furthermore, a family of curves obtained by our method closely resemble those produced by using oxygen consumption as the index of growth<sup>9</sup>. These results suggest that despite the almost immediate action of penicillin on cell wall mucopeptide synthesis the cells remain viable for some considerable time after cell wall synthesis is inhibited.

That the action of penicillin is so readily reversible is supported by the curves similar to that in Fig. 3 obtained

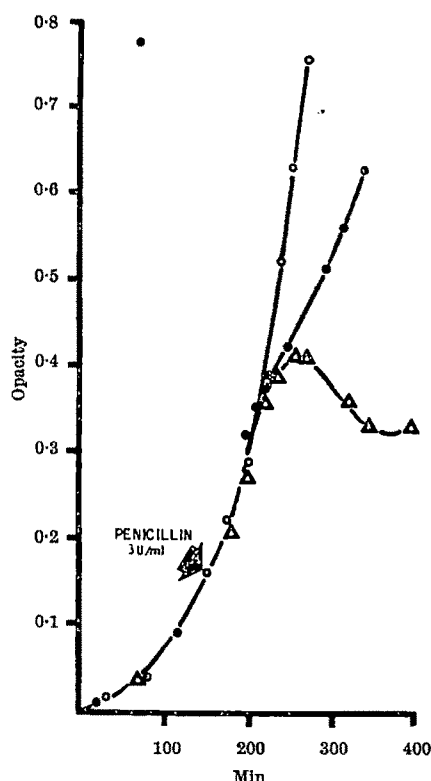


Fig. 2. The effect on the growth curve of the duration of exposure to penicillin. (○), 30 min; (●), 60 min; (△), 90 min.

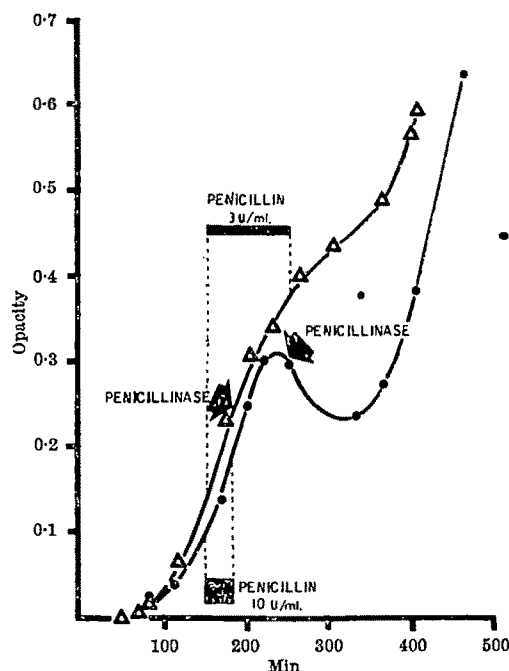


Fig. 3. A comparison of the effect of exposure to 3 U of penicillin/ml. for 100 min, and 10 U of penicillin/ml. for 30 min.

from a turbidimetric system measuring the effects of pulses of ampicillin on *Escherichia coli*<sup>10</sup>.

These results further emphasize the disparity in time between the effects of penicillin on cell wall synthesis and those secondary factors which lead to death and lysis of the cells.

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## Membrane Active Drugs and the Aggregation of Human Blood Platelets

by  
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The aggregation of human blood platelets is stimulated by ADP and other materials, and is known to take place in two stages. Studies with chlorpromazine, imipramine and related compounds indicate that they affect the second stage

HUMAN blood platelets can be made to aggregate by the addition of adenosine diphosphate (ADP), adrenaline, 5-hydroxytryptamine, thrombin and collagen, as well as by other agents (for review, see ref. 1). ADP is released from platelets by fatty acids<sup>2</sup>, thrombin<sup>3</sup> and collagen<sup>4</sup>, and the aggregation that they produce is inhibited by enzymes which remove ADP from the plasma<sup>5</sup>. These enzymes also inhibit aggregation by adrenaline and 5-hydroxytryptamine which, therefore, apparently act

through ADP released from the platelets. Macmillan<sup>6</sup> has observed that, in some samples of human citrated plasma, platelet aggregation occurred in two phases and he suggested that the added ADP caused the release from the platelets of more ADP which was responsible for the second phase. Haslam<sup>7</sup> has additional evidence that supports this conclusion.

Many drugs including antihistamines, local anaesthetics, tranquillizers and antidepressants in high concentrations

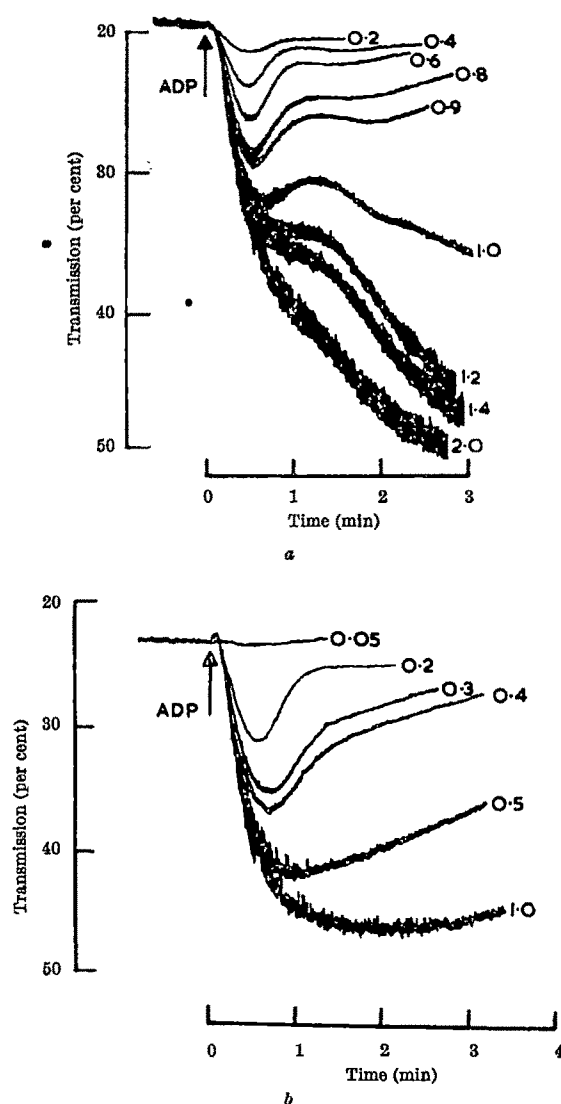


Fig. 1. Aggregation of platelets by ADP in platelet-rich plasma with (a) citrate or (b) heparin as anticoagulant. ADP was added at the arrow to give the final concentrations ( $\mu\text{moles/l.}$ ) shown by each curve.

(0.2–2 mmol/l.) inhibit the aggregation of human platelets by ADP (ref. 7). Promethazine inhibits the aggregation of rabbit platelets by 5-hydroxytryptamine at a much lower concentration (35  $\mu\text{moles/l.}$ ) (ref. 8). More recently, Ryšánek *et al.*<sup>9</sup> showed that aggregation of human platelets by adrenaline and by ADP can be inhibited by antidepressant drugs of the imipramine type at 50  $\mu\text{moles/l.}$  By using the turbidimetric method of Born<sup>10</sup> to follow aggregation continuously, we have established that these drugs at low concentrations act exclusively on the second phase. Some of the results have been reported briefly<sup>11</sup>.

Blood was obtained from healthy volunteers by venipuncture and collected in plastic centrifuge tubes containing 0.1 volume of a 3.8 per cent solution of trisodium citrate or 0.01 volume of a heparin solution (1,000 U/ml. saline). The blood was centrifuged at room temperature for 10 min at 226*g* to obtain platelet-rich plasma which was kept in siliconized glass vessels at room temperature. A sample of 1 ml. was pre-warmed to 37° C for 5 min and placed in a siliconized glass tube in a temperature controlled cell compartment of a modified EEL long cell absorptiometer similar to that used by Haslam<sup>2</sup> but stirred from below with a magnetic stirrer. Light transmission was recorded continuously on a 'Vitatron' 10 mV

recorder at a chart speed of 3 cm/min. The recorder scale was calibrated by setting the absorptiometer to 100 per cent transmission with each platelet-free plasma and adjusting the zero and sensitivity to give full-scale deflexion between 10 and 60 per cent. Reagents were added with microsyringes in volumes of up to 50  $\mu\text{l.}$

Fig. 1 shows superimposed the aggregation traces of normal human platelet-rich plasma with several concentrations of ADP, using citrate (Fig. 1a) or heparin (Fig. 1b) as anticoagulant. With citrate, aggregation was clearly separated into two phases with the plasma samples of twenty-seven out of thirty-one donors tested so far. The concentration of ADP at which the separation was seen, however, was highly critical and varied from one donor to the next. In the remaining four samples the traces were similar to those obtained with heparinized plasma, in which it was rarely possible to distinguish the two phases; the usual pattern was that of Fig. 1b.

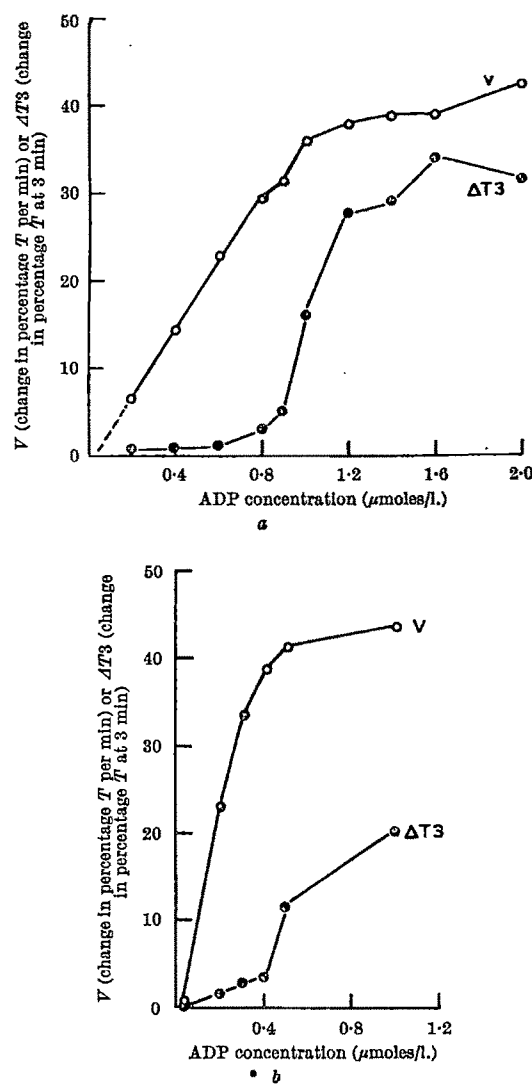


Fig. 2. Concentration-dependence of first phase ( $V$ ) and second phase ( $\Delta T_3$ ) of platelet aggregation induced by ADP in platelet-rich plasma containing (a) citrate and (b) heparin. For definition of  $V$  and  $\Delta T_3$ , see text.

To measure the two phases separately, we chose the initial rate of transmission change ( $V$ ) as an estimate of the first phase and the change in transmission 3 min after the addition of ADP ( $\Delta T_3$ ) as an estimate of the second phase. When the second phase was small, that is, with

low ADP concentrations (Fig. 1b), the initial aggregation reversed within 2.5 min so that by 3 min the contribution of the first phase to the overall effect could be ignored. In Fig. 2,  $V$  and  $\Delta T_3$  are plotted against the concentration of ADP with citrate (Fig. 2a) or heparin (Fig. 2b) as anticoagulant. At a given ADP concentration in heparin plasma the second phase was smaller than in citrate plasma, whereas the first phase was larger. The relation between  $V$  and the concentration of ADP was approximately hyperbolic with a small intercept on the abscissa (representing less than 0.1  $\mu$ molar ADP). By contrast, the plots of  $\Delta T_3$  were sigmoid.

The effect of desmethylinipramine on aggregation by ADP in heparin and citrate plasma is shown in Fig. 3. With citrate (Fig. 3a), low concentrations inhibited the second phase without affecting the first. With heparin plasma, the same concentrations had no effect on the initial velocity but were clearly inhibitory later on (Fig. 3b); this confirms the assumption that the second phase was present although masked by the first phase in the absence of inhibitor.

Adrenaline produced a two phase aggregation which differed from that seen with ADP in that the first phase was slower and did not spontaneously reverse; moreover, the rate and extent of the second phase were independent

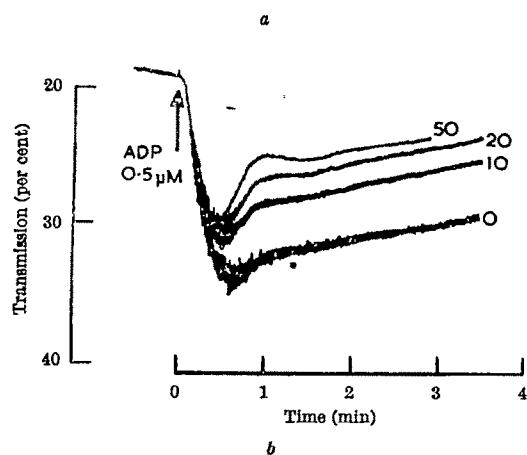
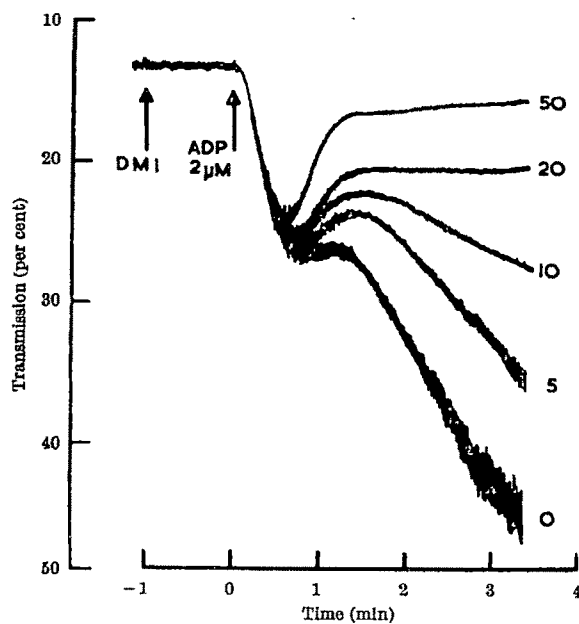


Fig. 3. Inhibition by desmethylinipramine of platelet aggregation induced by ADP in platelet-rich plasma containing (a) citrate and (b) heparin. Desmethylinipramine was added, 1 min before the ADP, to give the final concentrations ( $\mu$ moles/l.) shown by each curve.

of the concentration of adrenaline above 2  $\mu$ moles/l.; higher concentrations merely accelerated the onset of the second phase<sup>12</sup> (Fig. 4). Desmethylinipramine (Fig. 5) and the other drugs inhibited both phases of adrenaline aggregation though the effects were much more pronounced on the second phase. The first phase was irreversible, and therefore the two phases were measured by, respectively, the change in transmission ( $\Delta T_1$ ) at the point of inflexion of the curve and the further change ( $\Delta T_3 - \Delta T_1$ ) from this point to the point on the curve 3 min after the addition of adrenaline.

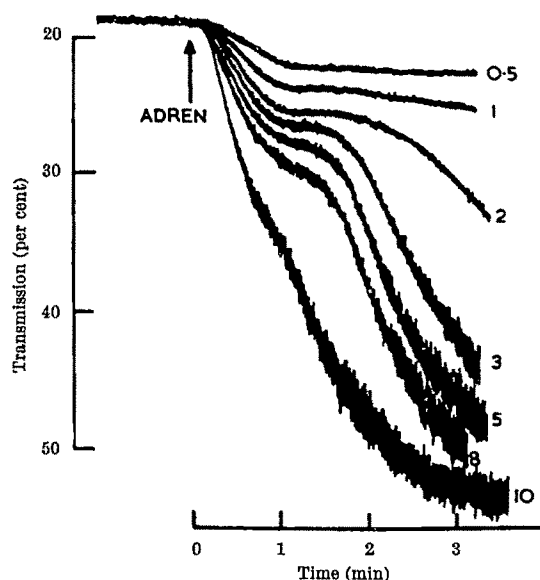


Fig. 4. Aggregation of platelets by adrenaline in platelet-rich plasma containing citrate. Adrenaline was added at the arrow to give the final concentrations ( $\mu$ moles/l.) shown by each curve.

Aggregation by 5-hydroxytryptamine was small, reversible and consisted of one phase only; higher concentrations inhibited<sup>13</sup>. Much lower concentrations of desmethylinipramine and the other drugs inhibited aggregation by 5-hydroxytryptamine than by ADP or adrenaline. All the drugs listed in Table 1 except diphenhydramine completely inhibited at 10  $\mu$ moles/l. the effects of 4  $\mu$ molar 5-hydroxytryptamine; chlorpromazine almost completely inhibited at 1  $\mu$ mole/l.

Suspensions of collagen for producing aggregation were prepared freshly each day by grinding dried collagen (Sigma (London), Ltd.) with saline in a glass mortar and centrifuging the mixture to remove the large particles. Collagen produced irreversible aggregation in a single phase after a lag period which was shortened by increasing concentrations. At 10  $\mu$ moles/l. and greater the drugs prolonged the delay and reduced the maximal rate of the aggregation which sometimes reversed a little; the most active drugs were nortriptyline and amitriptyline. Thrombin at a concentration too low to induce visible fibrin formation (0.1 M.I.H. U/ml.) caused a single-phase reversible aggregation which was not inhibited by chlorpromazine (50  $\mu$ moles/l.) or by desmethylinipramine (20  $\mu$ moles/l.).

Table 1 gives the percentage inhibition by drugs of both phases of aggregation induced by 2  $\mu$ molar ADP or adrenaline. For each aggregating agent the order of activity of the drugs was the same although the absolute inhibitions varied from plasma to plasma. Nortriptyline, amitriptyline and desmethylinipramine were the most powerful inhibitors of aggregation by ADP. Chlorpromazine, however, was as active against adrenaline and more active against 5-hydroxytryptamine.

The results show that chlorpromazine, desmethylinipramine and the other drugs in low concentrations inhibit the second but not the first phase of platelet aggregation by ADP. From their lack of effect on the first phase it appears that the drugs do not influence the mechanism by which ADP aggregates platelets. The second phase of ADP aggregation as well as aggregation by 5-hydroxytryptamine, adrenaline, thrombin and collagen apparently result, as already pointed out, from release of ADP from the platelets. It follows that the drugs probably act by inhibiting this release. Chlorpromazine, diphenhydramine and particularly promethazine have a "stabilizing" action on biological membranes; they inhibit swelling and contraction of isolated mitochondria and ion movements into liver slices<sup>15</sup> and they protect rats against the leakage of cell contents from the liver caused by poisons such as carbon tetrachloride<sup>16</sup>. The phenothiazines inhibit hypotonic lysis of erythrocytes<sup>17</sup>. Desmethylinipramine prevents the depolarization of frog sartorius muscle fibres produced by removal of calcium ions<sup>18</sup>.

Table 1. EFFECT OF DRUGS ON PLATELET AGGREGATION EXPRESSED AS PERCENTAGE INHIBITION OF CONTROLS

Drug	Final concentration ( $\mu$ moles/l.)	ADP (2 $\mu$ moles/l.) $\Delta T_3$	Adrenaline (2 $\mu$ moles/l.) $\Delta T_3 - \Delta T_1$
Chlorpromazine	50	1 (-2-2)	57 (42-68)
	10	6 (3-12)	29 (13-58)
Promethazine	50	-3 (-7-0)	62 (56-68)
	10	-1 (-2-1)	33 (29-37)
Desmethylinipramine	50	11 (1-25)	77 (65-95)
	10	5 (4-6)	53 (44-62)
Nortriptyline	50	-1 (-9-7)	84 (81-87)
	10	-1 (-7-5)	30 (21-39)
Amitriptyline	50	14 (5-27)	69 (45-89)
	10	7 (0-15)	54 (35-74)
Imipramine	50	13 (11-14)	59 (55-62)
	10	4 (1-7)	33 (32-35)
Diphenhydramine	50	5 (-10-14)	35 (25-51)
	10	2 (1-4)	25 (15-36)

$\Delta T_1$  and  $\Delta T_3$  represent the first phase,  $\Delta T_3$  and  $(\Delta T_3 - \Delta T_1)$  the second phase of aggregation (for definition of these parameters, see text). The figures are the mean and range of two to four experiments.

Imipramine and related compounds and, to a lesser extent, chlorpromazine inhibit the uptake of catecholamines by peripheral adrenergic neurones<sup>19</sup>. Both imipramine<sup>20,21</sup> and chlorpromazine<sup>21</sup> inhibit the active uptake of 5-hydroxytryptamine by platelets. This uptake is also inhibited by ouabain<sup>22</sup> which, we have found, does not inhibit aggregation by 5-hydroxytryptamine, ADP or adrenaline. This lack of effect indicates that the mechanism of action of chlorpromazine and imipramine on platelet aggregation is not related to inhibition of amine uptake or of the "sodium pump".

If the drugs act by inhibiting the release of ADP from platelets, their lack of effect on aggregation induced by thrombin implies either that it causes aggregation independently of ADP (ref. 23) or that it releases ADP by a different mechanism. Thrombin<sup>24</sup> and collagen<sup>14</sup> both cause degranulation of platelets but only aggregation by collagen is inhibited by chlorpromazine, etc. If both thrombin and collagen produce aggregation through degranulation, then they must do this in different ways.

If platelets release ADP when they aggregate, this could be the chain reaction postulated by Born<sup>25</sup> to account for the rapid growth of platelet aggregates into haemostatic plugs or thrombi. The low concentrations at which chlorpromazine and desmethylinipramine cause inhibition of

the second phase of platelet aggregation suggest that this effect may be produced *in vivo* in patients receiving these drugs. The inhibition of ADP release by drugs may be effective in the treatment of thrombotic conditions if compounds of this type can be found which produce no serious side effects on the nervous system.

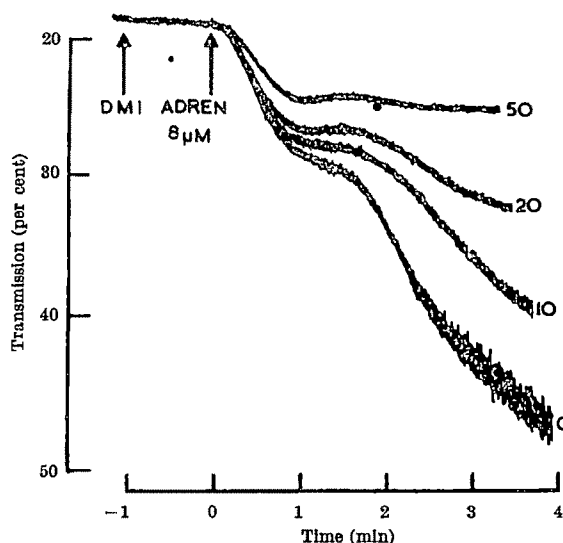


Fig. 5. Inhibition by desmethylinipramine of platelet aggregation induced by adrenaline. The desmethylinipramine was added 1 min before the adrenaline to give the final concentrations ( $\mu$ moles/l.) shown by each curve.

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## Pulse Labelled RNA associated with Ribosomes in *Escherichia coli*

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Pulse-labelling studies involving sucrose gradient centrifugation in changing concentrations of magnesium ions have been used to demonstrate that newly synthesized RNA, contrary to the current view, is not reversibly bound to 70S ribosomes but is attached even in low concentrations of magnesium ions to both 50S and 30S ribosomal sub-units

THE genetic analysis of the *lac* region of *E. coli* suggested the existence of short lived RNA (called messenger RNA) distinct from both ribosomal and transfer RNA (ref. 1). According to Jacob and Monod, genetic information is transcribed into an unstable messenger RNA which becomes temporarily attached to ribosomes where it serves as template for the synthesis of polypeptide chains. This hypothesis encouraged the search for messenger RNA which should be recognized as distinct from ribosomal RNA and in association with pre-existing ribosomal particles. There is evidence for the existence, in normal *E. coli*, of RNA molecules with these properties<sup>2</sup>. The problem was how to detect messenger RNA, which comprises only 2 per cent to 5 per cent of *E. coli* RNA. The solution was based on the assumption that messenger RNA shows a rapid turnover and therefore most of the RNA synthesized during a very short period would be messenger RNA. Growing cells of *E. coli* were pulse labelled with a precursor of RNA for 1/180 of the generation time, the cells were ground with alumina in a solution containing  $10^{-4}$  molar magnesium ions, and the nature of the pulse labelled RNA was investigated. The conclusion that the pulse labelled RNA was messenger RNA was based on two sets of evidence. (1) When bacterial extracts were treated with phenol most of the newly synthesized (pulse labelled) RNA had a sedimentation coefficient of 8S (whereas ribosomal and transfer RNA had sedimentation coefficients of 23S, 16S and 4S, respectively). (2) When the cell free extracts prepared in  $10^{-4}$  molar magnesium ions were subjected to sucrose density gradient centrifugation the 50S and 30S ribosomal sub-units were almost unlabelled and the majority of radioactivity moved with the 14S peak. When additional magnesium was added to extracts in  $10^{-4}$  molar magnesium ions about one third of the label sedimented with 70S ribosomes.

We have attempted a critical analysis of the results obtained by Gros *et al.*<sup>3</sup>, which shows that the data presented are not sufficient for the identification of pulse labelled RNA with messenger RNA.

The evidence that pulse labelled RNA of normal *E. coli* is distinct from ribosomal RNA is based on the finding that after extraction with phenol it sediments at 8S. The size of pulse labelled RNA which is extracted by phenol largely depends on the mode of extraction of nucleic acids. When the nucleic acids are extracted from *E. coli* which has been lysed by sodium dodecyl sulphate, the preparations of pulse labelled RNA contain, besides chains of low sedimentation rate, large components (16S to 30S) (ref. 3). When RNA is prepared by direct extraction of whole cells with phenol in a low ionic environment the pulse labelled fraction contains no small chains, and can be resolved, on methylated albumin columns, into two main components the molecular size of which correspond to sedimentation coefficients of 16S and 23S, that is, the molecular size of pulse labelled RNA was indistinguishable from that of ribosomal RNA<sup>4</sup>.

In view of these observations, it was assumed that the 8S material, originally designated as messenger RNA,

could be a product of the degradation of large molecules. Pulse labelled RNA of normal *E. coli* can be selectively degraded in conditions where the RNA of mature ribosomes is stable. This has been demonstrated both *in vivo* and *in vitro*<sup>5,6</sup>.

The evidence that pulse labelled RNA is messenger RNA is based on the finding that pulse labelled RNA exists free of ribosomes in extracts made in solutions of  $10^{-4}$  molar magnesium ions (sedimenting at 14S–16S) and that a fraction of the pulse labelled RNA sediments with 70S when the concentration of magnesium is raised to  $10^{-3}$  molar. We have already mentioned that the pulse labelled RNA in extracts made in low magnesium may be a degradation product, and so it is possible that the 14S pulse labelled RNA observed in extracts made in solution of  $10^{-4}$  molar magnesium ions is also a degradation product of large RNA molecules. It is important to decide whether or not the 14S pulse labelled RNA is a degradation product. Pulse labelled RNA may be messenger RNA, or ribosomal RNA which has been synthesized during the short period of labelling, or a mixture of both. In order to distinguish between these alternatives, preparations must be used in which the integrity of pulse labelled RNA is preserved.

The profile produced by bacterial extracts in a solution of  $10^{-4}$  molar magnesium ions has a shoulder of radioactivity of 30S, which is largely obscured by the overlapping and diffuse region of radioactivity with a peak at 14S. The radioactivity which sediments at 30S may represent the undegraded fraction of pulse labelled RNA which exists free of ribosomes in low magnesium and becomes attached to 70S ribosomal particles when the magnesium concentration is increased (messenger RNA). It may, however, represent pulse labelled RNA attached to 30S ribosomal sub-units in a low concentration of magnesium. In the latter case, the radioactivity found in 70S ribosomes on increasing the concentration of magnesium would not signify reversible attachment of pulse labelled RNA to ribosomes, but would result from the association of 50S sub-units with the labelled 30S ribosomal sub-units.

The presence in bacterial extracts of a large fraction of a possible degradation product of large molecules of pulse labelled RNA makes the interpretation of the results equivocal. For investigation of the nature of pulse labelled RNA which is associated with ribosomes, a system composed of ribosomes has several advantages over crude bacterial extracts, as long as a significant portion of the pulse labelled RNA sediments with ribosomes. This should be made clear for the following reasons. (a) Only pulse labelled RNA which is attached to ribosomes is considered; (b) the bulk of endonuclease activity present in bacterial extracts remains in the supernatant after the high speed centrifugation which is involved in the preparation of ribosomes; (c) the use of extracts in a solution containing  $10^{-4}$  molar magnesium ions, which may be detrimental to the integrity of pulse labelled RNA, is omitted; (d) if any portion of the pulse labelled RNA becomes degraded to chains of small



molecular size (8S–14S) in the course of the preparation of ribosomes, it would remain in the supernatant and not obscure the picture of pulse labelled RNA attached to ribosomes.

In this communication we present an analysis of pulse labelled RNA which sediments with ribosomes at  $10^{-2}$  molar concentration of magnesium ions. The results show that about 62 per cent of the total pulse labelled RNA sediments with ribosomes at a concentration of  $10^{-2}$  molar magnesium ions and that the pulse labelled RNA associated with ribosomes behaves as if it were an integral part of newly synthesized ribosomes rather than messenger RNA.

*Escherichia coli*, strain B, was grown in the synthetic medium of Davis and Mingioli<sup>7</sup> in a Dubnoff water bath at 37° C, shaken at 100 oscillations/min (the generation time was about 75 min). The cultures were grown overnight with limiting glucose (0.02 per cent) and reached an optical density of 0.07 (as measured in a spectrophotometer at 550 mμ). Overnight cultures were supplemented with 0.2 per cent glucose and the cells were grown for two generations. To 100 ml. of the exponentially growing cells 10 μc. of uracil labelled with carbon-14 were added. After 30 sec of labelling the cultures were made  $10^{-2}$  molar in sodium azide and poured over frozen and crushed  $5 \times 10^{-3}$  molar *tris* buffer, pH 7.4, containing  $10^{-2}$  molar magnesium ions (referred to as *tris*-Mg<sup>2+</sup> buffer). The cells were twice washed by centrifugation and then frozen. The frozen pellet was ground with twice its weight of alumina and extracted with several volumes of the *tris*-Mg<sup>2+</sup> buffer. Alumina and cell debris were removed by centrifugation at 30,000g for 20 min. For preparation of ribosomes the crude extracts were passed through two cycles of centrifugation at 35,000g for 20 min each and the ribosomes were pelleted and washed by two centrifugations in an ultracentrifuge at 150,000g for 60 min each. The final ribosomal pellet was taken up to  $5 \times 10^{-3}$  molar *tris* buffer, pH 7.4, containing the required concentration of magnesium ions. Three to five optical density units of ribosomes in 0.4 ml. of the appropriate buffer were layered on top of 4.4 ml. of linear sucrose gradient (5 per cent to 20 per cent w/v). The sucrose solutions were made in *tris* buffer and contained the specified concentrations of magnesium ions. The gradient was centrifuged at 37,000 r.p.m. for 2 h at 4° (unless stated otherwise). Samples of four drops each were taken. Volumes were made up to 1.0 ml. with the *tris* buffer and the ultraviolet absorption at 260 mμ and radioactivity (precipitable with trichloroacetic acid) were measured.

Table 1. DISTRIBUTION OF PULSE LABELLED RNA IN BACTERIAL EXTRACTS AND RIBOSOMES

Experiment	Preparation	Ribosomes O.D.	Ribosomal fraction (c.p.m.)	Radioactivity At 14S–16S (c.p.m.)	Ribosomal fraction percentage of total	Specific activity of ribosomal fraction
(1)	Cell free extracts	41.80	88,348	61,253	59.0	2.10
	Ribosomes	42.16	74,104	10,890	87.0	1.80
(2)	Cell free extracts	41.55	82,445	46,493	64.0	1.98
	Ribosomes	46.55	85,650	9,100	90.0	1.84

Table 1 shows that when bacterial extracts prepared in  $10^{-2}$  molar magnesium ions are centrifuged in sucrose density gradients containing  $10^{-2}$  molar magnesium ions 59 per cent to 65 per cent of the pulse labelled RNA sediments with 70S ribosomes and the remaining label moves free from ribosomes and sediments at about 16S–14S. The radioactivity which sedimented at 4S was not counted, because it apparently represented the newly synthesized transfer RNA. Specific activity of ribosomes prepared from the extracts was similar to that of the ribosomal fraction of crude bacterial extracts which indicated that in the course of preparation of ribosomes there was no loss of the pulse labelled RNA which was initially associated with 70S ribosomes. The table also shows that the

ribosomes obtained from bacterial extracts at a concentration of  $10^{-2}$  molar magnesium ions are relatively free of the 14S pulse labelled fraction.

The four parts of Fig. 1 show the analysis of sedimentation of ribosomes prepared from *E. coli* pulse labelled with uracil for 1/150 of the generation time. In  $10^{-2}$  molar magnesium ions (Fig. 1A) most of the radioactivity sediments with 70S ribosomes. Fig. 1B shows the profile of optical density and radioactivity of pulse labelled ribosomes in  $2 \times 10^{-3}$  molar magnesium ions. Here about 75 per cent of ribosomes are dissociated into 50S and 30S sub-units and the radioactivity is distributed among all three groups of ribosomes with distinct peaks at 70S, 50S and 30S. There is some difference between the specific radioactivities associated with the different groups of ribosomes; the highest is that of the 30S ribosomal sub-units (about 50 per cent higher than that of the 50S sub-units). Ribosomes run in sucrose density gradients containing  $4 \times 10^{-3}$  molar,  $6 \times 10^{-3}$  molar and  $8 \times 10^{-3}$  molar magnesium ions (not shown in Fig. 1) gave profiles of optical density and radioactivity similar in all respects to that shown in Fig. 1B, the only difference being the proportion of undissociated 70S ribosomes. The higher the concentration of magnesium ions the larger was the proportion of undissociated ribosomes and a correspondingly larger proportion of radioactivity sedimented at 70S.

Fig. 1C shows the analysis of ribosomes with pulse labelled RNA run in  $10^{-3}$  molar magnesium ions. The profile of optical density shows that the ribosomes are mainly dissociated into 50S and 30S sub-units. Of the three distinct peaks of radioactivity, two coincide with the peaks of 70S ribosomes and 30S ribosomal sub-units. The third peak of radioactivity, which at a concentration of  $2 \times 10^{-3}$  molar magnesium ions, sedimented at 50S, in  $10^{-3}$  molar magnesium ions, lags behind the 50S ribosome peak and sediments at about 47S–43S.

In  $5 \times 10^{-5}$  molar magnesium ions (Fig. 1D) the ribosomes are completely dissociated into 50S and 30S sub-units and the distribution of radioactivity differs considerably from that observed at higher concentrations of magnesium ions. Fig. 1D shows that the peak of radioactivity at 50S completely disappeared and that the region of radioactivity became rather diffuse with a peak around 30S and somewhat below it. This was a consistent finding in all our experiments.

We decided to examine in greater detail the sedimentation behaviour of pulse labelled RNA at a concentration of  $5 \times 10^{-5}$  molar magnesium ions. The behaviour at a low concentration of magnesium ions may merely reflect the dissociation of pulse labelled RNA which sediments at 50S at higher concentrations of magnesium ions, while the sedimentation rate of pulse labelled RNA which sediments with 30S ribosomal sub-units remains unchanged when the concentration of magnesium ions is decreased.

To test this interpretation, pulse labelled ribosomes were resolved into 50S and 30S sub-units by centrifugation in sucrose density gradients containing  $2 \times 10^{-3}$  molar magnesium ions (see Fig. 1B), isolated, made  $5 \times 10^{-3}$  molar with respect to *tris* buffer and  $10^{-2}$  molar with respect to magnesium ions and brought down by centrifugation at 60,000g for 18 h. The pellets of the 50S and 30S sub-units with pulse labelled RNA were suspended in  $5 \times 10^{-5}$  molar magnesium ions and run in sucrose density gradients containing  $5 \times 10^{-5}$  molar magnesium ions. The profiles of optical density and radioactivity are shown in Fig. 2. The main results were: (a) in  $5 \times 10^{-5}$  molar magnesium ions the radioactivity associated with 30S ribosomal sub-units is not displaced and sediments at exactly 30S; (b) the radioactivity associated with 50S ribosomal sub-units lags behind the peak of 50S ribosomal sub-units and sediments at about 40S–30S; (c) there is almost no pulse labelled RNA which sediments at a concentration of  $5 \times 10^{-5}$  molar magnesium ions which is free of ribosomal sub-units.

Our experiments demonstrate that: (a) in extracts prepared by grinding with alumina and elution in *tris* buffer containing  $10^{-2}$  molar magnesium ions about 65 per cent of the pulse labelled RNA is associated with 70S ribosomes and the remaining label sediments free of ribosomes; (b) the pulse labelled RNA associated with ribosomes in extracts made in  $10^{-2}$  molar magnesium ions can be quantitatively recovered with the ribosomal fraction prepared from the extracts by high speed centrifugation; (c) the pulse labelled RNA which sediments with ribosomes is not associated with 70S ribosomes as

such, but with both 50S and 30S ribosomal sub-units; (d) the label associated with the 30S ribosomal sub-units sediments at 30S from the concentration of magnesium used; (e) the label associated with 50S ribosomal sub-units sediments at 50S at concentrations of magnesium ions down to  $2 \times 10^{-3}$  molar. At  $10^{-2}$  molar magnesium ions and below it lags behind the 50S ribosome peak and sediments at 47S-30S.

Analysis of sedimentation shows that by lowering the concentration of magnesium ions the pulse labelled RNA

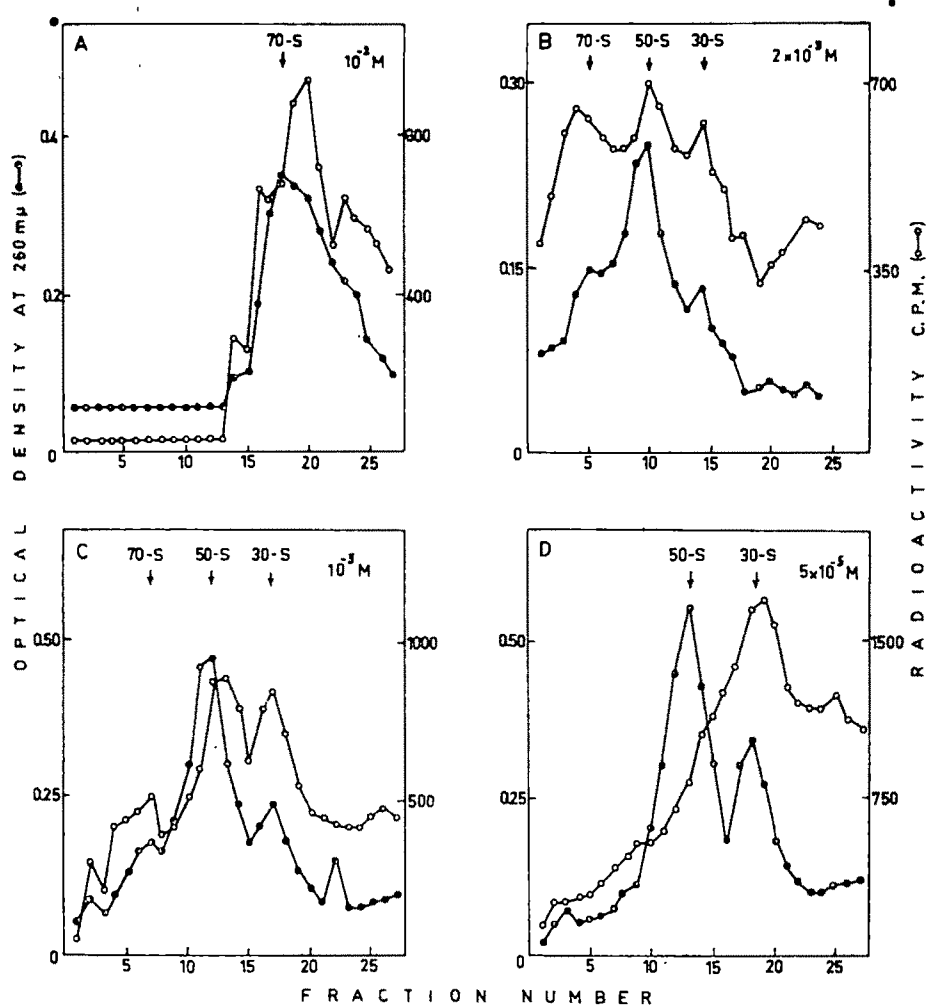


Fig. 1. Sucrose density gradient centrifugation of ribosomes from *E. coli* pulse labelled with uracil for 30 sec. The ribosomes were layered on top of 4.4 ml. of linear sucrose gradient (5 per cent-20 per cent w/w) in 0.005 molar *tris*-hydrochloric acid buffer, pH 7.4, containing magnesium acetate at (A)  $10^{-2}$  molar; (B)  $2 \times 10^{-3}$  molar; (C)  $10^{-3}$  molar; (D)  $5 \times 10^{-5}$  molar. Centrifugation was for (A) 60 min at 37,000 r.p.m., (B, C and D) 120 min at 37,000 r.p.m.

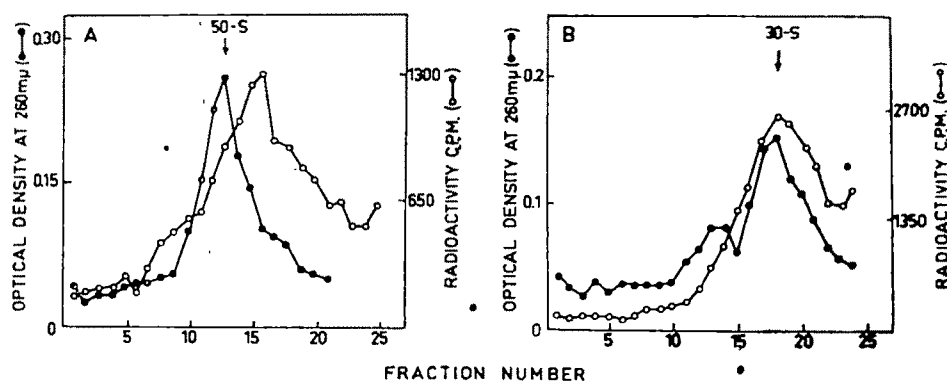


Fig. 2. Sucrose density gradient centrifugation of 50S and 30S ribosomes from *E. coli* after treatment with labelled uracil for 30 sec. The ribosomes were suspended and run in 0.005 molar *tris*-hydrochloric acid buffer, pH 7.4, containing  $5 \times 10^{-3}$  molar magnesium ions. (A) 50S ribosomal sub-units; (B) 30S ribosomal sub-units. Centrifugation was for 180 min at 37,000 r.p.m.

does not dissociate from ribosomes, but sediments with the 50S and 30S ribosomal sub-units. These results can be interpreted in two different ways: (1) the pulse labelled RNA is messenger RNA, but contrary to current ideas it is attached not to 70S ribosomes but to 50S and 30S ribosomal sub-units and it does not dissociate from ribosomal sub-units at a low concentration of magnesium ions; (2) the pulse labelled RNA represents the RNA portion of ribosomal sub-units which are synthesized during the period of labelling.

If the pulse labelled RNA was messenger RNA, its attachment to pre-existing ribosomal sub-units should have brought about an increase in the sedimentation rate of the resulting complexes, so that the slowest peak of radioactivity which represents messenger RNA attached to pre-existing 30S ribosomal sub-unit should sediment between 40S and 50S. The results showing that the pulse labelled RNA associated with 30S ribosomal sub-unit sediments exactly at 30S are most simply interpreted to mean that the pulse labelled RNA represents the RNA portion of the newly synthesized 30S ribosomal sub-units. In the same way, the pulse labelled RNA associated with 50S ribosomal sub-units represents the RNA portion of the newly synthesized 50S ribosomes.

The pulse labelled RNA which sediments at 50S at  $2 \times 10^{-3}$  molar magnesium ions begins to lag behind the 50S ribosome peak in a low concentration of magnesium. The displacement of radioactivity associated with 50S ribosomal sub-units observed at low concentrations of magnesium ions can best be interpreted in terms of conversion of the newly synthesized 50S sub-unit into a ribonucleoprotein particle which sediments more slowly. This interpretation is supported by the finding that mature 50S ribosomes of *E. coli* treated with EDTA could be converted into a component which sediments at 30S (ref. 8). A similar conversion of newly synthesized 50S ribosomal sub-unit seems to be caused by a decrease in the concentration of magnesium ions. 60S ribosome of yeasts can be converted into a 50S ribonucleoprotein particle simply by lowering the concentration of magnesium ions<sup>9</sup>.

The pulse labelled ribonucleoprotein particle which lags behind the 50S ribosomal sub-unit in  $10^{-3}$  molar magnesium ions has all the characteristics of the neosome<sup>10</sup>. Our results indicate that this ribonucleoprotein particle may not be a precursor of the 50S ribosomal sub-unit, but the newly synthesized 50S ribosome itself which becomes converted into a more slowly moving particle at a low concentration of magnesium ions.

It is not surprising that pulse labelled RNA of normal *E. coli* contains newly synthesized ribosomal RNA.

Different methods have resulted in the conclusion that pulse labelled RNA of *E. coli* was made up of 66 per cent of ribosomal RNA<sup>11</sup>. No fraction of the pulse labelled RNA associated with ribosomes which, on the basis of reversible association with pre-existing 70S ribosomes, could be designated as messenger RNA was detectable. If we had analysed the sedimentation behaviour of the pulse labelled RNA in two concentrations of magnesium only (in  $10^{-2}$  molar and  $5 \times 10^{-3}$  molar) we could have interpreted the results in terms of messenger RNA and would not have suspected that the pulse labelled RNA was associated with both 50S and 30S ribosomal sub-units. The evidence that the pulse labelled RNA associated with ribosomes is an integral part of newly synthesized ribosomes was provided by the analyses carried out in magnesium concentrations which were intermediate between the two extreme ones and by analysis of ribosomal sub-units with pulse labelled RNA isolated by sucrose density gradient centrifugation.

There is no direct evidence for the existence in normal *E. coli* of RNA molecules distinct from ribosomal RNA and reversibly attached to pre-existing 70S ribosomal particles depending on the concentration of magnesium ions.

The currently favoured view is that messenger RNA binds ribosomes into polysomes<sup>12,13</sup>. In bacterial extracts prepared by grinding with alumina no polysomes can be detected and the ribosomes exist in the form of monosomes. The reason for this is unknown. The evidence for the existence of polysomes in *E. coli* was provided by the use of gently lysed spheroplasts<sup>14</sup>. It is possible that the pulse labelled RNA which exists free of ribosomes in extracts made in  $10^{-2}$  molar magnesium ions represents messenger RNA which is unable in these conditions to attach to ribosomes and therefore to bind them in polysomes.

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## Substrate Hydrophobic Groups and the Maximal Rate of Enzyme Reactions

by  
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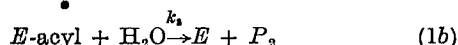
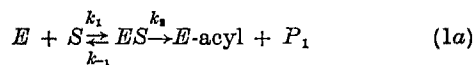
The maximum rate ( $V_m$ ) of certain enzyme reactions is increased as much as two to three times by a single additional carbon atom in a "chemically inert" hydrophobic region of the substrate molecule

THE rate of non-enzyme hydrolysis of a series of *n*-fatty acid esters of *m*-hydroxybenzoic acid is not influenced by the length of the acyl carbon chain between  $C_4$  and  $C_{10}$  (ref. 1). Apparently, the electronically insulated and relatively chemically inert carbon chain does not affect the ester bond. Nevertheless, the maximal rate ( $V_m$ ) of hydrolysis of these esters by various esterases usually increases with the length of the acyl carbon chain up to a certain length of chain and most often drops

sharply thereafter<sup>1-5</sup>. This "optimum" length is determined by the acyl moiety of the substrate<sup>3,6</sup> and is specific for each esterase tested. Below the optimum, however, the rate increase for each carbon atom is of the same order (by a factor of between two and three) for enzymes as different as liver esterase<sup>1</sup> and chymotrypsin<sup>3,6</sup>.

These observations indicate that, in contrast to non-enzyme hydrolysis, the "chemically inert" carbon chain plays an important part in hydrolysis by enzymes. Of

particular importance for the present discussion is the fact that the length of the carbon chain invariably affects  $V_m$ . On the other hand,  $K_M$  is not always affected. For example, with liver esterase  $V_m$  increases by a factor of between two and three for each additional carbon atom up to at least  $C_{10}$ , but there is little or no effect on  $K_M$  (ref. 1). As has been shown previously<sup>8</sup>, this indicates that  $K_M$  is a dissociation constant ( $k_{-1}/k_2$ ) and that  $k_2$  is the rate limiting constant for the overall reaction written as



Thus, the length of the carbon chain of the substrate influences the rate at which the products ( $P_1$ ,  $P_2$ ) are formed from the enzyme-substrate complex ( $ES$ ) but has little or no influence on the affinity of the substrate for the enzyme. Perhaps in this case the contribution of the acyl moiety to the affinity of the whole substrate molecule for the enzyme is negligible compared with that of the non-acyl moiety.

The quantitatively similar effect of the length of the carbon chain (below the optimum) on the  $V_m$  constant of different esterases suggests that a common underlying principle is involved. This principle must be sought in "physical" properties of the esters rather than in "chemical" properties because the former (for example, melting point) are influenced by the length of the carbon chain to a greater extent than the latter (for example, the rate of non-enzyme hydrolysis).


Because the rate of non-enzyme hydrolysis of the esters is not affected by the length of the carbon chain beyond  $C_3$ , it appears that the relative rates ( $V_m$ ) of enzyme hydrolysis of a homologous series of substrates  $>C_3$  depend only on the size of the hydrophobic group. A tentative explanation for the large effect of a single carbon atom on the rate of the enzyme reaction and for the occurrence of an optimum length of the carbon chain could be based on the assumption that the hydrophobic acyl group of the substrate enters into association with a particular hydrophobic region in the active centre of the enzyme. (Direct evidence has already been presented for the occurrence of such a region in chymotrypsin<sup>7,8</sup>.) In a hydrophilic environment the largest possible number of substrate carbon atoms could be expected to enter into association with such a hydrophobic region of the enzyme. Only the hydrophobic moiety of that substrate which contains the maximum number of carbon atoms that could be accommodated by this region would thereby be forced into a fixed position on the protein. Smaller hydrophobic groups as well as larger ones could take different positions with the same number of carbon atoms in contact with the enzyme. Thus the size of the hydrophobic region on the enzyme, which may differ from one esterase to the next, may influence the orientation of the substrate in the enzyme-substrate complex. The closer the correspondence of the size of this region to that in the substrate, the greater would be the restriction on the orientation of the latter. For the case where the deacylation constant ( $k_3$ ) is rate limiting, this would apply to the orientation of the acyl group attached to the enzyme by means of an ester bond. (An effect of the length of the  $n$ -acyl carbon chain on the rate of hydrolysis of acylated chymotrypsin has indeed been observed<sup>9</sup>).

This hypothesis would reduce the problem to the effect of intramolecular "strain" in the enzyme-bound substrates on the rate of the pertaining enzyme reactions (for references and discussion, see first edition of ref. 10). This may be further elaborated by assuming that the carbon chain of the substrate enhances the formation and stabilization of the hydrophobic area in the enzyme formed through juxtaposition of hydrophobic amino-acid

side chains which are remote in the unfolded protein. Depending on the number and the nature of the amino-acid side chains involved, some substrate carbon chains would be too short to stabilize effectively the hydrophobic area. Others would be too large to be limited to the stabilization of that particular protein conformation most favourable for the reaction.

The effect of the length of the carbon chain of the substrate on its affinity for an enzyme has been interpreted on the basis of decreasing solubility with increasing length of the carbon chain<sup>11,12</sup>. Decrease in the solubility of the chain could also affect intramolecular hydrophobic interaction in the enzyme-substrate complex, but could not account for the occurrence of an optimal length of the carbon chain.

Although the size of the postulated hydrophobic area on the enzyme would be only one of the parameters determining the rate of reaction, its importance as one of the determinants for specificity would be indicated by the fact that the optimum number of carbon atoms in a particular homologous series of fatty acid esters differs for different esterases<sup>1-5</sup>. Furthermore, in two series of  $n$ -fatty acids esters with different non-acyl moieties, the same optimum number of carbon atoms is found for a particular enzyme. For example, it has been shown<sup>3,6</sup> that although the absolute maximal rates of hydrolysis ( $V_m$ ) of  $n$ -fatty acid esters of *ortho*- and of *meta*-hydroxybenzoic acid by chymotrypsin are grossly different, for both series of substrates the highest relative  $V_m$  is found with the heptoyl ester. This point is further emphasized by the recent findings of Jones *et al.*<sup>13</sup>, who investigated the rate of hydrolysis by chymotrypsin of a homologous series of  $N$ -acylated amino-acid esters with  $n$ -alkyl side chains ( $R_2$ ). The highest rate of hydrolysis was found for  $R_2 = C_5H_{11}$ . Based on the general formula for chymotrypsin substrates,  $R_1-CH(R_2)-CO-R_3$  (ref. 14), the optimum  $R_2$ -chain of the fatty acid esters (for which  $R_1 = H$ ), is identical with that of the amino-acid esters. Becker *et al.*<sup>15</sup> found that the optimum number of carbon atoms in the  $R_2$ -group is the same with respect to the overall rate of acylation (equation 1a) of chymotrypsin by a series of  $n$ -alkyl substituted phosphonates (where P takes the place of the carbonyl-C in the general formula), as for the overall rate of acylation by fatty acid esters of hydroxybenzoic acid. (The relative overall rate of acylation, as compared with the acylation constant ( $k_2$ ), is given by the relative  $V_m/K_M$  ratios of true substrates<sup>5,9</sup>. The optimum number of C-atoms in the  $R_2$ -group of  $n$ -fatty acid esters, with respect to  $V_m/K_M$ , is 6 as compared with 5 for  $V_m$  (ref. 5). Becker *et al.*<sup>15</sup> found an optimum number of 6 carbon atoms for the  $R_2$ -group with respect to the overall rate of acylation by the  $n$ -alkyl phosphonates.)

In  $N$ -acylated phenylalanine esters which are hydrolysed by chymotrypsin at a high rate<sup>10</sup>, the  $R_2$ -group is represented by  $-CH_2\text{---}$  . For the formation of micelles in solutions of compounds with hydrocarbon chains of varying size, a benzene ring is equivalent to 3-4 straight chain carbon atoms<sup>16</sup>. The formation of micelles like the proposed interaction of the  $R_2$ -group with the enzyme depends on the formation of hydrophobic bonds. Thus in this respect, the size of the  $R_2$ -group in the phenylalanine esters also corresponds approximately to the optimum size of the  $R_2$ -group in the fatty acid esters. On the basis of the proposed hypothesis it is thus conceivable that a chief factor in the relatively high rate of hydrolysis of esters of aromatic amino-acid esters by chymotrypsin is that the size of the  $R_2$ -group of these substrates, in particular those of phenylalanine and tyrosine, corresponds to the size of the postulated hydrophobic area on the enzyme surface. The rate of hydrolysis of acetyl-tryptophan ethyl ester is about ten times less than that of acetyl-tyrosine ethyl ester<sup>10</sup>. Perhaps the tryptophan side chain is too large for the hydrophobic area on the

enzyme and therefore not limited to one particular orientation.

The observation that the optimum size of the  $R_2$ -groups is the same for esters of amino-acids, fatty acids and even of substituted phosphoric acid would suggest that in productive complexes the  $R_2$ -groups of these esters are attached to the same hydrophobic area on the enzyme surface. This, however, does not exclude the possibility that part of the chymotrypsin molecules form unproductive complexes through combination of an  $R$ -group with the "wrong" site on the enzyme<sup>17</sup>. As suggested by Jones *et al.*<sup>13</sup>, this could be the reason for the relatively low  $V_m$  of chymotrypsin with respect to the hydrolysis of the fatty acid esters of hydroxybenzoic acid, that is, the benzene ring in  $R_3$  would tend to combine with the site to which the  $R_2$ -group must be attached for reaction to occur. For the esters of amino-acids, this possibility would be reduced through the presence of the  $R_1$ -group that combines with a corresponding group in the multifunctional enzyme<sup>17</sup>. In fatty acid esters the  $R_1$ -group is absent and these esters, therefore, are merely "makeshift" substrates for chymotrypsin. It may be assumed, however, that true fatty acid esterases (and most other hydrolytic enzymes) are bifunctional in nature, that is, are adapted to bifunctional substrates, and do not require the  $R_1$ -group in the substrate. For such enzymes, the size of the hydrophobic side chains in the substrates would be an even more dominant factor for determining the rate of the reaction than is the case for chymotrypsin.

The effect of substrate hydrophobic groups on enzyme specificity is not limited to the type of enzymes discussed

here. A compilation by Dixon and Webb<sup>10</sup> shows that the rate of the reaction of a large variety of enzymes, including oxidases, depends greatly on the presence and length of  $n$ -hydrocarbon chains in the substrate and most often shows a definite optimum value for a carbon chain of particular length. This emphasizes the general importance of "chemically inert" regions in the substrates. In terms of the proposed hypothesis, it would mean that all these enzymes are endowed with a hydrophobic region in the active site. The optimum length of the carbon chain of the substrate would be related to the size of this region.

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## Immunological Relationship between Streptococcus A Polysaccharide and the Structural Glycoproteins of Heart Valve

by

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Streptococcal infections can cause rheumatic heart disease. Polysaccharide from group A streptococci may induce the production of antibodies by an animal which then react against its own valvular glycoproteins

THE part played by group A streptococcus in the pathogenesis of rheumatic fever is now generally recognized. The mechanism by which streptococcal infection induces the joint and heart disease, however, has not been resolved. Kaplan's recent investigations<sup>1,2</sup> have suggested that the myocardial lesions may arise from an immunological process which results from the cross-reaction between the streptococcal M protein and some protein constituents of the myocardium.

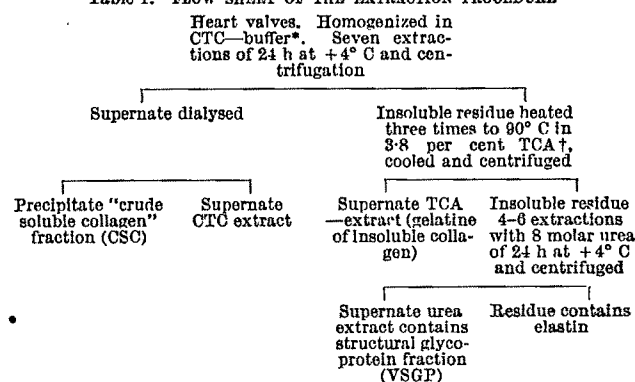
Our investigations tend to indicate that there is a pronounced immunological cross-reaction between the polysaccharide specific to the streptococcus A group and the structural glycoprotein(s) isolated from heart valves.

Valvular extracts were prepared according to the procedure of Robert *et al.*<sup>3</sup> from human and bovine cardiac valves. Human valves were obtained from various heart surgery clinics through the courtesy of Professor Dubost, or were obtained from cadavers within a few hours of death. Bovine valves were obtained from the slaughter house and frozen immediately to  $-20^{\circ}\text{C}$ .

The extraction procedure is depicted as a flow sheet in Table 1. The details of the method have already been re-

ported<sup>3,4</sup>. The structural glycoprotein fraction(s) of heart valves (VSGP) obtained by the urea extraction procedure contains several components; their physical and chemical

Table 1. FLOW SHEET OF THE EXTRACTION PROCEDURE<sup>a</sup>



\* Calcium chloride (1 mole/l.) + tris (0.05 mole/l.), citric acid (0.02 mole/l.) adjusted to pH 7.5.  
† Trichloroacetic acid.

properties will be described elsewhere. The dialysed urea extract precipitates slowly during storage in the cold; however, it can be redissolved in 8 molar urea and redialysed against a 0.9 per cent solution of sodium chloride for use. The structural glycoprotein fractions of other organs, such as aorta, cartilage and cornea, were prepared by the same procedure<sup>3,4</sup>.

Streptococcal extracts were obtained as follows: (1) a trypsinized suspension of group A haemolytic streptococcus, NY 75 strain, was treated with a 0.5 per cent solution of trypsin at pH 8 for 24 h; (2) group A streptococcal polysaccharide was prepared and purified according to the procedure of Fuller<sup>5</sup> and McCarty<sup>6</sup>; (3) group A streptococcal polysaccharide was linked to edestin by diazotization according to the method devised by Hämmerling<sup>7</sup>.

The anti-heart valve antisera used were obtained from rabbits by injecting the total homogenate of the heart valve or its CTC extract (see Table 1). Three 1-ml. injections were given weekly, which contained 5 mg of protein emulsified in an equal volume of Freund's complete adjuvant, for 3 weeks. After a rest for 1 week a booster injection of an equal dose was given and the animals were bled 1 week later.

The anti-streptococcal antisera were obtained by injecting over a period of 3 weeks 0.25 ml. of a suspension of  $10^8$  bacteria in complete Freund's adjuvants in the plantar soles, followed by fifteen daily injections of a water suspension of the bacteria at the same concentration, in progressively increasing dosage, from 0.25–1 ml. After a rest for 8 days, the animals were bled. All immune sera were kept at  $-20^\circ\text{C}$ .

The anti-polysaccharide-destin sera were obtained by injecting 15  $\mu\text{g/kg}$  of the protein-polysaccharide complex in Freund's complete adjuvant three times a week for 2 weeks intramuscularly, followed by three intravenous injections of 20  $\mu\text{g}$  of the complex during the subsequent week. Some animals received a booster injection 10 days later. These sera were tested against the edestin-polysaccharide complex as well as against its separate constituents.

The antibody titres were determined by the quantitative precipitation technique of Kendall-Heidelberger as described by Kabat<sup>8</sup>, using Markham's micro-Kjeldahl procedure<sup>9</sup>, and by a passive haemagglutination reaction using rabbit erythrocytes coupled with antigen by diazotized benzidine as described earlier<sup>10</sup>. The gelose double-diffusion experiments were performed according to a modified procedure using 0.9 per cent agarose plates in a 0.9 per cent solution of saline buffered to pH 6.0. The immunofluorescence experiments were performed according to Coon's direct technique. The gamma-globulin fraction of the antisera was first precipitated at 33 per cent saturation with ammonium sulphate and then labelled with fluorescein isothiocyanate, followed by purification on 'Sephadex G-25' column and fractionation on a DEAE cellulose column according to the technique of Lewy and Sober<sup>11</sup>. The globulins labelled with fluorescein were tested on slides with streptococci of groups A, C, G and H. The specificity of the antisera was tested by absorbing them to the homologous antigen or to antigens to be tested for cross-reactions. The absorptions were performed by adding 5–10 mg antigen as a lyophilized powder/ml. antisera, incubated for 24 h in the cold and

centrifuged. This treatment was repeated three or four times.

All rabbits immunized with the valvular extracts (total homogenate or CTC extract) developed antibodies against several components of the valvular tissue present in the CTC extract (soluble proteins) and in the total homogenate (soluble and insoluble components). All antisera reacted with the structural glycoprotein fraction (urea extract, VSGP) when tested by the haemagglutination and/or precipitation reaction. The titres obtained are indicated in Table 2. The sera obtained by immunization with the urea extract (VSGP) did not give precipitates with this extract in the usual conditions (24 h of incubation at  $+4^\circ\text{C}$ ) but did react in the passive haemagglutination reaction, giving titres between 1/128 and 1/1,256. The weaker antigenicity of the urea extract might arise from the alterations produced by the isolation procedure (see Table 1).

The precipitation and haemagglutination reactions were regularly nullified by previously absorbing the antisera on total valvular homogenates or on the CTC extract. Absorption to the urea extract (VSGP) of either the anti-total homogenate or of the anti-CTC sera reduced their titres by 40–50 per cent. This reduction was accompanied by the disappearance of one of the precipitation lines observed on agarose plates.

All the immunized animals produced antibodies in high titres, ranging from 200 to 1,060  $\mu\text{g}$  nitrogen/ml. serum. These antibodies were entirely absorbed on intact streptococci and also on the purified group A polysaccharide. For the cross-reactivity studies, we used the sera giving the highest titres. Table 3 summarizes the cross-reactions observed between anti-heart-valve antisera and the group A specific polysaccharide. This polysaccharide, when coupled to edestin, precipitates with the anti-valve antisera, giving titres of 25–38  $\mu\text{g}$  nitrogen/ml. serum. When the pure polysaccharide was used somewhat lower titres were obtained. No reaction was obtained with edestin.

Table 3. CROSS-REACTIONS BETWEEN AN ANTISERUM TO TOTAL BOVINE HEART VALVE HOMOGENATE AND THE GROUP SPECIFIC POLYSACCHARIDE OF STREPTOCOCCUS A

Antiserum absorbed to:	Precipitating antibodies to				Fluorescence of group A strepto- cocci treated with fluorescent antiserum	
	$\mu\text{g}$ Nitrogen/ml. of serum †	Strep. Bovine polysacc. VSGP edest.*	No. of lines in double gel diffusion	Strep. Bovine polysacc. VSGP edest.*	Strep. A	Strep. C, G, H ‡
No treatment	114	38	2	1	+++	±
Group A spec. strept. polysacc.	68	0	1	0	±	±
Bovine VSGP	0	0	0	0	±	±

\* Polysaccharide A-destin complex.

† Tested separately with all three strains.

‡ Total precipitate comprising antibody and coprecipitating antigens.

Absorption to VSGP eliminated all the antibodies reacting with the streptococcal polysaccharide. Absorption to the group A polysaccharide did not completely eliminate the reaction with VSGP, but it did eliminate one of the precipitation lines on Ouchterlony plates. The polysaccharide-destin complex also gave a precipitation line with anti-valve antisera which disappeared after absorption on VSGP or on the pure polysaccharide. The antisera labelled with fluorescein reacted specifically

Table 2. PRODUCTION OF ANTIBODIES IN RABBITS IMMUNIZED WITH BOVINE AND HUMAN HEART VALVE EXTRACTS

Immunizing antigen	No. of animals	Precipitating antibodies to:		No. of lines in double gel diffusion		Haemagglutinating antibodies to (inverse of haemagglutination titre):	
		$\mu\text{g}$ Nitrogen/ml. serum*					
		Bovine valve CTC	Bovine VSGP	Bovine valve CTC	Bovine VSGP	Bovine valve CTC	Bovine VSGP
Whole bovine valvular homogenate	12	56–250	20–114	3–5	1–2	32,000–64,000	80–1,280
CTC extract of bovine valve	7	93–152	24–62	3–6	1–2	20,480–40,960	40–80
CTC extract of human valve †	3	40–120	18–36	2	1	16,000–32,000	128
Bovine VSGP	6	±	± ‡	0	0	0	128–2,560

\* Determined on total precipitate (includes antibody and coprecipitated antigen).

† Anti-human CTC-sera were assayed with test antigens of human origin: human heart valve CTC extract and human heart VSGP.

‡ Precipitate too weak to be determined.



with the group A streptococcus and did not react with the other groups of streptococci tested. This reaction was also abolished by prior absorption of the sera to VSGP, or to the specific polysaccharide. Similar results were obtained with anti-VSGP antisera using the passive haemagglutination reaction: the titres were abolished or considerably weakened after absorption to group A streptococci or to the purified group A polysaccharide.

Absorption to pneumococcus type III specific polysaccharide failed to decrease any titre or to make any precipitation line disappear.

The results of the experiments on the cross-reaction between anti-streptococcal antisera and the structural glycoprotein obtained with bovine heart valve extracts are given in Table 4. Some of the experiments were repeated with human heart valve CTC extracts with essentially identical results. Precipitation reactions were regularly obtained by adding the urea extract of heart valves to anti-streptococcal antisera, giving titres of the order of 30–40 µg nitrogen/ml. serum. This precipitation reaction was abolished by previous absorption of the antiserum to group A streptococci or to the group A specific polysaccharide. The CTC extract of human heart valve also gave precipitates with the anti-streptococcal sera, though in a somewhat lower titre: about 20 µg nitrogen/ml. serum. This reaction was also abolished by previous absorption to group A polysaccharide as well as by the VSGP fraction.

The bovine and human VSGP fraction gave one or two precipitation lines on double diffusion against the anti-streptococcal antisera. These lines disappeared after absorption of the antisera to the VSGP preparation. The precipitation line given by the group A polysaccharide did not disappear after absorption of the anti-streptococcal antisera on to VSGP. This can be explained by the presence of antigenic determinants on the polysaccharide not shared with the preparation of the structural glycoprotein.

Cross-reactions of varying intensity also exist between the structural glycoprotein preparations of various organs<sup>2,4</sup>. The urea extracts of human aorta and of bovine cornea (KGAG) gave cross-reactions with the anti-streptococcal antisera as was demonstrated by the double diffusion method. These precipitation lines disappeared after absorption of the sera to the VSGP fraction.

Absorption to human erythrocytes of blood group A and B did not result in the disappearance of the precipitation lines. The results obtained by specific precipitation and double diffusion were confirmed by the immunofluorescent technique. Fixation of the antiserum labelled with fluorescein to group A streptococci was weakened by previous absorption to VSGP preparations and completely disappeared after absorption to group A streptococcal polysaccharide.

These results clearly demonstrate the existence of common antigenic determinants between streptococcal components, especially the group A specific polysaccharide and the glycoproteins present in the urea extract of heart valves and other tissues (structural glycoprotein fraction).

The immunization of rabbits with human or bovine heart valve extracts regularly induced the production of antibodies against several constituents of this tissue. Some of these antibodies cross-reacted with other tissue

extracts or with serum protein. The cross-reactions obtained between the extracts of several tissues prepared as described here, such as skin, cartilage, cornea, and the polysaccharide of streptococcus A should be emphasized. No cross-reactions, or only very weak ones, were obtained with myocardial extracts. Immunization with total valvular extracts or with the CTC extract yielded anti-VSGP antisera of a higher titre than immunization with the VSGP itself. Nevertheless the VSGP preparation always absorbed one of the antigenic components of the total valvular extract or of the CTC extract. These results are identical with those already reported for the structural glycoprotein of cornea (KGAG)<sup>3</sup>, and are explained by the presence of a soluble "precursor" form of the structural glycoprotein in the soluble fraction of the tissue extract (CTC extract)<sup>12</sup>.

The experiments clearly show the existence of a cross-reaction between antisera obtained by immunization with group A streptococcus or its specific polysaccharide and the structural glycoprotein fraction of heart valves. A significant fraction of the total anti-streptococcal antibody precipitated with the VSGP preparation. On the other hand, the purified group A streptococcal polysaccharide or its edestin complex reacted with the specific anti-VSGP-antisera and with the total anti-valvular antisera, eliminating the anti-VSGP-antibodies. The presence of specific precipitating as well as haemagglutination antibodies was confirmed by the double diffusion and fluorescent antibody techniques. They strongly indicate that the group A specific polysaccharide contains the common antigenic determinant responsible for the cross-reaction with VSGP.

The pronounced cross-reactions obtained with the structural glycoprotein fractions of several types of connective tissue and the weakness or lack of reaction with myocardial tissue tend to indicate that the antigen responsible for the cross-reaction with group A streptococcus is located in the connective tissue of the heart muscle. We obtained evidence strongly in favour of the identity of the structural glycoprotein fraction of heart valves with this cross-reacting antigen. It seems probable that the carbohydrate portion of the VSGP preparation carries the antigenic determinant which cross-reacts with the group specific polysaccharide of the streptococcus. On the basis of these data it is tempting to make the suggestion that the group specific polysaccharide and the structural glycoproteins of the heart play an important part in the production of the auto-antibodies observed in rheumatic heart disease. In this respect it is worth mentioning that recently we were able to demonstrate the presence of antibodies which react with the urea extract of heart valves (VSGP) in the sera of patients with rheumatic disease (to be published).

Thus, in conclusion, immunization of rabbits with extracts from bovine and human heart valves induced the formation of antibody which reacted with several antigens present in these extracts and specifically with a structural glycoprotein fraction extracted with urea (see Table 1). The main interest of the results reported here, however, is the evidence of a cross-reactivity between the valvular structural glycoprotein fraction and group A streptococcal polysaccharide. Group A anti-streptococcal sera were precipitated with the valvular extracts. We think that the cross-reacting antigen is the structural glycoprotein

Table 4. CROSS-REACTIONS BETWEEN AN ANTISERUM TO GROUP A STREPTOCOCCUS AND STRUCTURAL GLYCOPROTEINS

Antisera absorbed to	Precipitating antibodies to				No. of lines on agarose plates				Bovine cornea KGAG ‡	Fluorescence of group A streptococci treated with fluorescent antiserum
	µg Nitrogen/ml. § Polysacc. A*	Bovine VSGP	Polysacc. A*	Bovine VSGP •	Human VSGP	Human aorta SGP †	Human valve			
No treatment	238	43	1	2	1	1	2	1		++
Bovine VSGP	172	0	1	0	0	0	0	0		++
Streptococcal polysacc. A	0	0	0	0	0	0	0	0		0

\* Group specific polysaccharide of streptococcus A.

† Structural glycoprotein of human aorta.

‡ Structural glycoprotein of cornea, keratoglycosaminoglycane.

§ Total precipitate comprising antibody and coprecipitated antigens.

fraction. Anti-valvular antisera precipitated with group A specific polysaccharide and this cross-reaction was specific for this group of streptococcus. These results suggest that the group A streptococcal polysaccharide, on account of its antigenic relationship with the valvular structural glycoprotein, is involved in the production of the anti-valvular auto-antibodies observed in the sera of patients with rheumatic fever.

We thank Dr. Hämmerling for his help and for guidance in the preparation of the edestin-polyoside complex used in this study.

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## Dietary Retinol and Alpha-Tocopherol and Erythrocyte Structure in Rats

by

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The red blood cells of rats deficient in vitamin A become deformed when vitamin E is added to the diet, but are normal in control rats fed vitamin A

Lucy and Dingle<sup>1</sup> investigated the effects of retinol and alpha-tocopherol on rabbit erythrocytes *in vitro*. They showed that erythrocyte membranes are unstable when the cells are incubated in the presence of large amounts of retinol. This treatment causes internal vacuolization of the red blood cell and eventual haemolysis. Addition of alpha-tocopherol to the incubation mixture *in vitro* inhibited this lysis and stabilized the erythrocyte membrane.

Previous work from this laboratory by Roels *et al.*<sup>2</sup> has shown that changes in the stability of the liver lysosomal membrane from rats deficient in vitamin A caused by different dietary levels of alpha-tocopherol can be reversed by dietary retinol: low dietary alpha-tocopherol weakened the liver lysosomal membrane of rats deficient in vitamin A, whereas animals receiving high levels of dietary alpha-tocopherol and no retinol had unusually resistant liver lysosomal membranes. The addition of retinol to the diet brought the stability of the lysosomal membrane back to normal in both groups of animals.

To determine whether this *in vivo* effect of retinol and alpha-tocopherol on the lysosomal membrane is a more general phenomenon, applicable to other biological membranes, we have examined the effect of different doses of dietary vitamin A and vitamin E on rat erythrocytes. Examination with the electron microscope indicates that erythrocytes from rats deficient in vitamin A receiving high or normal doses of vitamin E are swollen and distorted, whereas erythrocytes from pair fed littermate controls receiving normal doses of vitamin A do not show this distortion.

Male albino Sherman rats 21 days old, weighing 40–44 g, were housed in individual cages and fed an experimental diet containing 18 per cent vitamin free casein; 68 per cent glucose; 5 per cent cellulose; 5 per cent peanut oil and 4 per cent Fox and Briggs salt mixture (all percentages by weight). In addition, 2 mg/kg thiamine hydrochloride; 4 mg/kg riboflavin; 4 mg/kg pyridoxine; 1,000 mg/kg choline; 1,000 mg/kg inositol; 300 mg/kg *p*-aminobenzoic acid; 100 mg/kg nicotinamide; 2.5 mg/kg folic acid; 0.05 mg/kg vitamin B<sub>12</sub>; 0.1 mg/kg biotin; 0.042 mg/kg ergocalciferol; 10 mg/kg vitamin K and 10 mg/kg calcium pantothenate were added to the mixture.

Vitamins A and E were administered twice weekly by mouth to the different groups of animals as shown in Table 1. There were ten pairs of animals in each group.

Table 1. RETINYL ACETATE AND ALPHA-TOCOPHEROL CONTENT OF THE OIL\* SUPPLEMENT ADMINISTERED BY MOUTH TWICE WEEKLY TO EACH EXPERIMENTAL ANIMAL

Group	Pair fed		Pair fed		Pair fed	
	Deficient	Control	Deficient	Control	Deficient	Control
Retinyl acetate†	–AE–	AE–	–AE	AE	–AE+	AE+
α-Tocopherol‡	0	120 µg	0	120 µg	0	120 µg
	0	0	1.75 mg	1.75 mg	17.5 mg	17.5 mg

\* Stripped corn oil was used to bring the vitamin supplements to 0.1 ml.

† U.S. Pharmacopeia vitamin A reference standard.

‡ Pure D,L-alpha-tocopherol.

The controls were pair fed with the rats on the vitamin A deficient diet as described previously<sup>3</sup>.

Small samples of whole blood were taken from the tails of the rats, fixed in buffered osmium tetroxide (pH 7.4), washed with distilled water and deposited on grids coated with carbon. After drying at room temperature, the preparation was shadowed with germanium and observed under an electron microscope.

Examination of erythrocytes from rats 3 weeks after starting the experimental diet indicated that the cells from –AE+ rats were distorted as shown in Fig. 1, whereas erythrocytes from the pair fed controls (AE+) were less distorted. Distorted cells in Fig. 1 appear spindle shaped or prolate. Normal cells in Fig. 2 have circular outlines and central depressions.

A frequency count was made using a haemocytometer to determine the proportion of spindle shaped cells occurring in each treatment. Erythrocytes from –AE+ rats contained 43 per cent spindle shaped cells, whereas a sample of erythrocytes from the pair fed control (AE+) contained 24.9 per cent distorted cells. The mean number of cells examined in each condition was 115.

Additional information about the shape of the erythrocytes was obtained using the electron microscope. Erythrocytes from –AE+ rats appear distorted (Fig. 3). The most severe anomaly is represented by spindle shaped cells which cast irregular shadows with undulating margins or sharp peaks. The form of the shadow indicates that some of the spindle shaped cells are folded once; the portion of the cell which has been folded over projects above

the plane of the cell and therefore casts a long and irregular shadow compared with the cells from the pair fed controls (AE+) which are shown in Fig. 4.

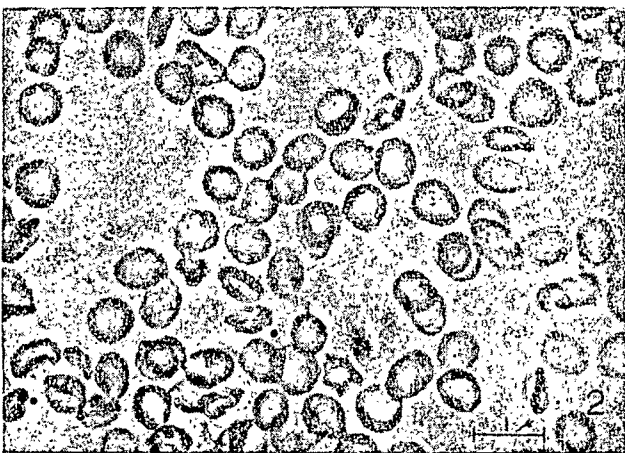
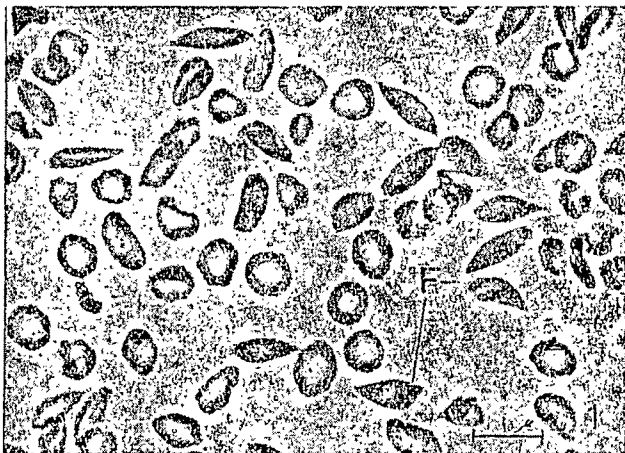
In all cases, observations were made over extended areas of the grid and representative clusters of cells were photographed. Even when the least distorted cells obtained from the experimental animals were compared with control specimens there was still a noticeable difference between the two samples.

Observations were subsequently made on blood samples obtained from the same animals at weekly intervals and the degree of distortion increased as the deficiency progressed.

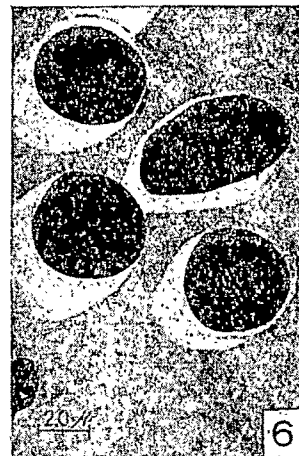
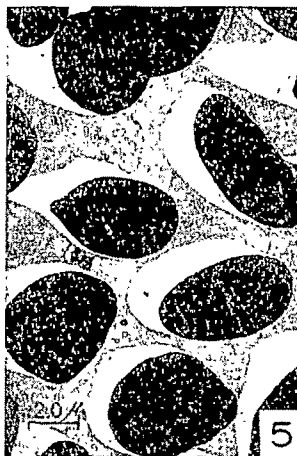
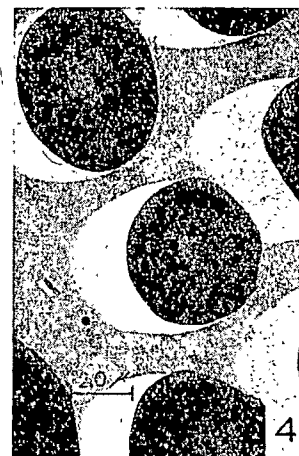
To gain additional evidence about the shape of the spindle shaped cells, clay models of erythrocytes were constructed and observed under oblique illumination from an electric lamp. Patterns of form and shadow similar to those in Fig. 3 were produced by folding models of normal cells along their diameter. When these models were rotated around their axes, shadows were produced much like those in Fig. 3.

Additional measurements were made to determine the size of cells in each experimental treatment. Spindle shaped erythrocytes from rats receiving -AE+ diets had a mean length of  $10.9\mu$  while normal erythrocytes from rats receiving AE+ diets had a mean diameter of  $6.6\mu$ .

Erythrocytes from rats receiving no vitamin A and normal levels of alpha-tocopherol (-AE) appear dis-



Figs. 1 and 2. Photomicrographs of whole erythrocytes fixed in osmium tetroxide. Fig. 1. Erythrocytes from rats receiving no retinol and high doses of alpha-tocopherol (-AE+) showing folded cells (F) which appear spindle shaped ( $\times 900$ ). Fig. 2. Erythrocytes from rats receiving normal doses of retinol and high doses of alpha-tocopherol (AE+). Most cells appear normal with circular perimeters and a central depression ( $\times 900$ ).



Figs. 3-6. Electron micrographs of shadowed whole erythrocytes. Fig. 3. Erythrocytes from rats receiving high doses of alpha-tocopherol and no retinol (-AE+) ( $\times 3,780$ ). Fig. 4. Erythrocytes from the pair fed control rat (AE+) ( $\times 3,600$ ). Fig. 5. Erythrocytes from rats receiving normal doses of alpha-tocopherol and no retinol (-AE) ( $\times 3,125$ ). Fig. 6. Erythrocytes from the pair fed control (AE) ( $\times 3,125$ ).

torted (Fig. 5), much like those from animals receiving high levels of vitamin E and no vitamin A (-AE+).

In this case, however, the distortion is much less severe. Folding of the cells is much less marked than in Fig. 3 and the perimeter of the cells more closely approaches a normal circular perimeter. When these erythrocytes from experimental rats are compared with those receiving normal amounts of vitamin A (AE) it is apparent (Fig. 6) that the latter cells are normal.

In most cases, the cells in Fig. 6 show a central depression and the perimeter is almost circular. These cells closely approximate the biconcave disk of the normal erythrocyte, but the tendency toward swelling and folding is not entirely absent.

Erythrocytes from animals receiving a diet low in vitamin E and without vitamin A (-AE-) or normal doses of vitamin A (AE-) contained slightly distorted cells. In addition to normal cells, abnormal erythrocytes appeared with angular margins and a tendency toward swelling.

The perimeter often appears somewhat angular.

Thin sections of erythrocytes were made from all three experimental conditions reported here. In all cases, no differences in internal structure were observed when erythrocytes from rats deficient in retinol were compared with those from the control receiving normal doses of retinol. No internal vacuoles were observed in any of the sections examined.

These results lead us to conclude that in the absence of dietary retinol, dietary alpha-tocopherol administered

in normal or high doses produces anomalies in the structure of the rat erythrocyte which can be counteracted when normal doses of vitamin A are included in the diet.

The severity of the distortion of erythrocytes from vitamin A deficient rats caused by dietary vitamin E appears to be related to the dose administered; normal doses of alpha-tocopherol result in less distortion than high doses. In both cases, the administration of normal levels of vitamin A inhibits this effect of vitamin E.

At present it is not possible to determine whether the effects of these vitamins result from alterations in the synthesis and development of erythrocytes in the haemopoietic tissue or result from changes in the blood plasma bathing the erythrocytes in the circulatory system.

Low dietary alpha-tocopherol caused slight distortion of the red blood cells, both in the absence and presence of dietary retinol.

These observations provide additional evidence in support of our hypothesis that retinol counteracts changes in the structure and/or function of biological membranes caused by different levels of alpha-tocopherol.

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## Metabolism of Methylcarbamate Insecticides by the NADPH<sub>2</sub>-requiring Enzyme System from Houseflies

by

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Certain resistant strains of houseflies are distinguished from susceptible strains by greater enzyme activity for the oxidation of several insecticide chemicals. This may contribute to the mechanism of resistance. Abdomens are a convenient source of this enzyme

THE toxicity of certain insecticidal chemicals is limited by their rapid rate of detoxication in insects. This limitation is often more significant with insecticide-resistant strains, but can frequently be overcome by simultaneous treatment with appropriate synergist chemicals. Repeated exposure of an insect population to an insecticide sometimes selects, as survivors, a strain that is resistant not only to the specific toxicant used and to related agents, but also to some apparently unrelated insecticidal chemicals. While several genetic and biochemical factors are undoubtedly involved in the development of insecticide resistance, one factor is probably the genetic selection of an enzyme system that degrades many types of insecticidal chemicals.

Microsomal enzyme systems are important in limiting the duration of action of drugs in mammals<sup>1</sup>, and many insecticidal chemicals are metabolized to non-toxic or toxic derivatives by the mammalian nicotinamide adenine dinucleotide phosphate (NADPH<sub>2</sub>) system which is reduced by the liver microsomes<sup>2-4</sup>. *In vivo*, insects rapidly carry out many of the same chemical reactions<sup>5,6,7</sup> that occur during incubation of insecticidal chemicals with the NADPH<sub>2</sub> system of liver microsomes. With *in vitro* conditions, however, insect enzyme preparations usually show very low activity in comparison with liver microsomes<sup>8</sup>. NADPH<sub>2</sub> systems of insect microsomes are known to carry out the following reactions (although the yield in reaction products is generally very low): oxidation of phosphorothionates to phosphates<sup>9-11</sup>; oxidation of dimethylphosphoramides<sup>12</sup> and methylcarbamates<sup>13</sup>; hydroxylation of naphthalene<sup>14,15</sup> and DDT<sup>16</sup>; epoxidation of aldrin to form dieldrin<sup>6,17</sup>.

For investigation of the biochemical genetics of resistance in insects to toxic chemicals, there is a need for *in vitro* insect enzyme systems able to degrade many types of insecticidal chemical. Insects readily accomplish such reactions *in vivo*, and so it seemed likely that the enzyme and NADPH<sub>2</sub> system of an insect would meet the need as long as the conditions used are optimal and the system is active when prepared from a convenient species (for example, *Musca domestica* L.); (b) shows differential activity when prepared from susceptible and resistant

strains; (c) acts on a variety of insecticidal chemicals; and (d) is sufficiently active to allow assay of degradation products even when they are prepared from only a few individuals and without extensive enzyme fractionation or purification. Such a system was found in a systematic investigation in which various parts or the entire bodies of adult houseflies were tested, as a source of enzyme with a variety of, for example, co-factors, temperatures, times of incubation, buffer composition, and pH. Various organ systems from adult houseflies were tested, without homogenization, for metabolism of a carbamate insecticide, and the mid-gut was the most active. With homogenates, the highest activity for metabolism of insecticidal chemicals was found with the NADPH<sub>2</sub> system of the abdomen, and this system can be used with or without isolation of the microsome fraction.

Twenty-four insecticides labelled with carbon-14 together with synergists were investigated; nineteen of these were prepared in this laboratory<sup>18-22</sup>. (The insecticides and synergists used are designated by their common or trade names; they are chemically defined either in a later part of this report or in a published list<sup>23</sup>.) The following carbamates labelled with carbon-14 were used as substrates for the enzyme system: 'Baygon' (2-isopropoxyphenyl methylcarbamate); carbaryl (1-naphthyl methylcarbamate), UC 10854 (3-isopropylphenyl methylcarbamate); HRS-1422 (3,5-diisopropylphenyl methylcarbamate); 'Banol' (2-chloro-4,5-xylyl methylcarbamate); 'Mesurol' (4-methylthio-3,5-xylyl methylcarbamate); 'Matacil' (4-dimethylamino-3-cresyl methylcarbamate); 'Zectran' (4-dimethylamino-3,5-xylyl methylcarbamate); 'Isolan' (1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate); dimetilan (1-dimethylcarbamoyl-2-methyl-4-pyrazolyl dimethylcarbamate). In most of the experiments, 'Baygon' and 'Matacil' labelled with carbon-14 were used.

For thin layer chromatography silica gel G chromatoplates, 0.25 mm thick, were used. Labelled compounds were detected on the plates by autoradiography, and authentic samples of unlabelled insecticidal chemicals and their metabolites with appropriate chromogenic agents<sup>2,18</sup>. Co-chromatography on thin layers, usually in two dimen-

sions using two different solvent systems, was used for metabolite resolution and tentative characterization. Six housefly strains were studied; these were three laboratory strains (including the SCR-strain<sup>16</sup>), and three strains resistant to carbamate (the Hokota strain which was selected initially for diazinon resistance<sup>24</sup> and later for resistance to 'Baygon', a strain selected with a mixture of carbaryl and synergist, and one selected with 'Isolan'). *L.D.*<sub>50</sub> values ( $\mu\text{g}$  of insecticide/female) were as follows, respectively, for the SCR and Hokota strains: 0.1 and >100 with 'Baygon'; 20 and >100 with 'Matacil'.

Enzyme preparation consisted of homogenization of whole adult flies, or parts of them, in 0.25 molar sucrose and 0.15 molar  $\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$  buffer (pH 7.5) in an ice bath using a glass and 'Teflon' homogenizer. In certain cases, differential sedimentation by centrifugation was used to prepare fractions, as follows: nuclei and debris at 800*g* for 20 min; mitochondria at 10,000*g* for 30 min; microsomes at 105,000*g* for 60 min. The soluble fraction was the supernatant which remained after sedimentation of the microsome fraction; the particulate fractions were washed once by resuspension and resedimentation. The concentration of homogenized whole flies, or parts of them, or centrifugal fractions, was usually 10 fly equivalents/2 ml. A typical incubation mixture, in a 25 ml. Erlenmeyer flask, consisted of approximately 0.1  $\mu\text{moles}$  of labelled insecticide or synergist, 5  $\mu\text{moles}$  of  $\text{NADPH}_2$  and 2 ml. of enzyme in the buffered sucrose solution. The substrate was initially added to the flask in hexane solution, the solvent was evaporated, and then the other reaction constituents were added. After incubation for 2 h at 30° C in air with shaking, the reaction mixtures were extracted four times with 10 ml. portions of ether and the components extractable with ether were analysed by thin layer chromatography. The solvent system varied with the labelled materials which were used. For one dimensional chromatography of the labelled methylcarbamates, the plates were developed first with benzene or hexane, to remove certain interfering unlabelled extractives toward the front, and then with one of the following solvent mixtures: ether, hexane and ethanol (77:20:3); benzene and ethanol (9:1); chloroform, ether and ethyleneglycol monomethyl ether (10:1:1); chloroform and acetonitrile (3:1). For two dimensional thin layer chromatography of the carbamates, the plates, which measured 20 cm  $\times$  20 cm, were developed first with the ether, hexane and ethanol (77:20:3) mixture and then with one of the other solvent systems.

Extensive metabolism of labelled 'Baygon' initially was not achieved with homogenates of the whole insect body of laboratory strains and with incubation in the presence or absence of  $\text{NADPH}_2$ . When the body regions were separated, however, the abdomen homogenates gave much greater activity in the presence of  $\text{NADPH}_2$  than did homogenates of the head, thorax or whole insect body. The activity of the abdomen homogenate was greater for any of three resistant strains than for any of three laboratory strains. Assay of centrifugal fractions of homogenate from the abdomen revealed that the activity resides almost entirely in the microsome fraction and that the activity of this fraction is not enhanced by addition of the soluble fraction. The microsomal activity was almost specific for  $\text{NADPH}_2$ ; reduced nicotinamide adenine dinucleotide ( $\text{NADH}_2$ ) was only slightly effective, and the oxidized forms of these cofactors ( $\text{NADP}$  and  $\text{NAD}$ ), flavin adenine dinucleotide, and flavin mononucleotide did not function as cofactors. Although 5  $\mu\text{moles}$  of  $\text{NADPH}_2$  were routinely used, activity was evident with 1–10  $\mu\text{moles}$  of  $\text{NADPH}_2$  and increased when larger quantities were used. Addition of homogenates of the head or the thorax, individually or in combination, to homogenates of the abdomen always resulted in remarkable reduction in the activity of the enzyme system. The biochemical nature of the inhibitory materials was not defined, but they are present largely

in the 800*g* sediment fraction (debris and more inhibitory when obtained from the head, thorax, and are relatively heat stable (c when held in a boiling water bath for 15 min); activity for enzyme systems which metabolize *in vitro* was achieved by using homogenates separated abdomens, or microsomes from separated abdomen homogenates or the whole homogenates as the enzyme source.

Similar findings were obtained for each methylcarbamate insecticide chemicals investigated. Enzyme activity is higher for the resistant susceptible strain, for the abdomen homogenate with the whole body homogenate, and  $\text{NADPH}_2$  as compared with no added  $\text{NAD}$ . Overall relationships are illustrated in Fig. 1, 'Matacil' as the substrate and the Hokota as resistant and susceptible strains, respectively.

Conditions of rearing and handling the fly preparation of enzymes, also affected its results. Great difference in activity was not found between male and female flies and within 1–15 days after emergence of adults, when these adults were fed only sugar; this is at variance with a published report of activity of fly microsome in hydroxylation of increases remarkably during ageing of the adult. Activity was higher for adult flies fed on mill for those fed on sugar and water; it is believed that micro-organisms in the digestive tract are not involved in carbamate metabolism, because flies fed containing 3 per cent penicillin G and 10 per cent streptomycin sulphate (w/v) for 4 days, before homogenization of the abdomens, had the same activity as those not so treated. The housefly enzyme of limited stability on incubation at 37° C, was chosen for incubation and only fresh preparations were used. The enzyme activity of homogenate from the abdomen was largely lost at 5° C or in a frozen state, but the activity of the thorax fraction, when frozen, was stable for this routine investigation, the flies were not sexed and used without anaesthetization 3–7 days after emergence during which period they were fed freely on milk, sugar and water.

A variety of different chemical reactions to the stated conditions, based on the identity of carbamate metabolites which have been tentatively characterized; (a) aromatic hydroxylation occurs at 4- or 5-position of carbaryl; (b) *O*-dealkylation of 'Baygon' to yield 2-hydroxyphenyl methylcarbamate; (c) *N*-dealkylation occurs with 'Matacil' to yield 4-methylamino, 4-amino and 4-formamido derivatives (Fig. 1) as well as the 4-methylformamido derivative 'Zectran' also forms an analogous series of (d) *N*-methyl hydroxylation to form the *N*-hydroxy derivatives occurs with 'Matacil' (Fig. 1) and 'Baygon', 'Banol' and 'Zectran'; (e) sulfoxidation of 'Mesuro' to the corresponding sulfoxide.

The  $\text{NADPH}_2$  system from the fly abdomen was active in the conversion of other types of chemicals and synergists to more polar products indicated by thin layer chromatography and thin layer chromatography. In each case, little if any metabolism without added  $\text{NADPH}_2$ , compared with the addition of  $\text{NADPH}_2$ . Two chlorinated insecticides, aldrin and DDT, and an organophosphorus insecticide, 'Imidan' (*o,o*-dimethyl *S*-phthal phosphorodithioate), were metabolized. 'Matacil', aldrin and 'Imidan', but not DDT, was active when enzyme preparations from the Hokota strain were used. Aldrin yielded primarily dieldrin but also several products, while 'Imidan' gave several metabolites including small amounts of the oxygen analogue as a metabolite. The list of different chemical reactions in the stated conditions can be expanded by a



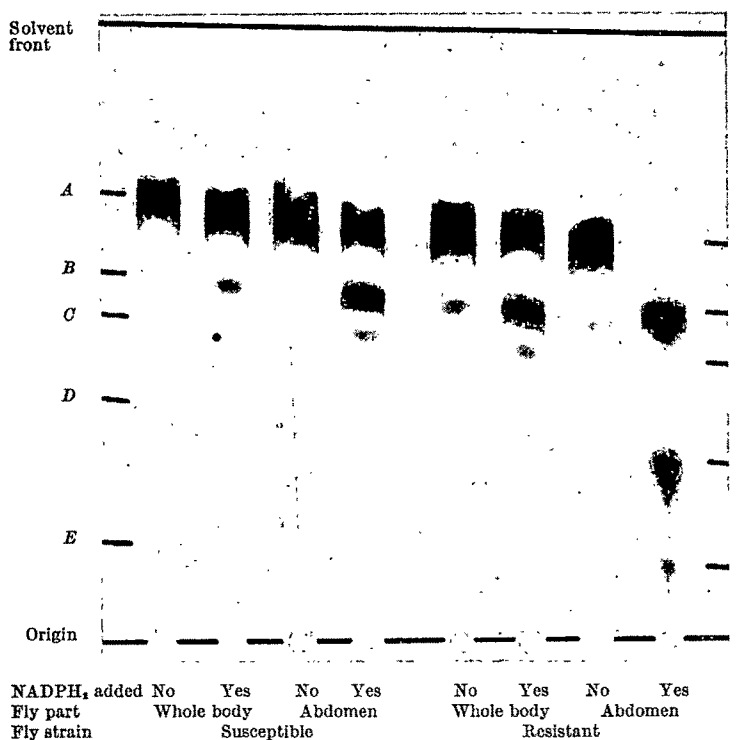
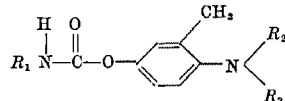


Fig. 1. Autoradiogram showing *in vitro* production of metabolites of  $^{14}\text{C}$ -matacil by homogenates of whole bodies or abdomens of susceptible and resistant houseflies.

The extraction solvent was ether; the developer for the thin layer chromatogram was hexane followed by a mixture of ether, hexane and ethanol (77 : 30 : 3). Carbon-14 was in the carbonyl position; analogues of



were used with structures as follows:

	A ('Matacil')	B	C	D	E
$\text{R}_1 =$	$\text{CH}_3$	$\text{CH}_3$	$\text{CH}_2\text{OH}$	$\text{CH}_3$	$\text{CH}_3$
$\text{R}_2 =$	$\text{CH}_3$	$\text{CH}_3$	$\text{CH}_3$	$\text{H}$	$\text{H}$
$\text{R}_3 =$	$\text{CH}_3$	$\text{H}$	$\text{CH}_3$	$\text{H}$	$\text{CHO}$

of the identity of metabolites tentatively characterized, as formed from these chemicals: (a) phosphorothionate oxidation converts 'Imidan' to the corresponding oxygen analogue, based on co-chromatography in two dimensions with benzene and ethyl acetate (9 : 1 and 1 : 2); (b) epoxidation forms dieldrin from aldrin, as confirmed by co-chromatography using hexane and hexane-benzene (4 : 1) for the two dimensional development; (c) alkyl hydroxylation of DDT to form dicofol in small yield was indicated (comparisons for metabolite identification based only on  $R_F$  values, in two dimensions, with hexane and carbon tetrachloride). Extensive metabolism also took place with two botanical insecticides, rotenone (unpublished results of Fukami and Casida) and pyrethrin (unpublished results of Yamamoto and Casida) and with three synthetic D-trans-chrysanthemumate esters<sup>22</sup> (the allethrolol ester—allethrin; the 2,4-dimethylbenzyl ester—dimethrin; and the 3,4,5,6-tetrahydropthalimido-methyl ester—phthalthrin). The following six methylenedioxyphenyl compounds, which are active as insecticide synergists, were also metabolized by the NADPH<sub>2</sub> system of the fly abdomen (unpublished results of Esac and Casida): piperonyl butoxide; sulphoxide (two diastereoisomers, separately investigated); myristicin (5-allyl-L-methoxy-2,3-methylenedioxybenzene); safrole; and dihydrosafrole. It is not known whether the conditions which were found to be optimal for 'Baygon' metabolism are also those optimal for metabolism of the aforementioned compounds, or whether one or more

enzymes are involved in metabolizing these varied types of insecticide chemical and synergists.

The activity of the system, using homogenates of abdomens from the Hokota strain, was such that one abdomen (average fresh weight of 6 mg) resulted in significant metabolism of 0.1  $\mu\text{mole}$  of 'Banol', 'Matacil', 'Mesurol', or 'Zectran' when incubated for 1 h; ten abdomens destroyed 0.1  $\mu\text{mole}$  of each of these four carbamates and also destroyed 1  $\mu\text{mole}$  of phthalthrin in 2 h. The activity of the homogenate of the resistant fly abdomen or microsomes is much greater, for destruction of methylcarbamate insecticides, than that of an equivalent fresh weight of rat liver homogenate or microsomes, respectively.

Detoxication processes constitute only one of several mechanisms involved in resistance. In certain cases, the pathway of degradation of insecticides *in vivo* differs from that in a selected *in vitro* enzyme system. Most of the metabolic changes already mentioned involve conversions to products of reduced insecticidal activity, but this is not always the case as, for example, in aldrin epoxidation, phosphorothionate oxidation, and hydroxylation or oxidation of certain substituted-phenyl methylcarbamates<sup>2-4,7,13,28</sup>. It is therefore difficult to generalize on the potential significance of the microsomal-NADPH<sub>2</sub> system as a resistance mechanism.

These enzyme systems from flies should be useful in future investigations of relations between structure, metabolism and toxicity of insecticidal chemicals, as well as of the basis for synergism and the biochemical genetics of resistance mechanisms. It may also be appropriate for comparative biochemical studies on the mechanism of microsomal hydroxylation reactions.

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## LETTERS TO THE EDITOR

## PLANETARY SCIENCE

## Neutron produced Phosphorus-32 in the Barwell and St. Severin Meteorites

NUCLEAR reactions induced by neutrons in meteorites have been discussed by a number of authors<sup>1-6</sup>. A few radioactive and stable isotopes, produced mainly by thermal and epithermal neutrons, have been measured<sup>7-16</sup>, but there is not much direct information on the flux of fast neutrons ( $E \gtrsim 1$  MeV). The most sensitive indicator of fast neutrons, the phosphorus-32 produced by  $^{32}\text{S}(n,p)$  reactions, has been used only once<sup>11</sup>, partly because of the short half-life of phosphorus-32, 14.3 days. Furthermore, in the one measurement of phosphorus-32, only the activity of the bulk sample was determined, that is, no attempt was made to distinguish between the component produced by spallation and that produced by the  $^{32}\text{S}(n,p)$  reaction.

We were able, through the kindness of Dr. Eva Paneth, Prof. Sylvester-Bradley of the University of Leicester, and Dr. Max Hey of the British Museum, London, to obtain a sample of the Barwell meteorite which fell on December 24, 1965, and were able to measure the amount of phosphorus-32. Details of the experimental procedure used to extract a number of radioactive isotopes from various mineral fractions of the meteorite will be given in a later paper. In this report we wish to give the results for phosphorus-32 only.

Sulphur occurs in normal chondrites predominantly in the form of troilite, so that this mineral will contain the phosphorus-32 produced by the  $(n,p)$  reaction in addition to that from the spallation of the iron in the iron sulphide. In the metal phase, on the other hand, only phosphorus-32 produced by spallation will be present. Thus separate analyses of the two phases should allow the two fractions to be distinguished. Complications, however, must be expected if the troilite and/or the metal phase are extremely fine-grained ( $d \lesssim 1\mu$ ), that is, if their average grain size is comparable with the range of the recoil phosphorus-32. Fortunately, it rarely happens that most of either or both phases occurs in such a way; instead, it is occasionally even possible to separate mechanically large troilite nodules and metal particles in sufficient quantities to allow an accurate determination of the activity of the phosphorus-32.

In the case of the Barwell meteorite, the separation was carried out by fractional dissolution. First, in order to dissolve the metallic iron-nickel, the powdered sample (164.3 g) was treated under vacuum for 2 h with 1.4 l. of an aqueous solution containing 150 g of ammonium-peroxydisulphate. This was followed by a 30 min treatment with 50 c.c. of bromine in 2.5 l. of water which dissolved all the troilite and a small amount of olivine. In the ammonium persulphate step 56 mg of phosphorus carrier were added in the form of ammonium hydrogen phosphate while during the bromine treatment sufficient native phosphorus went into solution, as expected. Table 1 gives the amounts of the relevant elements (and silica) which were dissolved in the two steps. (The calcium to phosphorus ratio found in the bromine fraction supports the idea that in normal chondrites phosphorus occurs as calcium phosphate and not as schreibersite.) In both cases the phosphorus was precipitated as ammonium phosphomolybdate, radiochemically purified and converted to  $\text{Mg}_2\text{P}_2\text{O}_7$ . The chemical yields were 55 per cent for the metal and 81.5 per cent for the troilite. The activity was determined with a

'Sharp' low beta flow counter and followed for several half-lives. As can be seen from Fig. 1, the samples counted were radiochemically very pure. Furthermore, there is no indication of any contribution to the activity from phosphorus-32. Because of the short range of the  $\beta$ -particles, compared with the sample thickness, this is to be expected.

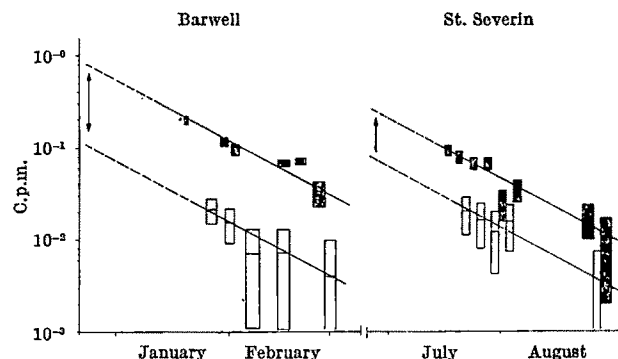


Fig. 1. Phosphorus-32 activities in troilite (black bars) and metal-fraction (open bars) of Barwell and St. Severin as a function of time. The least square fit curves were calculated with a half-life of 14.3 days. The arrows indicate the date of fall of the meteorites.

From the measured activities, extrapolated to the time the meteorite fell (Table 1; last column), and the amounts of nickel-iron and sulphur dissolved, the rates of production of phosphorus-32 from the spallation of iron ( $R_{\text{spall}}$ ) and the  $^{32}\text{S}(n,p)$  reactions ( $R_{n,p}$ ) can be calculated (Table 2).

As we completed these measurements, another stone meteorite—the amphoterite St. Severin—fell on June 27, 1966. Again, we fortunately obtained a suitable sample two weeks after the date of the fall, this time through the prompt and generous support of Dr. Jacques Labeyrie, Centre National de la Recherche Scientifique, Gif-sur-Yvette. In this case a chemical separation of the metal and troilite phase was not necessary as our sample contained some fairly large troilite inclusions which were taken out mechanically. A few grams of metal phase were separated with a magnet. The purpose of measuring the phosphorus-

Meteorite		Iron	Nickel	Sulphur	Calcium	Phosphorus	Silica	(dpm) <sub>t=0</sub>
Barwell	Metal	14.22	1.86	1.38	0.070	—	1.04	1.3 ± 0.6
	Troilite	4.96	—	2.28	0.256	0.130	2.14	2.64 ± 0.26
	Bulk analysis	21.90	1.1	2.24	1.37	0.08	39.35	—
St. Severin	Metal	2.23	0.94	0.045	—	—	0.024	0.25 ± 0.10
	Troilite	2.20	—	1.26	—	—	—	1.10 ± 0.10
	Bulk analysis	22.6	0.59	3.21	—	—	—	—

The amounts of relevant elements and silica dissolved as "metal phase" and "troilite" (in grams). The results of the "bulk analysis" are given in weight per cent. (In case of St. Severin an aliquot of the pulverized sample (170 g) was used for the bulk sulphur analysis. The troilite taken out mechanically before crushing the sample is included in the result.) The last column gives the phosphorus-32 activities in disintegrations per min at the time of fall of the two meteorites.

Meteorite	$R_{n,p}$	$R_{\text{spall}}$	$R_{\text{total}}$
Barwell	1,200 ± 180	-20 ± 40	27 ± 4
St. Severin	780 ± 100	68 ± 40	25 ± 4

The production rates of phosphorus-32 by spallation reactions on nickel-iron ( $R_{\text{spall}}$ ) and due to the  $^{32}\text{S}(n,p)$  reaction ( $R_{n,p}$ ), given in atoms per min and kg of NiFe and S, respectively. In the last column the total activity in disintegrations per min per kg meteorite is listed. In both cases only the contribution due to  $R_{n,p}$  was taken into account. The inclusion of the non-significant  $R_{\text{spall}}$  values would be misleading.

32 in the metal phase was not to determine  $R_{\text{spall}}$  but to make sure that the activity in the troilite comes predominantly from the  $^{32}\text{S}(n,p)$  reaction.

Both samples were dissolved in bromine water, after addition of phosphorus carrier; all further processing was identical to that described above. The chemical yield was 77 per cent for the metal phase and 56 per cent for the troilite. The results for both meteorites show unambiguously that the activities of phosphorus-32 in the troilite phase are produced by  $(n,p)$  reactions on sulphur-32. The values for the rates of production of phosphorus-32 allow some important conclusions. According to Lingenfelter *et al.*<sup>16</sup>, 88 per cent of all neutrons produced in chondritic material are evaporation neutrons with a differential spectrum  $N(E)dE = E e^{-2E/\theta} dE$ , where  $\theta$  is the average energy; the remaining 12 per cent are knock-on neutrons with a differential spectrum  $N(E)dE = \beta E^{-2} e^{-180/E} dE$ . Using these spectra with  $\theta = 3$  MeV<sup>17</sup> and the cross-section data for phosphorus 32 produced by the  $(n,p)$  reaction in the relevant energy region<sup>18-22</sup>, an effective cross-section of  $\sigma(n,p) \sim 130$  mb. is obtained. This together with the  $R_{n,p}$  values of Table 2 yields a neutron flux of 8.2 neutrons/cm<sup>2</sup> sec for Barwell and of 5.2 neutrons/cm<sup>2</sup> sec for St. Severin. (With  $\theta = 2$  MeV the effective cross-section would be  $\sigma(n,p) \approx 70$  mb. and the fluxes correspondingly higher. In both cases the change of the neutron energy spectrum due to the slowing down of the neutrons was neglected, as only a few collisions bring the neutrons below 2 MeV, where the cross-section is already negligibly small.)

These different values do not necessarily reflect a difference in intensity of the cosmic radiation as the neutron flux is especially sensitive to the pre-atmospheric size of a meteorite and the position of the sample within it.

As expected, the production rates of phosphorus-32 from spallation of iron cannot be given with any degree of accuracy, partly because of the small amounts of metal available and the predictably low yield<sup>3</sup>. Furthermore, as metallic nickel-iron is frequently found adjacent to troilite, an appreciable portion of the activity in the metal may be introduced by recoil of phosphorus-32 produced by the  $(n,p)$  reaction from sulphur-32 (see above).

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## Ozonesonde for Rocket Flight

It is well known that the vertical distribution of the atmospheric ozone shows marked variations with latitude, season and weather conditions. Ozone measurements had usually been made primarily with balloons using instruments due to Paetzold and Piscalar<sup>1</sup>, Brewer and Milford<sup>2</sup> and Regener<sup>3</sup>, and by a few rocket probes using various types of solar spectrometers<sup>4</sup>. The balloon sondes do not reach the stratopause. Recently, a rocket-borne ozonesonde<sup>5</sup> which utilized the chemiluminescent principle for ozone detection was developed and flown with the *Arcas* rocket at White Sands Missile Range (32° N.), New Mexico. A serious disadvantage of these earlier rocket-borne sondes is that they cannot be fired during daylight because of the effect of stray light on the photomultiplier output. There is a pressing need for an ozonesonde which can be deployed at any time of day.

This rocket ozonesonde, a self-pumping type, consists of three main parts: power supply, sample bottle including photomultiplier tube and chemiluminescent detector, and telemetry circuit. The photomultiplier tube and the associated high voltage supply circuitry are potted in black silicone rubber and mounted inside the bottle, as is shown in Fig. 1. The channel for the flow of air into the bottle is made from 'Teflon' and provides two 90° turns to eliminate stray light. The chemiluminescent detector is mounted across the photomultiplier tube. Ozone in the environment flows over the detector and the photons produced by the destruction of ozone molecules on the chemiluminescent material are monitored by the photomultiplier tube, the output signal from which is transmitted on a carrier frequency of 1,680 Mc/s (ref. 6).

The ozonesonde is deployed from an *Arcas* rocket above the stratopause. The bottle empties itself at high altitude and gases flow into it as the instrument descends on a radar reflecting parachute 15 ft. in diameter. The intensity of the emitted light is directly proportional to the flux of ozone entering the detector, and this in turn is equal to the product of the ozone concentration and the rate of flow. Thus the flow rate into the detector must be known. As the instrument falls through an atmosphere of increasing density, the pressure inside the bottle tends to come into equilibrium with the external pressure, so that the net flow of air (c.c./sec) into the bottle through the inlet channel depends on both the external and internal pressures\* and is equal to

$$\frac{V_i T_a}{P_a T_i} \left| \left( \frac{dP_i}{dt} - P_i \frac{d \ln T_i}{dt} \right) \right|$$

where  $V_i$  is the volume of the bottle;  $T_i$  the air temperature and  $P_i$  the pressure inside the bottle;  $T_a$  the ambient external temperature; and  $P_a$  the ambient external pressure;  $t$  is the time.

This expression can be simplified considerably if one assumes  $P_i = P_a$  and  $T_i = T_a$ . As the instrument falls, the bottle will be cooled continuously, so that the second term, which is an order of magnitude less than the first term, will always add to the flow rate. The ozonesonde is calibrated before launch by the use of an ozone generator<sup>3</sup>. Ozonized air of known concentration is injected into the bottle at a known flow rate and sensitivity is set in the proper range.

The rocket borne ozonesonde\* was flown on June 15, 1966, at 2215 U.T. and deployed at 65 km altitude. Radar tracking of the parachute provided the altitude and rate of fall as the function of time. Ozone concentration was sampled continuously. The results are shown in Fig. 2, as the variation of ozone partial pressure with altitude.

The total ozone measured by the instrument was compared with the measurement taken by Dobson's spectrophotometer at this latitude. The agreement is fairly good.

\* The rate of flow is independent of the characteristics of the inlet because the latter has a large diameter.

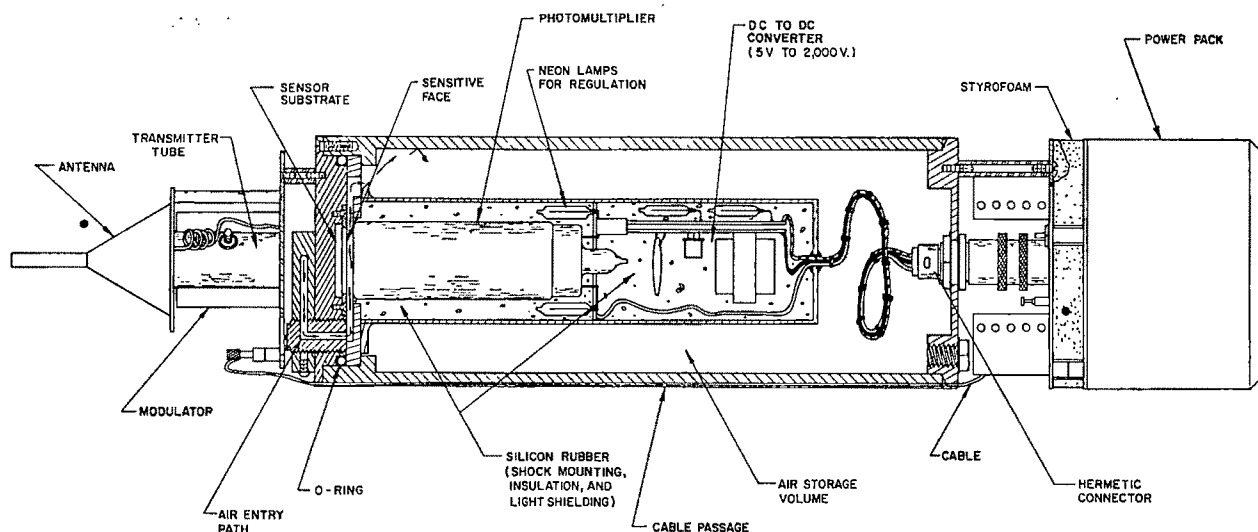


Fig. 1. Schematic diagram of rocket-borne ozonesonde.

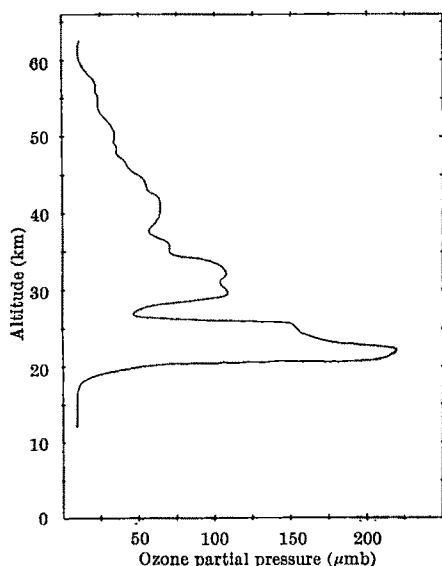


Fig. 2. Vertical distribution of ozone at White Sands Missile Range on June 15, 1966, 2215 U.T.

I wish to acknowledge the help received from the staff of the Schellenger Research Laboratories, Texas Western College, El Paso, for the fabrication of the instrument.

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### Kink-bands and Related Geological Structures

MARSHALL's renewed criticism<sup>1</sup> of my paper<sup>2</sup> on kink-bands again argues that kink-bands are the exact equivalents of shear planes, at least in terms of angular relationships. In 1964 there was scant experimental evidence for my contention that kink-bands in foliated rocks,

unlike shear planes, tend to form at angles of more than  $45^\circ$  to the direction of the maximum compression<sup>2-4</sup>. Field evidence<sup>2</sup> was considered suspect "in view of the difficulty of locating  $P_{\max}$  in any but a perfectly symmetrical, truly conjugate system of kink-bands"<sup>1</sup>.

The more recent experimental work of Paterson and Weiss<sup>5</sup>, which is of wide implications for structural geology, has now demonstrated conclusively that kink-band and shear-plane systems have different geometries. Specimens of Nelligen phyllite, compressed parallel to the foliation, showed well developed sets of conjugate kink-bands, symmetrical about the foliation. The authors<sup>5</sup> obtained 475 determinations of the angle between the undeformed foliation and the kink-plane (the angle  $\alpha$  of Fig. 4, ref. 2). The mean value was  $58^\circ$  and the variation shown in their histogram is quite small. The result compares well with the modal value reported<sup>2</sup> ( $55^\circ$ - $60^\circ$ ) for natural kink-bands, with the same symmetrical geometry, in the slates of the Ards Peninsula.

It may be that in these results Marshall finds room for application of the hypotheses of second-order shearing, either that of McKinstry<sup>6</sup> (as quoted in his letter of 1964, ref. 3) or that of Moody and Hill<sup>7</sup>. Some other treatment seems desirable. As yet, however, no complete theoretical explanation of the high  $\alpha$  values has emerged. Two suggestions have been offered. Paterson and Weiss<sup>5</sup> cite the work of Taylor<sup>8</sup> on the propagation of faults and write that they "would expect an analogous calculation" to show that in an ideal foliated body compressed parallel to the foliation, kink-bands would be "inclined at  $60^\circ$  to the direction of compression as opposed to  $45^\circ$  in Taylor's problem".

My own suggestion<sup>2</sup> is as follows. In an ideal foliated body, kink-bands, like shear planes (in that they constitute a zone of yielding and in that they effect a displacement), would also propagate at an angle of  $45^\circ$  to the direction of compression, as the same theoretical argument<sup>8</sup> seems relevant to both structures. In kink-bands the geometry of shearing is such, however, that frictional effects will tend to increase rather than decrease the  $45^\circ$  angle with  $P_{\max}$ . This is because shearing takes place not on the kink-plane but on the intra-kink-band foliation. The orientation of the latter changes during shearing, but its mean position is approximately perpendicular to the band<sup>2</sup>. Hence the necessary approximation and the positive sign in my statement, "kink-bands might be expected to make an angle of about  $90 + \phi/2$  degrees with the same major principal stress"<sup>2</sup>, which I applied only to the case of the symmetrical conjugate set. Both hypotheses are in accord with the experimental results so

far obtained. Further experiments to check their validity thoroughly would not be difficult.

Incidentally, Marshall<sup>1</sup>, in attempting to summarize my ideas for criticism, has distorted the original and has attributed to me views which I have not expressed and do not hold. To take a single point, his observations on the final position of the rotating internal shear planes do not invalidate, but in fact support strongly, my contention that the mean orientation of these planes during shearing is at about  $90 - \phi/2$  degrees with the maximum principal pressure<sup>2</sup>.

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<sup>7</sup> Moody, J. D., and Hill, M. J., *Geol. Soc. Amer. Bull.*, 67, 1207 (1956).

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### Volcanoclastic Rocks in Tenerife, Canary Islands

IGNIMBRITES are commonly of rhyolite, rhyodacite or dacite composition, less commonly of andesite composition, and rarely of basalt composition. Ignimbrites associated with sodic-alkaline volcanism are rare and known examples are restricted to the Kenya field, although doubt has been cast<sup>1-3</sup> on their ignimbrite origin<sup>4</sup>, and it has been suggested that they are more akin to froth flows. A preliminary examination of a series of rocks from Tenerife indicates that in this, another sodic-alkaline environment, a number of different types of volcanoclastic flow may be distinguished.

The volcanic rocks of the Canary Island Archipelago are dominantly alkaline, and in the area of the Las Canadas caldera, Tenerife undersaturated basalts and phonolites form a capping to the basement alkali-olivine basalts and ankaramites. Within the walls of the caldera, and on the outer flanks, are exposed a series of volcanoclastic rocks which are divided in the field into ignimbrites, pumice flows and eutaxites.

The ignimbrites are nowhere more than 12 ft. thick, yet in vertical section they display zonal characteristics commonly associated with much thicker flows. Generally four major zones can be distinguished: (1) a basal incoherent or weakly coherent zone; (2) a zone of maximum welding with abundant fiamme; (3) an indurated zone caused by post-depositional recrystallization; and (4) a surface incoherent zone.

Locally, within one of the ignimbrites, a second fiamme zone may separate zones 3 and 4, which suggests that the unit is not a simple cooling unit, and a marked increase in crystal content above zone 2 further suggests that some crystal settling had occurred in the chamber from which the flows were emitted, so that the initial "pulses" tapped the top part of the chamber which was depleted of crystals. The zones rich in crystals contain mainly intratelluric anorthoclase, with rare brown biotite, aegirine-augite, brown amphibole and sphene.

The secondary recrystallization begins just above the zone of maximum welding and is represented by axiolitic and spherulitic growths of alkali-feldspar within the pumice fragments, and a clouding of the glassy groundmass. The disrupted nature of the matrix is clearly evident, tricuspidate shards and elongate pumice fragments being seen in various stages of welding and compaction.

Pumice flows have been located only on the outer flanks of the volcano, where a number of flows from a series 20-30

ft. thick. Distinct changes are seen in vertical section, from the basal to the topmost flows, which are due to variations in the amount of pumice in individual flows. The basal flow is a mixture of flowing greenish-grey pumice and partially vesiculated black obsidian. The flow above this is completely pumiceous, with a base of incoherent grey pumice, a thin zone of maximum welding containing distorted and flattened pumice fragments, and a thicker upper zone of welded but undistorted pumice lumps. Above this the flows show a rapid increase in lava content which culminates in the topmost flow which contains only a small amount of pumice and is almost a true phonolite lava.

Together these pumice flows may represent a complete cycle of activity; the basal flows indicate the first stages of vesiculation, the flow above this represents the major phase of vesiculation and the topmost flows the waning stages when the gas had almost been depleted and only true lava was being emitted.

A third type of volcanoclastic flow is represented by the eutaxites. These were originally described from Tenerife<sup>5</sup>, when the term eutaxite was used to describe "a banded or flamed appearance" imparted to the rocks by alternations of either glassy and cryptocrystalline layers or cryptocrystalline and microcrystalline layers. The term has gained favour in ignimbrite nomenclature and is used to describe the flowing appearance given to a rock by the intermingling of pumice shreds and disrupted groundmass. Often if the pumice is completely flattened the "eutaxitic" texture is even more noticeable.

According to Ross and Smith the eutaxites of Tenerife "seem almost certainly to have been welded tuffs or at least welded clastic rocks"<sup>6</sup>. Re-examination of these rocks shows that although they may have the megascopic textural features of ignimbrites, that is a distinct flowing nature caused by irregular alternations of streaks of different colour, microscopically they show no criteria by which their ignimbrite origin may be proved.

In these rocks from Tenerife the eutaxitic texture appears to be formed by the alternation of areas of primary microcrystallization and glass, the crystalline areas being composed of unoriented alkali feldspar laths and shreds of aegirine. To what extent such textural relationships can be allied to McCall's hypothesis for the Kenyan eutaxites is not yet known, but the statement of Fritsch and Reiss<sup>7</sup>, that "in the Canadas Mts. these peculiar rock types occur in the superficial parts of lava streams, the main mass of which are phonolitic homogeneous rocks", suggests that there may be similarities between the eutaxites of Tenerife and the rocks described from Kenya.

Several preliminary conclusions may, therefore, be drawn from the types of flow the features of which are briefly outlined above. (1) Ignimbrites which display features commonly assumed to be the result of deposition from an emulsion of gas and solid can occur in an environment of sodic-alkaline volcanism. (2) The ignimbrites have an average thickness of only 12 ft. and so a large overload pressure is precluded. If temperature and overload pressure are the two dominant factors which produce the zonal arrangements commonly seen in ignimbrites, then for the rocks from Tenerife the temperature of emplacement must have been high, so that the shards and pumice fragments were plastic enough to deform under a small overload pressure. (3) The series of pumice flows may represent a "cycle of vesiculation". The crude zonal arrangement seen within the completely pumiceous unit suggests that it may have been deposited in a similar way to an ignimbrite, although it was different in that vesiculation was not followed by complete disruption of the magma. (4) The eutaxites of Tenerife are probably not true ignimbrites, and they are certainly not similar to the ignimbrites described as such here. Further investigation may indicate that they have close affinities with the eutaxites of McCall<sup>8</sup>, because the crystalline areas appear to be of primary origin and are not similar to the

areas of secondary recrystallization within pumice fragments, which are a common feature of many true ignimbrites.

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### Shock Metamorphism in the Carswell Circular Structure, Saskatchewan, Canada

PETROGRAPHICAL features such as multiple sets of deformation lamellae in quartz, isotropic quartz and feldspar, and characteristic microfracture patterns, are generally believed to be diagnostic of hyper-velocity impact<sup>1</sup>. These features can be produced experimentally by shock pressures of tens or hundreds of kilobars, which, it is assumed, can only be generated in nature by the hyper-velocity impact of large missiles. All these features occur in the Carswell circular structure, however, in circumstances which cast doubt on this theory.

The Carswell structure (Fig. 1), centred near 58° 24' N., 109° 30' W., occurs in the flat-lying Athabaska sandstone of Proterozoic age. The Carswell dolomite is known as a "toroidal" inlier downfaulted several thousand feet into the Athabaska formation. An exposure of pre-Athabaska

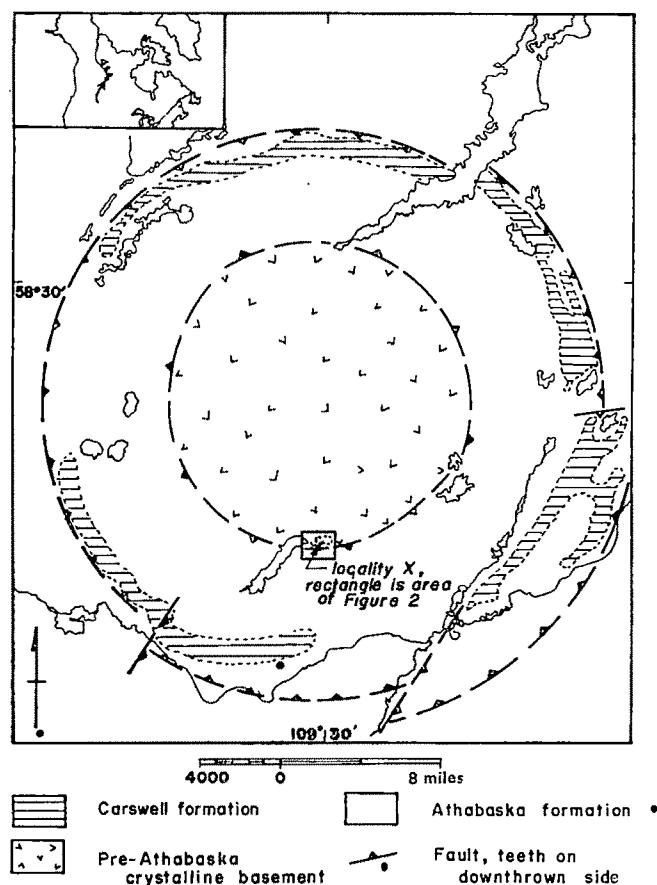


Fig. 1. Geological sketch of the Carswell circular structure.

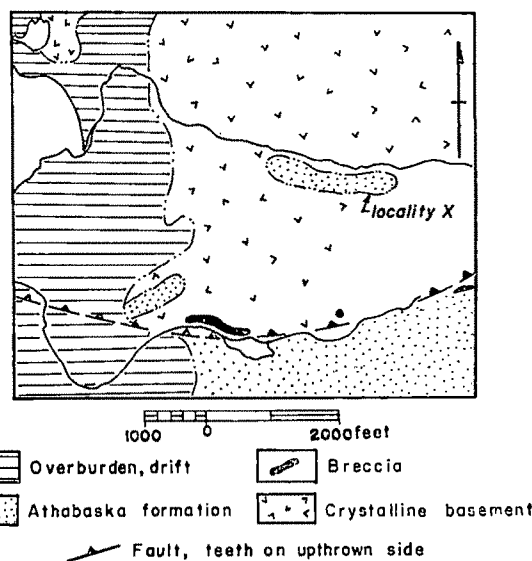


Fig. 2. Detailed geological sketch of small area of the Carswell structure.

crystalline basement, roughly circular and about 12 miles in diameter, occupies the centre of the structure. This outlier is the only one known in the 13,000 square miles covered by the Athabaska formation. Movement some hundreds of feet along a fault has juxtaposed basement rocks against the upper part of the Athabaska formation. Numerous small outliers of the lowest beds of the Athabaska formation red pebble conglomerates and sandstones are scattered over the pre-Athabaska surface.

At point X in Fig. 1, an outlier of Athabaska sandstone lies on the basement, and at the contact there is an unconformity which is well exposed. A detailed geological sketch of the area is shown in Fig. 2; Fig. 3 is a photomicrograph of quartz-garnet rock from the basement. This shows multiple sets of numerous deformation lamellae in the quartz grains. Initiation of fractures is seen in the lower right part of the field, and in the upper left corner the lamellae disappear into an area of amorphous material. According to the criteria of Short<sup>2</sup>, this rock has undergone moderate to strong shock metamorphism. Examples of such shock metamorphism are found sporadically over all the basement rocks.

Fig. 4 is a photomicrograph of Athabaska sandstone from immediately above the contact. There is no unusual



Fig. 3. Biotite-garnet-quartz gneiss, from lower side of contact at locality X, Fig. 1. Plane polarized light,  $\times c. 25$ .

deformation of the well cemented pebble sandstone, and the rare cracks which can be seen are typical of grains of the Athabaska formation.

Two thousand five hundred feet south of this area, breccia occurs in a fault zone which separates the basement from the upper part of the Athabaska formation (Fig. 2). The breccia contains fragments of shocked basement and unshocked Athabaska formation, and typical shock metamorphic features such as veinlets of heterogeneous glass, and biotite showing kink-bands. The Athabaska formation on the south side of the fault is undeformed, although before faulting it was several hundred feet nearer to the centre of the structure than the moderately shocked north wall. Shock deformation has not been detected in the Athabaska formation.

If the Carswell structure is an eroded impact scar, as suggested by Innes<sup>3</sup>, either maximum pressures were low in the Athabaska formation and increased abruptly in the underlying basement, or impact took place before the Athabaska sandstone formed and the glass-bearing breccias developed after formation. The latter alternative separates the supposed "impact metamorphic" phenomena into two categories occurring at widely different times, while the former appears to violate the laws of shock wave propagation. The shock impedance of the sandstone and basement is virtually identical below a few hundred kilobars (specific gravity of the sandstone 2.49, specific gravity of basement 2.48–2.52), so that the peak pressure of a shock propagating downwards must diminish as some power of the distance from the centre.

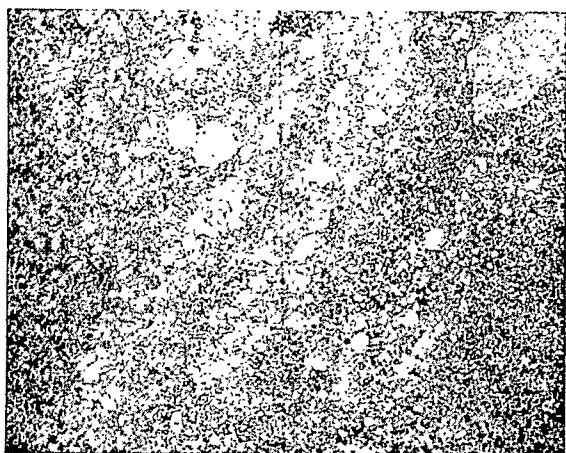


Fig. 4. Pebble sandstone with graded bedding, from upper side of contact at locality X, Fig. 1. Well rounded, unfractured larger grains of quartz and the partly sutured finer material are both typical of the Athabaska formation. Black areas are haematite cement. Plane polarized light,  $\times c. 13$ .

The phenomena can be explained by assuming that a strong shock wave propagated upwards through the basement, and was reflected, or considerably reduced, at the interface with the Athabaska formation—a model which attributes shock metamorphic effects to terrestrial processes. Alternatively it might be concluded that identical criteria are perhaps produced by causes other than shock pressure.

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## PHYSICS

### Symmetry of the Time Axis and Solar Observations

I SHOULD like to offer two comments on Stannard's<sup>1</sup> recent interesting postulate that there exists a counter-world to compensate for the asymmetries of nature that we find in our own world. The first comment proposes that observations do not exist to support his postulate, but that the counter-world suggested by the observations is of a somewhat different character. The second is a reconciliation between the two possible counter-worlds; Stannard proposes to add the faustian world to the present laws of physics to eliminate the asymmetries, whereas the observations suggest that the present asymmetries of physics have arisen because the present physical laws reflect an observation of the faustian world. In particular, our present physical laws could be said to be based on the assumption that the faustian universe is a contracting universe.

It is first necessary to outline the alternate counter-world suggested by the observations. Although this picture differs from Stannard's, I shall borrow the name "faustian". Our world is the real world and the counter-world is the "faustian" world. These worlds are relative in the sense that to the extent that real man can construct the faustian world mathematically, it is indistinguishable from the real world, but in so far as man can observe the faustian world, it is a real world that moves backwards in time. I do not wish, however, to say that the faustian world is moving backwards in time; such a statement has no meaning. Rather the real and faustian worlds are separate spaces and are related to one another as the Hermetian quaternion ( $ix, iy, iz, ct$ ) is related<sup>2</sup> to the metric quaternion ( $x, y, z, -ict$ ). Nevertheless in both worlds there is an absolute direction in time: a universe that expands and in which matter diffuses is said to move orthotropically; a universe that contracts and in which gases unmix is said to move paratropically. Both directions are absolute in either world, and indeed it is because of the preferred direction in time that the counter-world must be postulated.

The alternative picture to Stannard is very simple. I assume that real spontaneous emissions are "caused" by the absorption of faustian photons and that absorption of real photons "causes" the emission of faustian protons. I further assume that for every emission of a real photon from real matter, there is a corresponding absorption of a faustian photon into faustian matter. Between the two worlds there is no energy exchange—energies which are called real are transferred between real atoms by real photons; energies which are called faustian are transferred between faustian atoms by faustian photons. Although there is no energy exchange, there is nevertheless an exchange of causality. I now indicate how this causality exchange would be seen in observations of real atoms, not in thermal equilibrium.

In calculating the relative ratio of emission to absorption  $\epsilon/a$  for a particular bound-bound level transition, it is customary to write

$$\epsilon/a = N_e/N_a \neq e^{-h\nu/kT} \quad (1)$$

where  $N_e/N_a$  is the relative population ratio of the emitting atoms to absorbing atoms the energy difference of which is  $h\nu$ . For equilibrium (Planckian) radiation, or for a high rate of collisional excitation and de-excitation, the population ratio is given by  $e^{-h\nu/kT}$ . If, however, we replace the usual photon vacuum state of emission and absorption—the state to which and from which real photons are created and annihilated—by the faustian photon state, it is easy to show that the ratio

$$\epsilon/a = e^{-a} N_e/N_a \neq e^{-a} e^{-h\nu/kT} \quad (2)$$

in general where



$$e^{\alpha} = \frac{1 + \hat{N}}{\hat{N}} \quad (3)$$

is the ratio of final and initial state factors, that is, treating faustian photons as bosons. We additionally note that as  $\hat{N} \rightarrow \infty$ , the usual rules of physics are operative, and in effect, the faustian photon state may be replaced by the vacuum state. On the other hand, if  $\hat{N} \rightarrow 0$ , the ratio goes to zero, that is, spontaneous emissions cease.

The first evidence of a diminution of spontaneous emission in a non-equilibrium situation is indirect. If one applies the conventional equations and formulae of statistical mechanics in the non-equilibrium situation of the Fraunhofer line forming region of stars—low matter density and strong non-equilibrium radiation field—one can calculate large radiative imbalances which in turn cause large departures of  $N_e/N_a$  from the Boltzmann equilibrium ratios  $e^{-h\nu/kT}$ . Such departures are not observed—except in the sense that a systematic march of increasing temperature into the star is claimed to be derived if the Boltzmann distributions are assumed. The argument as to whether the atom populations are those given by the assumed Boltzmann distributions or those given by the theoretical calculations is a subject of much lively debate—the celebrated L.T.E. *versus* non-L.T.E. dilemma of modern solar physics<sup>3-5</sup>. The complete controversy, however, could be eliminated (an agreement between theory and observation could be said to exist) if the effect of the depletion of faustian photons could be incorporated into the non-equilibrium theory: spontaneous emissions (depending on the faustian photon density) would accordingly be theoretically reduced, and the presently predicted radiative imbalances diminished.

The second evidence is more direct. In the deeper and denser regions of the Sun—the photosphere—where the continuum radiation originates, the atom populations which effect the continuous radiation can be assumed to be given by their Boltzmann distributions because of the high rate of collisions. (The radiation field remains as before, non-Planckian.) And on this account Kirchhoff's law is traditionally used in the analysis of the solar photosphere, that is,

$$S_{\nu} = 2h\nu^3/c^2(e^{h\nu/kT} - 1)^{-1} \quad (4)$$

Our new picture demands that Kirchhoff's law be replaced by a source function conjectured by Wildt<sup>6</sup> of the Kothari-Singh<sup>7</sup> form

$$S_{\nu} = 2h\nu^3/c^2(e^{\alpha}e^{h\nu/kT} - 1)^{-1} \quad (5)$$

where the quantity  $\alpha$  is defined in equation (3). This source function can be distinguished from the Kirchhoff source function through a comparison of the empirical solar opacity to the theoretical solar opacity. The comparison is in the nature of the removal of a discrepancy of 300 per cent in the relative ratios of empirical to theoretical opacity over the wavelength range  $\lambda\lambda 5,000-24,000$  (ref. 7).

*The contracting universe.* In contradistinction to Stannard's suggestion, the present picture suggests that it is the absence of faustian matter that will decrease the emission of a thermal detector (or atom). Thus, the problem of the present or new picture of the counterworld is to explain why spontaneous emission is the natural state of affairs. That is to say, why are the traditional rules of physics approached as  $\hat{N} \rightarrow \infty$  and not as  $\hat{N} \rightarrow 0$ ? We examine this question by a reconsideration of Obler's paradox.

Obler's paradox simply asks: Why is the night sky not as bright as the average star (or the Sun)? And the paradox is only adequately answered by concluding that the universe must be expanding. Our paradox, however, is the reverse. Why, in traditional physics, that is,  $\hat{N} \rightarrow \infty$ , is the faustian night sky radiation so bright? And in the same logic of Obler's paradox, we must conclude that the faustian universe as seen by an orthotropically moving observer, is seen as a paratropically moving universe,

that is, it is a contracting universe. Thus, stars, instead of being brighter than the background night sky radiation, are instead merely occasional cool regions (or faustian photon sinks) superposed over an extended hot bright faustian sky. Indeed, it is on account of this intense faustian radiation that—for many conditions—Dirac's historic assumption that negative energy states are all occupied is possible.

Thus Stannard's picture of faustian stars being "cool" against the "night sky" radiation is correct, but the present picture would suggest that the "night sky" observation is in our physical laws rather than as a direct observation. With respect to direct observation, however, the surfaces of stars are cool in the sense that there are insufficient faustian photons to cause spontaneous emission. That is to say, stars produce orders of magnitude more real photons than they absorb—perforce in compensation they emit far less faustian photons than they annihilate. And therefore it is in the observation of the breakdown of the traditional physical laws on the surface of stars that is the primary observation.

Finally, instead of ending in a note of speculation, I should like strongly to urge that the counter-world, defined as the space orthogonal to ours, be designated as the faustian counter-world. There may exist other places in the mathematics where other counter-worlds can be postulated, but the orthogonal counter-world makes some sense in being called faustian. Further, this counter-world seems to hold the most theoretical promise as a working tool; there is no difficulty, for example, in giving a mathematical explanation as to why the real world has an electric current but no magnetic current.

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## Technique for Detecting Lead Particles in Air

TECHNIQUES for counting airborne lead particles would be useful in studies of air pollution. No available technique detects lead particles less than  $5\mu$  in diameter, and particles larger than this reside in the atmosphere so briefly that they are probably not a hazard to health. The present methods for measuring lead in the air<sup>1</sup> give only the mass of lead per unit volume. The effect on human beings of a particulate suspension cannot be assessed from mass alone, as capture in the lungs depends on particle size<sup>2</sup>.

This communication describes preliminary trials of a new method of detecting lead particles, based on the activity of lead iodide as an ice nucleating agent<sup>3</sup>.

We detect ice nuclei with an acoustical counter<sup>4</sup>. This instrument, developed at the National Center for Atmospheric Research (NCAR), Boulder, Colorado, counts the active ice nuclei in the air on a continuous real time basis at any desired temperature. In our experiments, air entering the counter is first passed through a small mixing chamber containing iodine crystals. Lead particles are thus converted into lead iodide which is active as an ice nucleus at sizes probably extending down to  $0.01\mu$  diameter, depending on the temperature. The lower the temperature of the cold chamber, the smaller the active particles.

Laboratory trials have established that lead particles (generated by heating lead beyond its melting point) do indeed form lead iodide in the mixing chamber, and that

these do cause ice crystals to form in the cold chamber of the counter. Lead particles without iodine do not act as ice nuclei, and iodine alone, in air free from particles, has no activity as an ice nucleus, at least at temperatures above  $-15^{\circ}\text{C}$ . Burning unleaded gasoline (white gas) produces no ice nuclei with iodine, while the exhaust from an automobile burning leaded fuel produces many nuclei.

In trials outside the laboratory with the counter at a temperature of  $-15^{\circ}\text{C}$ , the iodine-ice nucleus count is low or zero in the clean air which blows from the mountains west of Boulder, but when the wind becomes easterly and blows across the city, the count becomes very high, sometimes exceeding the capacity of the counter (the maximum counting rate is 10,000 particles/min, corresponding to 1,000 particles/l.).

Fig. 1 shows the records obtained on two consecutive days near a busy intersection in the centre of Boulder. The instrument was mounted in an automobile parked about 25 yards south-east of the intersection. On October 25 the wind was light and generally easterly, so that the instruments were upwind of the intersection. There were intermittent periods of calm. The air temperature was warm enough to create considerable vertical transport by convection, and the surface air could be expected to become diluted with cleaner air from higher levels. The counts at  $-14^{\circ}\text{C}$  were quite low at midday but rose through several orders of magnitude as the afternoon progressed. The increase was probably due to a slackening in the rate of vertical mixing as the temperature fell from 1400 h on. Traffic was moderate throughout the afternoon until the evening rush started, just before 1700 h. Shortly after 1700 h the count rate went off the scale and remained off. To get back on to the scale the counter was set at a warmer temperature ( $-12^{\circ}\text{C}$ ). Next morning the early rush, combined with the stable inversion conditions characteristic of that time of day and a dead calm, produced a very high rate of count. At  $-14^{\circ}\text{C}$  the count rate was off scale and at  $-12^{\circ}\text{C}$  it was near the top of the scale. This day was also warm and, as the temperature rose rapidly, the count decreased greatly. By 1130 h it had dipped below 1 particle/l., so the counter was cooled down to  $-14^{\circ}\text{C}$ . The count at this temperature was just coming back on scale, and occasionally going off scale. These results suggest that this technique may be useful in monitoring air pollution. It remains to be shown, however, that what is being counted is indeed lead. Other substances can also react with iodine to form ice nuclei. Silver would do so, but is unlikely to be present in suffi-

ent concentrations in outside air. Terpenes also form ice nuclei with iodine<sup>6</sup> and are likely to be present.

Some of this work was carried out with the collaboration of Dr. Ottavio Vittori, director of the Osservatorio di Monte Cimone, Italy, while he was visiting the United States. His assistance and encouragement are acknowledged. The co-operation of J. Rosinski, G. Langer and A. D. Gibson of NCAR was essential to the entire study.

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### Aspects of Similarity for Air-entraining Water Flows

Air is often entrained naturally by flowing water—for example, in the plunging waters of mountain streams, waterfalls, bores, breakers, hydraulic jumps and subterranean siphons—and, in these and similar situations, appears generally to be beneficial. The quality of the water, certainly, is improved by air entrainment and very few hazards tend to arise with these naturally occurring flows.

Air entrainment is, however, also encountered in many artificially contrived situations and its various influences must then be carefully studied. Engineering projects in which air entrainment exerts a significant influence include open channels with fast-moving water flows, which require depths adequate to accommodate air entrainment<sup>1</sup>; "morning-glory", air-entraining, spillways which individually must have the capacity to discharge the estimated flood and its entrained air<sup>2</sup>; spillway discharge tunnels which require adequate ventilation (to suppress tendencies to surge); siphon spillways, especially those which are deliberately air controlled (to allow the close regulation of the levels of lakes or rivers<sup>3</sup>); flood control gates, or weir structures (which need substantial and adequate ventilation<sup>4</sup>); vortex-tube drop shafts<sup>5,6</sup>; air lift pumps; and air exhausting, water jet pumps.

Regardless of whether air entrainment must be induced or discouraged, adequate knowledge of the flow pattern is essential for proper engineering design. Unfortunately, it seems that water flows which entrain air are still not yet amenable to satisfactory theoretical treatment and, in consequence, resort has been widely made to the use of hydraulic models. Even with these, however, the problems of correct scaling have still not been satisfactorily resolved. It is well known that air entrainment studies on simple Froude models, tested in the Earth's atmosphere, are subject to significant error<sup>2,4,7</sup>. Escande has demonstrated that such models necessarily entrain insufficient air<sup>8</sup> and it has been suggested that this type of test should be conducted in suitably scaled "model atmospheres". Tests recently undertaken at the Imperial College of Science and Technology on a full-size siphon under atmospheric conditions and on a one-sixth linear scale model air controlled siphon, initially under atmospheric conditions and finally in a one-sixth scale model atmosphere, indicate that significant inherent scale effects still persist, perhaps because of the incorrectly scaled, yet influential, surface tension forces and viscous forces.

Studies of the formation of vortices and swirls at intakes<sup>9</sup> and, more recently, studies with air-controlled siphons<sup>10</sup>, have indicated that the use of full scale velocities and pressures might provide useful similarity

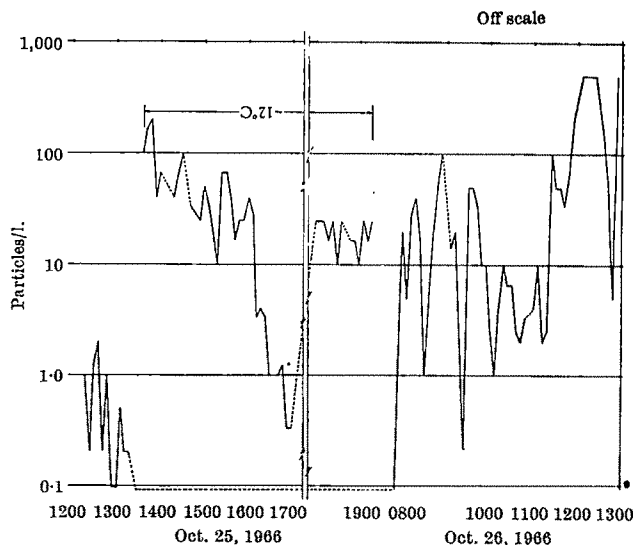


Fig. 1. Ice nucleus concentration in iodine-treated air (Boulder, Colorado, October 25-26, 1966); — — — indicates instrument off data collected at  $-14^{\circ}\text{C}$  unless noted.

criteria for studies of air entraining water flow and, in fact, it now seems that there may be some theoretical foundation for such notions. In considering incipient air entrainment in vortices, it has recently been emphasized<sup>11</sup> that when prototype and model fluids are identical the use of full scale velocities as a criterion of similarity is tantamount to ignoring the influence of inertia forces. Thus, when viscous and surface tension forces are together considered to dominate the pattern of flow and are identically scaled, the following ratios will necessarily be identical (in corresponding prototype and model situations)

$$\frac{\text{Viscous forces}}{\text{Surface tension forces}}; \text{ or } \frac{\left(\mu \frac{dv}{dy}\right)(\text{area})}{\sigma l};$$

or, alternatively,

$$\frac{\text{Weber numbers } \left(\frac{\rho v^2 l}{\sigma}\right)}{\text{Reynolds numbers } \left(\frac{\rho v l}{\mu}\right)}$$

will be the same in model and prototype. Thus for dynamic similarity between the viscous forces and the surface tension forces

$$\left(\frac{\mu v}{\sigma}\right)_{\text{model}} = \left(\frac{\mu v}{\sigma}\right)_{\text{prototype}}$$

or, when the same fluids (for example, air and water) are in use

$$v_{\text{model}} = v_{\text{prototype}}$$

(where  $\mu$  is the coefficient of absolute viscosity and  $\sigma$  is the coefficient of surface tension).

Precedent for the deliberate neglect of the influence of inertia forces, in order to achieve identical scaling of other pairs of significant forces, has been previously created in the resolution of various other flow systems—for example, those in which viscous and gravitational forces are considered to be dominant and where similarity can be attempted by equating ratios of

$$\frac{\text{Gravitational forces}}{\text{Viscous forces}} \text{ or, in effect, of}$$

$$\frac{\text{Reynolds numbers } \left(\frac{\rho v l}{\mu}\right)}{\text{Froude numbers } \left(\frac{v^2}{gl}\right)}$$

Such situations arise, for example, during hydraulic model studies of geological (or tectonic) movements<sup>12</sup>; in studies of hydraulic models of flow patterns in glass furnaces<sup>13</sup>; and, generally, in studies of free surface laminar flows under the influence of gravity<sup>14</sup>.

The influence of inertia forces has also been deliberately neglected, and the ratio  $(\mu v/\sigma)$  introduced, when contemplating various other situations in which viscous and surface tension forces are dominant, for example, when considering the distortion of drops in a shear flow during the formation of emulsions<sup>15</sup>; when considering the sintering and rounding of sharp edges by heat in glass technology<sup>16</sup>; when defining the boundary conditions of cavities in thin (lubricating) films<sup>17</sup>; when describing the surface waviness in annular two phase pipe flow<sup>18</sup>; and when contemplating the atomization of liquid fuels<sup>19</sup>.

The ratio  $(\mu v/\sigma)$  has not, however, to our knowledge, previously been applied to the study of air entraining water flows. Nevertheless, air entraining water flows necessarily carry air bubbles and in some cases the air bubbles can be introduced into the main flow with drops of water. Neither air bubble size nor water drop size is sensitive to linear scale and yet each is very much dependent on surface tension forces and doubtless also on viscous forces. Significantly, the ratio  $(\mu v/\sigma)$  (that is, viscous force/surface tension force) is independent of

linear scale and it seems likely that this ratio may be usefully adopted as a criterion of similarity for studies of air entraining water flows. Support for this suggestion stems from the studies on the formation of vortices and swirls<sup>9</sup> and on the air regulation of siphonic flows<sup>10</sup>.

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## Transport Processes and Thermodynamic Equilibrium

THERE is an error in my article in *Nature* under the above title<sup>1</sup>. The error occurs at the point where the chemical potential  $\mu$  is introduced as an undetermined multiplier and is in equation (7), the restriction on the volumes of the various parts of the system in a virtual reversible infinitesimal displacement of the system at constant total volume, mass, and entropy. The restriction given, that is,

$$\sum_i (\delta V_i + v_i \delta M_i) = 0$$

is appropriate when there are no long range gravitational or electrostatic potential forces, whereas for the increment in the total energy arising from such forces to be  $\sum_i \phi_i \delta M_i$ ,

a much stronger restriction must be applied, namely, for every region  $i$ ,

$$\delta V_i + v_i \delta M_i = 0$$

The erroneous restriction would lead to the conclusion that there is a uniform pressure throughout the system, which is true only if there are no long range forces. Using the correct restrictions to eliminate  $\delta V_i$  from the expression  $-p_i \delta V_i$  for the work done on the  $i$ th part of the system, it is then necessary to introduce only two undetermined multipliers  $T$  and  $\mu$  to deal with the restrictions on total mass and entropy. This results in the two conditions of equilibrium, namely, uniform temperature and a constant value, through the system, of

$$\mu = u_i - T s_i + p_i v_i + \phi_i$$

• Using this equation and following the method of ref. 1 it is easy to show that in a virtual displacement involving a deformation as well as a change of mass of the whole system

$$\bullet \quad dU = T dS + dW + \mu dN$$

where

$dW = -\sum p_i dV_i$  is the total mechanical work

performed on the system, and  $dV_i$  is the increment in volume of part  $i$  arising from both the deformation and the change of mass, and  $\mu$  is independent of the point in the system at which  $dN$  has been introduced.

The remainder of the article is not affected by this error.

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## THE SOLID STATE

### Early Fading of Thermoluminescence Induced by Radiation in Lithium Fluoride

IN a previous report<sup>1</sup> the fading of thermoluminescence induced by ionizing radiation in lithium fluoride has been discussed, but little attention has been paid to fading that may occur during the first 24 h after irradiation. Knowledge of, or elimination of, early fading will be of interest when it is desired to read out, with a precision of a few per cent, a group of samples soon after irradiation. Some users of lithium fluoride wait 24 h until the rate of fading has decreased enough so that fading will be negligible during the measurement of all the samples. Under certain circumstances, however, this delay is inconvenient.

Curve A of Fig. 1 indicates a typical curve of fading for 'Con-Rad Type 7' powder from a batch which had been exposed to a "standard" annealing of 1 h at 400° C plus 24 h at 80° C before use. The earliest measurement was taken 1 min after 3.5 min of exposure. It is clear that if the readout of a sizable series of samples irradiated simultaneously as a group is begun soon after irradiation, the fading can be more than a few per cent by the time the final sample is read out. It is also clear that the rate of fading decreases with time, so that one could decide, on the basis of such a fading curve, to delay readout until the amount of fading over the time span for reading all the samples is tolerable. We have found the effect to be quantitatively about the same over a range of exposures

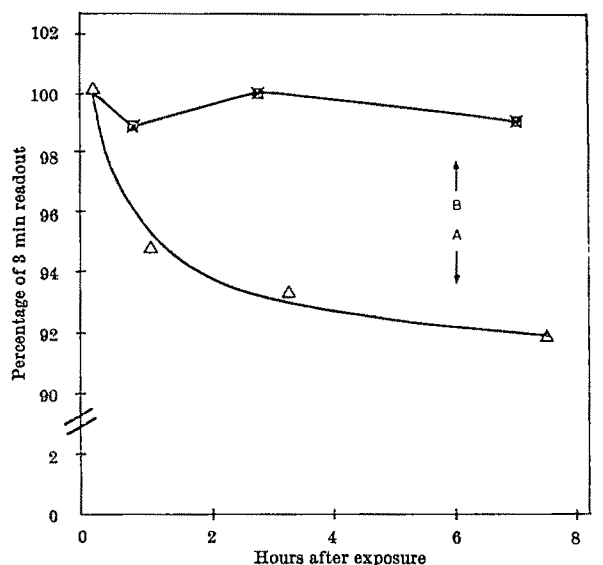


Fig. 1. Fading curves. The earliest readouts are at 3 min after exposure and are normalized to 100 per cent. Curve A, normal readout cycle; curve B, with readout cycle altered so that the photomultiplier does not function during the first 4.5 sec of the heating cycle.

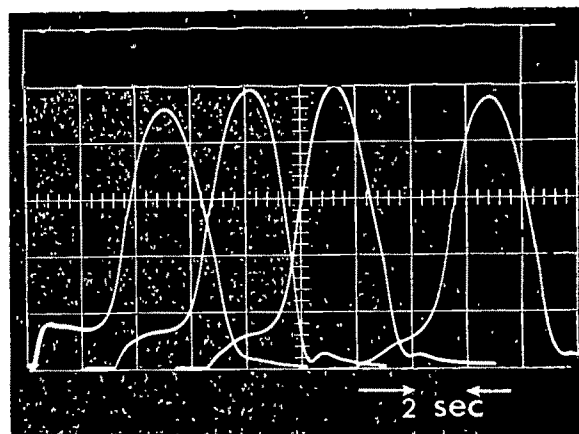


Fig. 2. Glow curves showing the decrease of the low temperature peak as the time between irradiation and readout is varied. From left to right, the delay between exposure and readout is 1 min, 8 min, 30 min, and 1.5 h. ('TLD 100', cobalt-60.) The time scale runs from left to right. The corresponding readouts were 548, 527, 518, 514. Curves were made using a 'Fluke 840A' electronic galvanometer and an oscilloscope with camera. (The electronic galvanometer requires almost 1 sec to reach 90 per cent of full response.)

from 10 r. to 1,000 r. of X-rays and  $\gamma$ -rays, independent of radiation quality and independent of exposure rate up to 250 r./min, the highest rate we studied. The two types of powder ('TLD 100' and 'Con-Rad Type 7') behaved in essentially the same manner. It is obvious that in instances of long exposure, early fading will be difficult to observe.

By recording glow curves we have shown that early fading is caused principally by decay of the low temperature peaks described by Zimmerman and associates<sup>2</sup>. Fig. 2 is a series of glow curves with the zero times displaced for the sake of clarity. The curves were made 1 min, 8 min, 30 min and 1.5 h after exposure, and the low temperature peak is seen to decrease progressively with time. (We use a 'Con-Rad TLD' reader and either lithium fluoride 'Con-Rad Type 7' or 'TLD 100' powder for radiation dosimetry.)

Zimmerman and associates<sup>2</sup> have also shown that the low temperature peaks are not present if the powder is annealed after irradiation for 10 min at 100° C, but early fading was not discussed specifically in relation to the low temperature peaks.

Curve A of Fig. 3 is a fading record for powder not annealed after irradiation. Each point on these curves represents an average of three readings. Curve B is for powder irradiated identically but annealed in the capsules for 10 min in the top of a double boiler at 100° C. The absolute readings were 10 per cent less than the highest readings of the unannealed powder, but the fading was not more than 1 per cent over the 8 h period studied. While this post-annealing removes the early fading, it does introduce some inconvenience.

Another method we have used to avoid early fading is to programme the readout cycle so that the phototube current circuit is held open by a time-delay relay until the low temperature peaks have been passed. The delay needed depends on such factors as heating current, initial temperature of planchet, and so forth. Fig. 1 shows fading records for powder readout with a normal cycle (curve A) and for powder readout with a 4.5 sec delay (curve B). Fading of the readout has been reduced to approximately 1 per cent. The absolute readings which used the delay were 8 per cent less than the highest readings which used the normal readout cycle. This percentage will vary with the amount of delay and the length of time that has elapsed between exposure of powder and readout. This arrangement permits minimal inconvenience and elapse of time between the end of irradiation and readout, but if the heating rate of the powder is changed, there will be

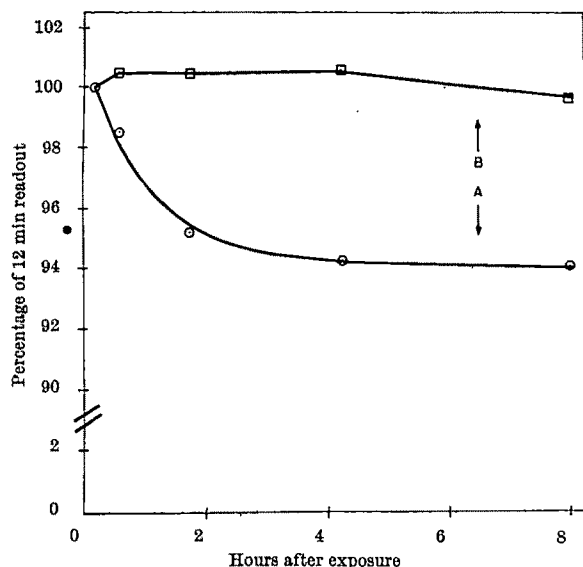


Fig. 3. Fading curves. The earliest readouts are normalized to 100 per cent. Both curves begin 12 min after exposure. Curve A, normal readout; curve B, with 10 min post irradiation annealing at 100° C. Each point is an average of three values.

a change in the fraction of the glow curve that is programmed out and, thus, a change in reader calibration. (Details of the modification of our reader will be sent on request.)

Two other methods for elimination of effects of early fading might be (1) to incorporate a short annealing at 100° C as the first part of the readout cycle, and (2) to correct the readings on the basis of a fading curve for the same batch of lithium fluoride powder.

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### Etching of Dislocations in Crystals of Aromatic Hydrocarbons

STUDIES of the defect structure of single crystals of aromatic hydrocarbons have revealed that "as grown" crystals contain a high concentration of dislocations<sup>1,2</sup>. These defects have a marked effect on the physical properties of the solid<sup>3,4</sup>. It is therefore desirable to have some method of determining dislocation concentrations in these solids so that the magnitude of this effect can be assessed. Previous attempts to study the dislocation structure of aromatic hydrocarbon solids by thermal etching<sup>5</sup>, electrolyte etching<sup>6</sup>, and enzyme etching<sup>7</sup> have produced oriented but ill-defined etch patterns. This communication reports the results of some attempts to etch such crystals using solvent and chemical etches.

A variety of possible chemical etchants were tried on very pure single crystals of anthracene and naphthalene grown from the melt by the Bridgman technique. Bromination, nitration and oxidation produced ill-defined patterns. Sulphonation with fuming sulphuric acid (65 per cent sulphur trioxide) diluted 1 : 10 with concentrated sulphuric acid proved most suitable. Freshly cleaved crystals were dipped in the acid, rapidly washed with water and dried. The vigorous reaction yielded a dark

brown reaction product which dissolved in the water to leave a clean etched surface. With anthracene the reaction was instantaneous; longer immersion periods (10 sec) were required for naphthalene. Well-defined rhombic etch pits were produced on the (001), *ab*, surfaces of anthracene crystals (Fig. 1). Attempts to etch other crystal faces were unsuccessful. When the etched crystals were examined between crossed 'Polaroids' with the beam normal to the (001) surface the isogyre was observed to be parallel to the long axis of the pits. For anthracene and naphthalene (monoclinic crystals) the optic plane is (010) and the *b* axis,  $\langle 010 \rangle$ , is a principal axis<sup>8</sup>. The isogyre will therefore be parallel to the  $\langle 100 \rangle$ , *a*, direction. Hence the long and short axes of the etch pits are aligned parallel to the *a* and *b* axes respectively. Close examination of the pits under high magnification showed that the pits may be inclined along the  $\langle 001 \rangle$  direction. Thus it is possible that the pits lie at the emergent ends of dislocations lying parallel to the  $\langle 001 \rangle$  direction. The possibility that dislocations of this orientation exist in naphthalene has been suggested by Gordon<sup>8</sup>. An excellent mirror image relationship was found between etch patterns produced on adjacent cleaved surfaces (Fig. 1).

Etch pits produced on naphthalene (001) surfaces using the same technique were initially similar in shape and orientation to those observed with anthracene. Because of the high volatility of the solid the pits rapidly developed into polygonal pits or lost their shape completely. Etching, followed by rapid photography, enabled reasonably well-defined patterns to be obtained. At a later stage the pits merged together and were not distinguishable. Fig. 2 shows an etched naphthalene surface at an intermediate stage.

Solvent etches, either water or mixtures of organic solvents with water, gave etch patterns similar to that shown in Fig. 3. The pits were conical in shape and showed no particular orientation with respect to the crystal axes. With naphthalene these etch pits were more stable than those produced by the acid etch; however, with time these pits again developed and became diffuse.

Table 1		
Crystal	Treatment	Dislocation content (cm <sup>-2</sup> )
Anthracene	As grown	10 <sup>8</sup> -10 <sup>7</sup>
	Annealed 200°, 100 h	10 <sup>8</sup> -10 <sup>6</sup>
	Anthraquinone doped, annealed as above	10 <sup>8</sup>
Naphthalene	As grown	10 <sup>8</sup> -10 <sup>6</sup>
	Annealed 78°, 280 h	10 <sup>8</sup> -10 <sup>4</sup>

Dislocation counts were made on etched crystals which had been subjected to various treatments. The details are given in Table 1.

As grown crystals of anthracene and naphthalene contain a high concentration of dislocations. This no doubt results from the strain induced during growth in a closed vessel. Lengthy annealing periods at temperatures close

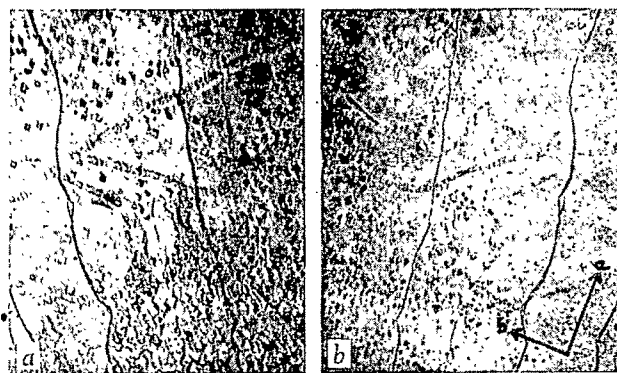


Fig. 1. Adjacent cleaved (001) faces of an anthracene crystal, sulphuric acid etch ( $\times c. 135$ ).



Fig. 2. Naphthalene crystal (001) face approximately 30 sec after sulphuric acid etching ( $\times c. 135$ ).



Fig. 3. Naphthalene crystal (001) face etched with cold water. Oblique illumination ( $\times c. 135$ ).

to the melting point of the solid can result in a marked reduction in dislocation content. Intentionally doped crystals contain a higher concentration of dislocations than the pure crystals. A noticeable factor in the case of the doped crystal was the greatly increased number of sub-grain boundaries compared with the pure crystal. These observations are in accord with the results of recent investigations of the effects of doping on the physical properties of aromatic solids. More detailed publications dealing with these aspects will appear shortly.

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## APPLIED SCIENCE

### Buffer Electrolytes for Fuel Cells

SULPHURIC acid, phosphoric acid and concentrated caustic solutions are the electrolytes most frequently used in the development of fuel cells, but they have disadvantages which justify the investigation of substitutes. The chief disadvantage of caustic electrolytes is their inability to reject carbon dioxide when a carbonaceous fuel is completely oxidized. Considering electrolyte invariance and ionic concentration polarization only, Williams and Gregory<sup>1</sup> recommend strong acid electrolytes for low temperature fuel cells. Acids, however, present a severe corrosion problem. Thus, fuel cells using acid electrolytes require noble metal catalysts, not only for catalytic activity, but also to withstand corrosion. The electrode supports must also either be a noble metal or a costly non-noble corrosion resistant metal such as tantalum. Cairns and MacDonald<sup>2</sup> have shown the feasibility of highly soluble carbonates as invariant electrolytes for fuel cells, although temperatures ranging from 130° to 200° C appear to be necessary. This communication shows that other choices of electrolyte are possible which may eliminate some of these problems.

A suitable electrolyte must exhibit many of the properties of strong acids and bases. In particular, a property of acids and bases frequently overlooked in fuel cell work is their excellent buffering ability. This is a necessary property because the potentials of both electrodes of a fuel cell depend directly on the electrolyte pH at the interface. Changes in pH, caused by current flow, at the interface are manifested as polarizations over and above activation and ohmic polarizations. Thus, buffering is necessary to minimize interfacial pH changes, so that buffers are the only possible substitutes for strong acids or bases.

Buffers can be selected which function at a pH where carbon dioxide is rejected. The electrolyte reactivity which plagues caustic pH electrolytes is then no longer a problem. In addition, corrosion is generally less severe in the intermediate pH range, so that buffers could make it possible to use non-noble metals as fuel cell components.

It remains to be shown, however, that appreciable activities can be obtained in the intermediate pH range. Although little work in fuel cell development has dealt with intermediate pH electrolytes, barring specific effects at a given pH, such as adsorption limitations, there is no *a priori* reason why activity should not be elicited over the entire pH spectrum. Tests were therefore conducted on the activities obtainable with buffer electrolytes using a typical fuel and oxidant.

Methanol is a carbonaceous fuel that has been anodically oxidized at a platinum black electrode, in strong acid and base electrolytes. Table 1 shows that methanol is also active in the weakly acid or nearly neutral pH range as well as in the weakly alkaline region. Carbon dioxide was rejected in all cases.

Table 1. ANODIC OXIDATION OF METHANOL AT 60° C OVER pH RANGE OF TEN UNITS

Geometric current density m.amps/cm <sup>2</sup>	Volts polarized from theoretical methanol*			
	3.7 molar H <sub>2</sub> SO <sub>4</sub>	1 molar each KH <sub>2</sub> PO <sub>4</sub> K <sub>2</sub> HPO <sub>4</sub>	1.8 molar Na <sub>2</sub> SO <sub>4</sub>	1 molar each KHCO <sub>3</sub> K <sub>2</sub> CO <sub>3</sub>
	pH 25° C = 0.7	pH 25° C = 0.6	pH 25° C = 7	pH 25° C = 10.7
10	0.46	0.40	0.58	0.30
25	0.48	0.46	0.64	0.33
50	0.50	0.51	0.67	0.40
75	0.51	0.53	0.71	0.44
100	0.52	0.50	0.73	0.46

\* Polarization is defined as the departure of the electrode potential from the reversible value under the same conditions of temperature, pressure and concentration.

The poor performance of methanol in sodium sulphate solution contrasts sharply with the anodic activity shown in the phosphate buffer at practically the same pH, and in the carbonate buffer 4 pH units removed. These results



show that buffering capacity rather than pH is the important criterion for electrolyte selection.

Stirring the electrolyte has no effect on the performance in 3.7 molar sulphuric acid, but can improve performance in phosphate and carbonate buffers by as much as 40 mV at 100 m.amp/cm<sup>2</sup>. This difference is due to the greater buffering capacity of 3.7 molar sulphuric acid and not to limitations on methanol diffusion. The improvements in performance when an unstirred but more concentrated buffer is used are the same as those accomplished by stirring in a more dilute buffer solution. Thus, with a soluble fuel in concentrated buffer solution, concentration polarization is virtually eliminated.

Although electrode structure is more critical with gaseous than with soluble reactants when buffer electrolytes are used, respectable levels of performance can still be attained. Table 2 shows the performances achieved with oxygen on an interface maintaining platinum-'Teflon' electrode.

Table 2. CATHODIC PERFORMANCES IN BUFFER ELECTROLYTES AT 60° C

Geometric current density m.amp/cm <sup>2</sup>	Volts polarized from theoretical oxygen			
	8.7 M H <sub>2</sub> SO <sub>4</sub>	1 M KH <sub>2</sub> PO <sub>4</sub> 1 M K <sub>2</sub> HPO <sub>4</sub>	2 M RbH <sub>2</sub> PO <sub>4</sub> 2 M Rb <sub>2</sub> HPO <sub>4</sub>	1 M KHCO <sub>3</sub> 1 M K <sub>2</sub> CO <sub>3</sub>
10	0.22	0.35	0.34	0.30
50	0.31	0.47	0.46	0.42
75	0.32	0.55	0.52	0.47
100	0.33	0.61	0.56	0.53
150	—	—	0.66	—

The poorer oxygen performances with the buffer electrolytes are mainly due to the greater concentration polarizations incurred with these electrolytes and diffusion electrodes. Increasing the buffer concentration, of course, reduces concentration polarization, and substantial improvements in performance at a cathode can be effected by increasing the buffer concentration (Table 2).

In comparing the performance of methanol and oxygen in the various electrolytes, the following conclusions can be drawn. Where the electrode is in direct contact with the bulk electrolyte, as when a soluble fuel is used, buffer electrolytes can be substituted for strong acids with little additional concentration polarization. At an electrode that has to maintain a three-phase interface, however, ionic mass transfer can occur solely by molecular transport (diffusion and conductance). Because of the abnormally high mobility of hydrogen and hydroxide ions, acids and bases are, therefore, more efficient than buffers in minimizing concentration polarization at interface maintaining structures.

A fuel cell using buffer electrolytes could therefore not match the power density the same cell would exhibit if a strong acid or base served as the electrolyte. In addition to the greater concentration polarization exhibited in buffer, the ohmic loss in the cell would be higher than these losses would be with acid or basic electrolytes. Assuming that activation polarization is identical in all the electrolytes, the cell operating with buffer electrolyte obviously has a smaller power output. Fuel cells of lower power density, however, could be acceptable substitutes, provided the buffers would allow the use of inexpensive non-noble metal catalysts.

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## Mechanism of Coal Decomposition

DAVIES<sup>1</sup> has studied the thermal decomposition of a bituminous coal, and has laid particular emphasis on the heat flux to the coal particles. Individual spheres of coal were suspended from a recording balance in a stream of hot nitrogen. To begin with, the weight record showed a constant rate of loss and in the final part the rate of weight loss decreased with time. The initial constant rate of weight loss was found to be directly proportional to the heat flux and was zero at about 400° C.

For a system with heat transfer by forced convection, the heat flux to a particle is given by

$$Q = hA(T_g - T_s) \quad (1)$$

where  $h$  is the heat transfer coefficient;  $A$ , the surface area of the particle;  $T_g$ , the gas temperature; and  $T_s$ , the surface temperature of the particle.

In the experiments reported here, particle diameter  $D$  and therefore area  $A$ , gas temperature  $T_g$  and gas flow rate were varied in order to vary the heat flux.

For the initial period of constant rate of loss of weight the surface temperature was estimated to be constant at about 400° C, by extrapolation from measurements with thermocouples at different distances below the surface of slabs of coal. A surface temperature of 400° C has also been reported for coal combustion during the initial "volatile" combustion period<sup>2</sup>.

The heat transfer coefficient,  $h$ , was obtained from the correlation<sup>3</sup>

$$\frac{hD}{K} = 2.0 + 0.6 (Re)^{1/2} (Pr)^{1/3} \quad (2)$$

where  $K$  is the particle thermal conductivity;  $Re$ , the Reynolds number; and  $Pr$ , the Prandtl number.

The heat transfer coefficient was changed by varying the gas temperature in the range 350°–550° C; by varying the rate of gas flow in the range 40–400 ft.<sup>3</sup>/h; and by varying particle diameter in the range 0.25–1 in.

To test the hypothesis that loss of weight depends directly on heat transfer, the initial, constant, rate of weight loss was plotted against (1) the gas temperature, for constant Reynolds number and particle diameter; (2) the square root of the Reynolds number, for constant particle diameter and gas temperature; (3) the particle diameter, for constant gas temperature and Reynolds number.

Straight lines were obtained in each case, an indication that the initial rate of weight loss is determined by heat transfer.

Most of those who have studied the kinetics of isothermal coal decomposition have suggested that chemical reactions are rate-controlling. Some of the "activation energies" quoted, however, 2–15 kcal/mole, appear to be too low to refer to the breaking of chemical bonds. Higher values have been obtained but they have come mainly from the analysis of weight losses measured only after the loss of most of the volatile material. Peters<sup>4</sup>, however, suggested that the rate of weight loss is controlled by the rate of a physical process. He also noted the similarity between weight loss curves for coal decomposition and the drying of granular material.

To summarize, it is suggested that the controlling factor in the initial stages of isothermal decomposition of bituminous coal particles is the rate at which heat can be transferred to the particles. This heat is absorbed during endothermic degradation reactions and in the removal of liquid and gaseous products.

The rate of weight loss is given by the equation

$$\frac{dW}{dt} = \frac{hA(T_g - T_s)}{\lambda}$$

where  $\lambda$  is 1,700 cal/g and  $T_s$  is 400° C for the coal used.

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## CHEMISTRY

### Thermoluminescence from Recombination of Organic Ions

THE trapping of positive and negative ions in  $\gamma$ -irradiated organic glasses at 77° K has been extensively studied by Hamill *et al.*<sup>1</sup> The products of the recombination of ions are of considerable interest in the elucidation of reaction mechanisms in radiation chemistry. We have shown previously<sup>2</sup> that ion recombination in solutions of naphthalene in a hydrocarbon glass (2 parts methyl-cyclohexane : 3 parts isopentane) leads to luminescence on warming—thermoluminescence (TL)—or on stimulation by infra-red radiation (IRSE). This work has been extended and we report here some new results.

A more careful estimate of the thermoluminescence yield leads to a  $G$  value for emitted quanta of the order of magnitude of 0.1: our previous estimate<sup>2</sup> of 10<sup>-6</sup> (based on the manufacturer's data for the photomultiplier sensitivity) was in error—the result partly of a faulty photomultiplier circuit and partly of a numerical mistake in the calculations: the new value depends on a comparison of the intensity of thermoluminescent emission with a standard scintillation solution containing carbon-14 (ref. 3). The  $G$  value for trapped ions is about 1: because the luminescence yield of excited naphthalene is low<sup>4</sup>, the majority of the recombining ions probably produce excited states. Luminescence is therefore a useful tool for studying ion recombination in this system.

Further study of the emission spectrum has shown that in addition to naphthalene fluorescence and phosphorescence, emission from the excimer<sup>5</sup> ( $C_{10}H_8$ )<sub>2</sub><sup>+</sup> also occurs. This band was at first thought to be caused by the solvent, but the band is narrower than the emission spectrum of the impure solvent and its intensity increases rapidly with increasing concentration (maximum used was 10<sup>-2</sup> molar). The excimer band is stronger in TL than in IRSE; this probably accounts for the apparent difference in phosphorescence/fluorescence ratio described previously<sup>2</sup> as the filter used to measure fluorescence intensities also transmitted the excimer band.

In addition to  $C_{10}H_8^-$  and the  $C_{10}H_8$  radical, we have now detected  $C_{10}H_8^+$  (ref. 6) in the visible absorption spectrum after irradiation. At low solute concentrations (for example, 10<sup>-4</sup> molar) in hard glasses, trapped electrons can be detected by their infra-red absorption<sup>1,2,7</sup>, but at 10<sup>-2</sup> molar concentration the only negative species observed was  $C_{10}H_8^-$ ; therefore it seems reasonable to suppose that luminescence results from recombination of  $C_{10}H_8^+$  and  $C_{10}H_8^-$  which diffuse together on warming the solvent. Because emission is observed both from excimers and single molecules, it is possible that the negative ion dissociates into  $C_{10}H_8$  and an electron during the diffusion process (perhaps as a result of the field of the positive ion). The previously suggested reaction between  $C_{10}H_8^-$  and a solvent cation, however, cannot yet be ruled out. The concentration dependence<sup>2</sup> of the yield of TL remains puzzling.

Johnson and Albrecht<sup>8</sup> have recently shown that  $CO_2^-$

may play an important part in the photolysis of similar glassy solutions because carbon dioxide is not removed by the usual freeze-pump-thaw degassing procedure. Experiments with added carbon dioxide and with solutions degassed by bubbling pure nitrogen through them show that our previous results were not affected by the presence of carbon dioxide: naphthalene competes efficiently for the electrons. Addition of large amounts of carbon dioxide (several hundred mm of mercury), however, removes the  $C_{10}H_8^-$  absorption leaving  $C_{10}H_8^+$ : TL is somewhat weakened and consists of phosphorescence alone. This suggests that recombination of  $C_{10}H_8^+$  with  $CO_2^-$  can lead to excitation, but there is insufficient energy available to produce the excited singlet state. The singlet and triplet excitation energies are 3.9 and 2.6 eV. The electron affinity of carbon dioxide (or, rather, the vertical ionization potential of the ion) has been estimated to be between 2.3 and 2.9 eV<sup>9</sup>. The ionization potential of  $C_{10}H_8$  is 8.1 eV in the gas phase<sup>9</sup>: it may be 1–2 eV less in solution. This leaves 4.2–5.8 eV for recombination, which may be further reduced if coulombic energy is lost in collisions during the approach of the ions.

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### Kinetics of Proton-transfer Reactions

THE kinetics of proton-transfer reactions have been studied, using a number of methods, by Eigen *et al.*<sup>1</sup> and by Ertl and Gerischer<sup>2</sup>, and this communication reports the application of yet another technique—pulse radiolysis—to the study of the reaction between  $H_3O^+$  and  $OH^-$ .

Pulse radiolysis of water and aqueous solutions produces transient changes in composition, and the subsequent relaxation of the system can be studied in various ways, for example, by measuring the electrical conductivity. The latter method is, of course, applicable only to reactions involving ionic species. In irradiated water the ionic products are  $H_2O^+$ ,  $H_2O^-$  and  $OH^-$  and the behaviour of the hydrated electron ( $H_2O^-$ ) has been extensively studied by absorption spectroscopy<sup>3</sup>. Conductimetry, however, can be applied to all these ions and thus, in conjunction with pulse radiolysis, can be used to study fast homogeneous reactions involving  $H_3O^+$ . The overall effect is the sudden production of these three ionic species (along with uncharged radicals) and the relaxation of the  $H_3O^+$  concentration can be followed under appropriate conditions.

In dilute ammonia the hydrated electron is relatively long lived, and if the concentration of  $OH^-$  is substantially larger than those of the  $H_3O^+$  and  $OH^-$  produced by

radiation, the observed decay in conductivity can be ascribed to the pseudo-first order reaction of  $\text{H}_3\text{O}^+$  with  $\text{OH}^-$ . Pure water and dilute aqueous ammonia were accordingly irradiated with 2.5  $\mu\text{sec}$  pulses of 4 MeV electrons from the linear accelerator at Wantage Radiation Laboratories, and the change in electrical conductivity with time was recorded. Doubly distilled water was used. In the second stage mildly oxidizing conditions were maintained in the still pot, carbon dioxide was rigorously excluded and a stream of purified argon was used to purge carbon dioxide from the system. The distillate was condensed in a silica condenser, collected in a silica flask saturated with hydrogen and blown through the conductivity cell with hydrogen under pressure. 0.05 molar ammonium hydroxide was added to bring the final concentration of  $\text{OH}^-$  to  $1.5 \times 10^{-6}$  molar. As produced, the water had a specific conductivity of about 1.3 times the value computed for pure water and the value was only slightly higher in use. Adequate purity in the radiation chemical sense was inferred from the observation that irradiation had no long term effect on the conductivity. Indeed, conductivity changes due to successive electron pulses  $\sim 3$  msec apart were identical within the limits of reproducibility of the accelerator. Attempts to clean up the water by pre-irradiation produced no change in the conductivity waveform.

The conductivity cell, made of 'Pyrex' and platinum, had three electrodes of 0.001 in. platinum and a window of 0.02 in. 'Pyrex' all perpendicular to the axis of the electron beam (Fig. 1a). The electrodes were 2.5 mm apart, the first being close up against the window, and the electron beam was 1 cm in diameter at the window. The change in conductivity was measured by applying a pulse of about 800 V to the outer two electrodes and then comparing the current received by the central electrode with that induced in a circuit simulating the cell. The difference signal due to the change in conductivity was displayed on a Hewlett-Packard oscilloscope.

A typical oscillogram is shown in Fig. 1b. It can be seen that long term effects after the initial decay due to the loss of  $\text{H}_3\text{O}^+$  and  $\text{OH}^-$  are negligible. The fact that the conductivity increase has disappeared after 60  $\mu\text{sec}$  when hydrated electrons are still present is due to the almost identical equivalent conductivity of  $\text{H}_2\text{O}^-$  and  $\text{OH}^-$  (ref. 4). This means that replacement of  $\text{OH}^-$  ions present before the pulse by  $\text{H}_2\text{O}^-$  after the initial decay produces no change in the conductivity. The plot of log conductivity change against time is shown in Fig. 1c (taken from the oscillogram in Fig. 1b). A straight line is obtained indicating pseudo-first order kinetics. The value of the

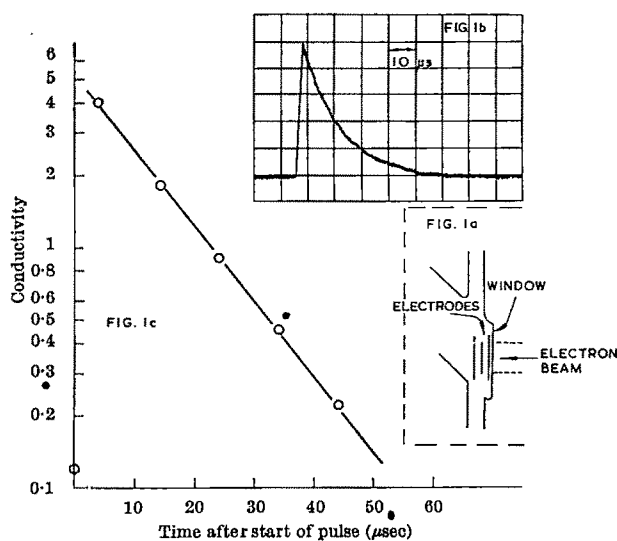


Fig. 1.

Table 1

$\text{OH}^-$ (moles/l.) $0.93 \times 10^{-4}$	$\text{H}_3\text{O}^+$ (moles/l.) $0.94 \times 10^{-7}$	$t$ ( $\mu\text{sec}$ ) 15	$k\text{H}_3\text{O}^+ + \text{OH}^-$ (moles $^{-1}$ sec $^{-1}$ ) $7.2 \times 10^{10}$
1.03	$0.94 \times 10^{-7}$	13.5	7.5
1.03	$1.7 \times 10^{-7}$	13.5	7.2
2.82	$1.7 \times 10^{-7}$	4.8	7.4
3.75	$1.7 \times 10^{-7}$	3.8	7.0

Mean 7.3

For these experiments the temperature was  $15^\circ \pm 1^\circ \text{C}$  and at this temperature Ertl and Gerischer obtain  $k = 1.16 \times 10^{11}$  moles $^{-1}$  sec $^{-1}$ .

rate constant obtained in this way is listed in Table 1 along with other values at different concentrations and compared with the value given by Ertl and Gerischer.

It is clear that the value obtained in these pulse radiolysis experiments is appreciably lower than that from other methods and it is pertinent to examine possible sources of error, although it seems unlikely that a linear plot (Fig. 1c) would be obtained if the observed decay were due to a combination of processes. In some cases curvature was observed at longer times but could be attributed to much slower processes (due presumably to traces of oxygen) and allowed for in the calculation of the rate constant.

Second order reactions would contribute little to the observed decay as all the radiation-produced species would be at the same or at lower concentrations than  $\text{H}_3\text{O}^+$  as listed in Table 1 and calculated from the conductivity peak in pure water. As the  $\text{OH}^-$  concentration falls due to reaction with  $\text{H}_3\text{O}^+$ , more is produced from the undissociated ammonium hydroxide, but though the relaxation time<sup>6</sup> is smaller than the time constant of the  $\text{H}_3\text{O}^+ - \text{OH}^-$  reaction, the amount produced would be too small to make an appreciable difference to the observed decay.

The applied field of about 2 kV/cm was too low to cause field dissociation of water or ammonium hydroxide. The ions are not produced uniformly, but it is difficult to see how a slightly uneven distribution could give too low a value of  $k$ .

At the moment, then, it does not seem possible to reconcile the results reported here with those obtained using other techniques. Further work is under way to improve the signal to noise ratio and to study other systems.

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## Glow Phenomenon of Chromium Oxide

CHROMIUM oxide is used extensively as a catalyst, both as the pure oxide and as an essential component of various multicomponent systems. The unsupported chromium oxide gel usually undergoes a strongly exothermic transformation, or "glow phenomenon", when heated in air at a temperature of about  $400^\circ \text{C}$ . The evolution of heat is accompanied by the development of the crystalline phase of  $\alpha$ -chromium oxide and a diminution in catalytic activity<sup>1,2</sup>; the existence of the glow phenomenon therefore imposes severe limitations on the use of the unsupported oxide as a catalyst.

The reason why crystallization of the hexagonal form occurs at the relatively low temperature of  $400^\circ \text{C}$  is still not clear. Comparison with aluminium oxide suggests that a temperature of over  $1,000^\circ \text{C}$  would be required before drastic crystallization of the corundum structure occurs, and it would appear that there is a special mechan-

ism for chromium oxide. According to Burwell *et al.*<sup>3</sup>, if a chromium oxide gel is heated cautiously the glow phenomenon can be avoided, and once safely taken to a temperature greater than 400° C the gel is then more stable than the material dried at a lower temperature. Differential thermal analysis was used, to show that a chromium oxide gel heated in an atmosphere of nitrogen fails to exhibit an exothermic peak up to a temperature of 600° C, but that the exothermic reaction occurs if air is admitted to the sample at this temperature<sup>4,5</sup>. It was concluded that the crystallization of chromium oxide is facilitated by an oxidizing atmosphere. It has been established<sup>6</sup> that chromium (IV) is formed (at least at the surface) on heat treatment of a chromium oxide gel, although no adequate explanation has been given for the part played by the higher oxidation state in connexion with the glow phenomenon.

We have investigated changes in the bulk and surface properties of various chromium oxide gels in an attempt to establish the conditions in which the glow phenomenon can be prevented, and to elucidate the mechanism of the process. It is now evident that the treatment advocated by Burwell is inadequate unless certain other conditions are fulfilled.

The differential thermal analysis curve in Fig. 1 was determined on a chromium oxide gel which was prepared by heating an aqueous solution of chromium (III) nitrate with urea<sup>3</sup>; the curve was obtained by heating the sample in an oxygen atmosphere at the rate of 5°/min. A broad endothermic peak is intersected by two exothermic peaks, one at about 230° C and the other at about 280° C, and the glow phenomenon is manifested by the sharp exothermic peak at 400° C. No significant change was observed by heating in air in place of oxygen, by using a dry or damp atmosphere, or by varying the rate of heating from 2°–10°/min. Heating in an atmosphere of nitrogen, however, did give a different differential thermal analysis curve (Fig. 2); the exothermic peak at 280° C is almost eliminated and the glow phenomenon is delayed until a temperature of about 580° C is reached. Change in the rate of heating again had little effect, and a curve similar to that shown in Fig. 2 was also obtained *in vacuo*. Results of differential thermal analysis on other chromium oxide gels were broadly similar to those shown in Figs. 1 and 2, although the exact location and the intensity of each peak depended on the preparation of the gel. It is clear that heating in nitrogen or *in vacuo* does not eliminate the glow phenomenon, but does suppress it until the temperature reaches at least 500° C; admission of air or oxygen, on the other hand, causes the glow to occur if the temperature is above 400° C.

In view of these observations, it was of interest to investigate heat treatment in a reducing atmosphere to

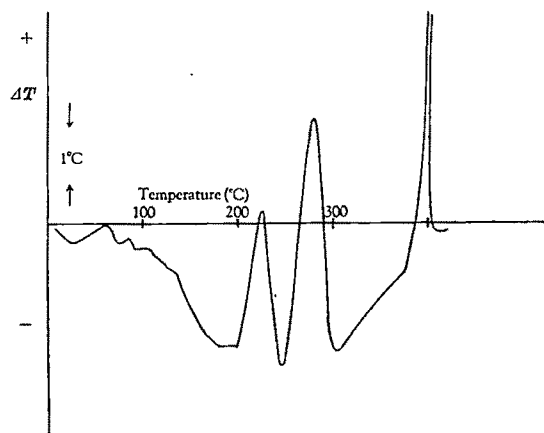


Fig. 1. Differential thermal analysis of chromium oxide gel in oxygen (rate of heating, 5°/min).

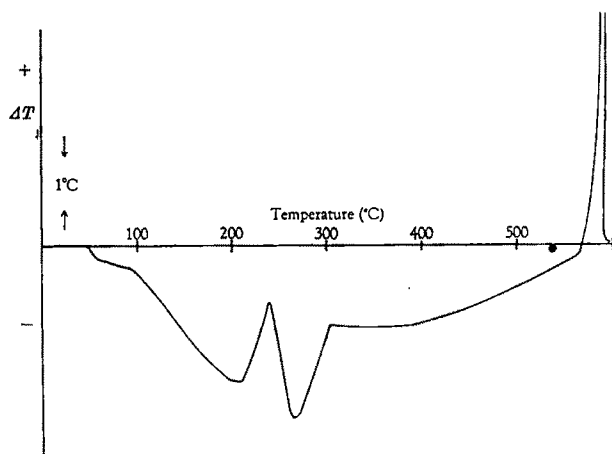


Fig. 2. Differential thermal analysis of chromium oxide gel in nitrogen (rate of heating, 5°/min).

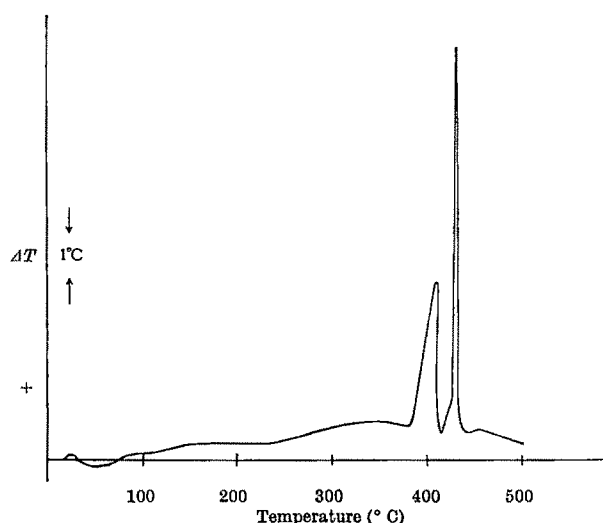


Fig. 3. Differential thermal analysis of chromium oxide gel in air (rate of heating, 5°/min), after initial heat treatment in oxygen for 8.5 h at 300° C.

ascertain whether the glow could be avoided. The phenomenon occurred at about 420° C, even after 3 h in the temperature of 300°–350° C. This result suggests a different mechanism from that applying when the crystallization occurs in an oxidizing atmosphere. We soaked the sample in an oxidizing atmosphere at a temperature lower than 400° C, to permit as much oxidation as possible below the "glow" temperature. It was then possible to prevent the glow phenomenon if the sample was held for 3 h at 350° C, but not if the preliminary heat treatment was maintained for up to 8.5 h at 300° C. Fig. 3 shows that the exothermic transformation at 400° C is resolved in these conditions into two peaks, one at 405° C and the other at 420° C, which indicates a two stage process.

The conversion of chromium oxide gel to macro-crystalline  $\alpha$ -chromium oxide appears to occur most readily (that is, to require the minimum amount of thermal energy) when the solid is subjected to a steadily increasing temperature in an oxidizing atmosphere. In such conditions, the oxidation of Cr (III) to Cr (VI) (and possibly also to one or both of the intermediate oxidation states) must promote the glow phenomenon. Radiochemical exchange experiments suggest that surface oxidation probably takes place at temperatures below 300° C, but there is clearly an additional process which probably involves penetration of oxygen through the outer layers of the solid. This oxidation process occurs fairly slowly at 350° C and rapidly at 400° C, and the difference in rate

is perhaps the most important factor in the control of the glow phenomenon. It appears that the slow oxidation at 350° C provides insufficient localized heating to overcome the energy barrier associated with the reorganization of the solid structure to the lattice of the  $\alpha$ -chromium oxide, whereas the more rapid release of heat at 400° C is sufficient to initiate the crystallization process, which in turn liberates more heat and results in an overall reduction. In accordance with these ideas, the glow phenomenon when it takes place in an oxidizing atmosphere is made up of two stages, which usually overlap giving rise to a large and continuous release of energy. When the secondary oxidation is reduced in extent or in rate, however, the two stages may be separated (Fig. 3).

It is impossible to state the proportion of the chromium oxide system which is converted to the higher oxidation state, but probably the catalytic activity (at least for certain reactions), the black appearance and the semiconductor properties all depend on the presence of two or more oxidation states in a defect structure. Prevention of the glow phenomenon is, therefore, important, not only because it results in crystal growth and loss of specific surface area<sup>3,7</sup> (recent work indicates that the rate of loss of surface area is not especially high at 400° C), but essentially because the overall reduction to  $\alpha$ -chromium oxide alters profoundly the electronic properties of the gel.

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### Recovery of Iodide- $I^{131}$ from Aqueous Solutions of pH 5

I HAVE found an unexpected dependence on the amount of carrier iodide<sup>1,2</sup> of the recovery of radioactive iodide by solvent extraction of its aqueous solutions to which sodium nitrite has been added. The percentage recovery of the radioactive iodide by the solvent is inversely proportional to the amount of iodide in solution if the pH is between 4 and 7. The relationship is linear for the range of concentrations of iodide which were tested, 0.01  $\mu$ g to 0.1  $\mu$ g, and the coefficient of variation of experimental results at the mid-point of the range was  $\pm 4$  per cent.

The procedure used was initiated by the addition of 1.0 ml. of a normal sodium nitrite and 4.0 ml. of sodium acetate and acetic acid buffer, pH 5.0, to an aqueous solution containing 10  $\mu$ c. of iodide- $I^{131}$  and a known amount of iodide as potassium iodide; the final aqueous volume was 14 ml. Solvent extraction was then carried out using 4.0 ml. of benzene and the radioactivity in this extract was compared with that of the initial radioactive iodide solution.

The result is given by this relationship:

reciprocal of percentage recovery of  $I^{131}$  =  $S$  per cent<sup>-1</sup>  $\mu$ g<sup>-1</sup> ( $I$  -  $\mu$ g) +  $K$  per cent<sup>-1</sup>

$S$ , the sensitivity, varies from 0 per cent<sup>-1</sup>  $\mu$ g<sup>-1</sup> at pH 4 to 16 per cent<sup>-1</sup>  $\mu$ g<sup>-1</sup> at pH 5, while at pH 7, although the sensitivity is high, the recovery was too low for accurate measurement. The value of  $K$  at pH 5 is 0.7 per cent<sup>-1</sup>.

When acetate buffer at pH 5 was used, results identical to those given in experiments conducted at the same pH without buffer were obtained.

The radioactive iodide was shown to act in its intended capacity as a radioactive label. The only effect of an increase in the amount of radioactive iodide was an increase in the blank value,  $K$ , in accordance with the known carrier content of the radioactive iodide solution. The change which was observed in the value of the sensitivity,  $S$ , from 9 per cent<sup>-1</sup>  $\mu$ g<sup>-1</sup> for freshly diluted radioactive solution, to 16 per cent<sup>-1</sup>  $\mu$ g<sup>-1</sup> for solution which had been diluted for 21 days, implies the degradation of the radioactive iodide into other species which do not take part in the reaction. The radioactive iodide solution was prepared in 0.02 normal sodium hydroxide by dilution of the commercially supplied solution which contains sodium thiosulphate as a stabilizing agent.

The function of the benzene was shown to be that of an extracting solvent and not that of an active reagent<sup>3</sup>. Other solvents were tried, but absorption on glassware decreased the linearity and accuracy of the results. Reproducible results could only be obtained by a strict control of the time sequence of operations. It was also necessary to wash the glassware with "chromic acid" and to follow this with a thorough rinsing with distilled water. The interference of chloride was found to be negligible in one experiment, in which 40 mg of chloride, as sodium chloride, was added to each of the iodide solutions.

I suggest that the basic mechanism of the procedure is the oxidation of iodide by nitrite to elemental iodine. The inverse relationship between the recovery of radioactive iodide and the amount of iodide implies that an isotopic dilution effect occurs and that a constant amount of iodine is extracted. The sensitivity of the procedure and the linearity of the results suggest the possibility of developing an analytical method for the determination of sub-microgram quantities of iodine.

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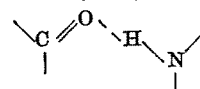
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### Electronic Absorption Bands associated with the Hydrogen Bondings of Thymine and Uracil

IN the course of investigations of the electronic absorption spectra of crystals of DNA bases, we have found that an unusual band appears in addition to the usual  $\pi \rightarrow \pi^*$

transition when the crystal has the

hydrogen bonding. This absorption band is observed in thymine monohydrate, thymine, uracil and 5-ethyl 6-methyl uracil crystals; however, it is not found in calcium thymidylate and barium uridylate<sup>1</sup>. In the former class of crystals, hydrogen bondings of the type



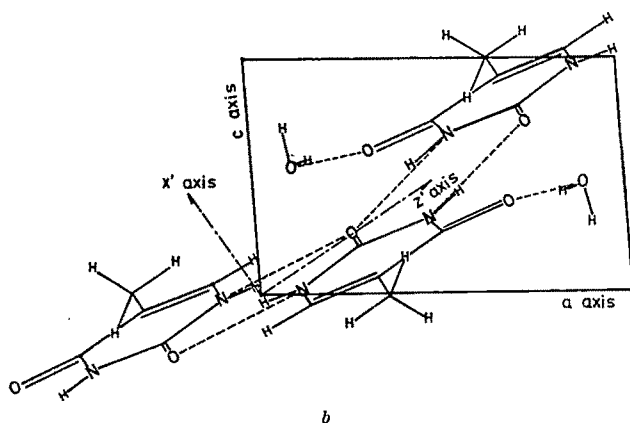
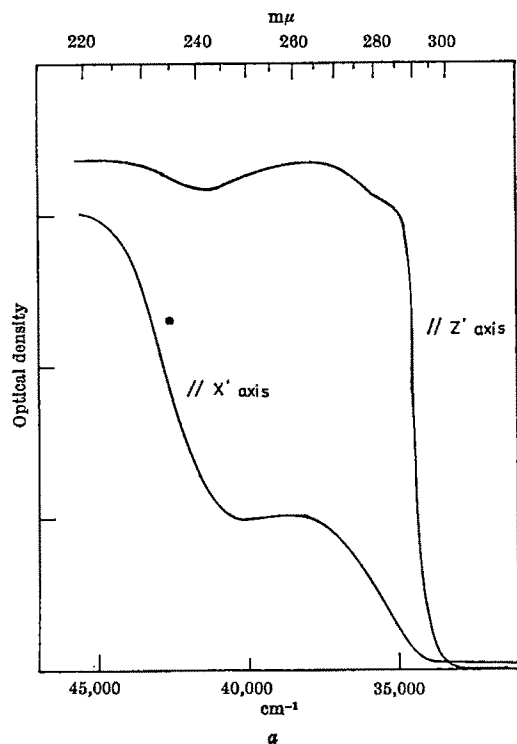
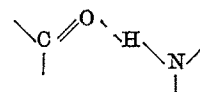


Fig. 1. *a*, Crystal absorption spectrum of thymine monohydrate taken with (010) face, and light polarized parallel to the  $Z'$  and  $X'$  axis—the former axis is the slow direction and the latter the fast optic axis. *b*, Projection of thymine molecules on to (010) plane. The optic axes are shown.

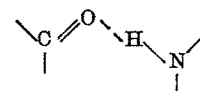
are present, while in the latter such bondings are not present, because the bases are separated from each other. Fig. 1*a* shows a typical absorption spectrum for a crystal of thymine monohydrate; for comparison, Fig. 2 shows the absorption spectrum of calcium thymidylate. Both compounds show similar absorption spectra in solution at about 2640 Å, and corresponding bands occur at about the 2600~2750 Å region in all such crystals. The extra band at 2850 Å in thymine monohydrate crystals is considered to be a new type, because it is observed only in the particular crystalline state. It was possible to determine the direction of the transition moment of the 2650 Å band ( $\pi \rightarrow \pi^*$  transition) from the polarization measurement of all these crystals. It was found to be inclined at about 20° from the  $N_1$  - -  $C_4$  line towards  $N_3$  atom (compare with Fig. 3). This result is nearly in agreement with the earlier results of Stewart and Davidson<sup>2</sup> on 1-methyl thymine, who regarded this angle to be either 14° or -19°.

The new specific band at 2850 Å has a different polarization property, as shown in Fig. 1. The direction of the

transition moment has been found to be in the direction of the hydrogen bonding between a pair of



groups. From a comparison with the 2650 Å band it would seem possible that the molar extinction coefficient of this band might be several thousand. With regard to the assignment of this band, the following points should be considered. (i) The new band can only be detected when the system has hydrogen bonding of the type



between the base pairs, and therefore it should be intimately connected with the hydrogen bonding. Alternative explanations, such as the Davydov-type band splitting in crystals, can be rejected because the band is observed both along the  $b$ -axis and  $a,c$ -axis directions, and this could not occur when the crystalline selection rule is dominant. (ii) The electronic origin of this transition might be not a  $\pi \rightarrow \pi^*$  type, because the parent absorption band is only slightly influenced by the occurrence of the new band. This suggests that the band does not arise from the  $\pi$ -electron interaction between the base pairs, but it is probably related to the non-bonding electron of

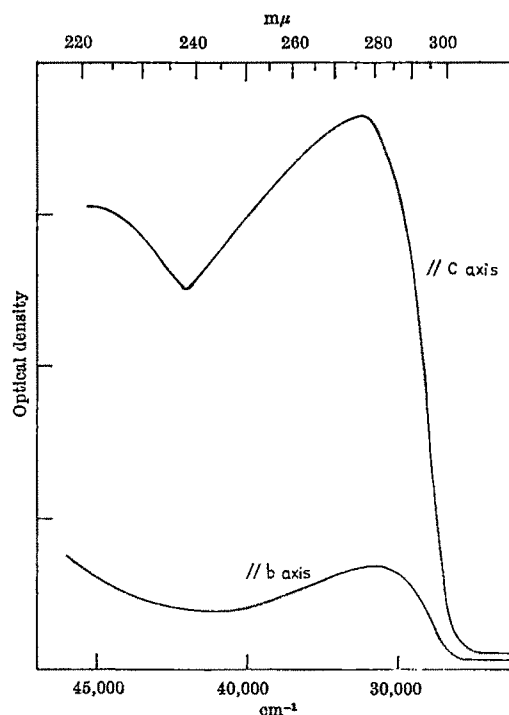


Fig. 2. Crystal absorption spectrum of calcium thymidylate taken with (010) plane and light polarized parallel to the  $b$  and  $c$  axes.

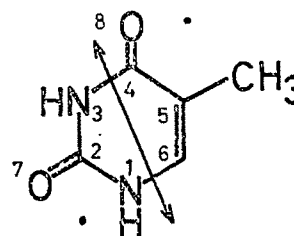
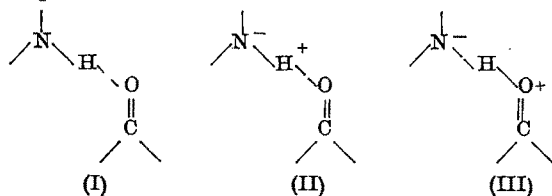


Fig. 3. The direction of transition moment of 2600 Å band ( $\pi \rightarrow \pi^*$  transition).



the oxygen atom and  $\sigma$ -orbital of the N—H bond. (iii) The  $n \rightarrow \pi^*$  transition of the carbonyl group should have a different polarization perpendicular to the ring plane. All these considerations lead us to the conclusion that the band is intimately related to the charge transfer interaction in hydrogen bonding.

The significance of charge transfer force in hydrogen bonding was first pointed out by Nukasawa, Tanaka and Nagakura<sup>3</sup> and further discussions are given by Tsubomura<sup>4</sup> and Coulson and Danielsson<sup>5</sup>. The charge transfer band in hydrogen bonding system was first recognized by Nagakura<sup>6</sup> in hydrogen maleate ion. In the present system the resonance structures of (I), (II) and (III) will be important,



and as a result of the resonance interaction between these structures, both stabilized ground and excited states will be produced. The transition between the ground state and the excited state will be possible when the electric vector of light vibrates along the hydrogen bonding. The location of the new band will depend on both the diagonal energy levels of structures (II) and (III) relative to (I) and the off-diagonal matrix elements. The numerical estimates of these values are rather difficult at the moment; however, the observed value of 2850 Å is certainly reasonable on the basis of the foregoing interpretation. We may therefore designate it an "intermolecular charge transfer absorption of hydrogen bonding" because structure (III) will be predominant in the lowest excited configuration.

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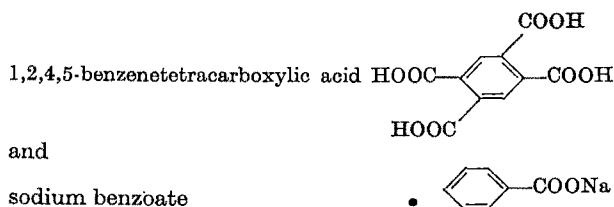
## MOLECULAR STRUCTURE

### New Approach to Structure Studies in Organic Chemistry

We have already reported some applications of electron spectroscopy in the study of chemical binding<sup>1</sup>. We showed that the shifts of the electron lines of an element can be correlated with its chemical state of oxidation. Our method makes use of Auger and photo electrons produced by X-rays and is called ESCA (electron spectroscopy for chemical analysis). Most of the results reported were obtained from the element sulphur, and electron spectra from this element have, for example, been used to solve a specific problem concerning the molecular structure of cystine S-dioxide<sup>2</sup>.

For a more general study of structure problems in organic chemistry by the ESCA method special importance

is naturally attached to the element carbon. This communication reports the first results to show that electron spectra from carbon are actually related to the molecular structure. We have chosen the compounds



both of which were irradiated with aluminium X-radiation. Fig. 1 shows the electron spectra obtained from these compounds. Electron lines from the constituent elements sodium oxygen and carbon are seen in the spectra, and the counting rate is high, although the elements all have low atomic numbers and hence small photoelectric cross sections. The line widths obtained are essentially the inherent widths of atomic levels.

The most striking observation is the fact that carbon gives two well separated lines, the energy difference being 3.8 eV in 1,2,4,5-benzenetetracarboxylic acid and somewhat less in sodium benzoate. The relative intensities of the two lines in the compounds are 4:6 and 1:6 respectively, which means that the low energy line corresponds to the carboxyl carbon atom and the other to the benzene carbon atom. The electron spectrum of carbon thus maps out very clearly the relative number of carbon atoms in the benzene ring and in the carboxyl groups that are attached to the ring. The finding that the carboxyl line has lower energy in the electron spectrum is consistent with the fact that the electronegativity of oxygen is higher than the electronegativities of both carbon and hydrogen. Because oxygen is more electronegative, there will be less negative charge within a certain atomic distance around the carbon nucleus in a carboxyl group than within the same distance around a carbon nucleus in the benzene ring. The electrostatic shielding of the 1s electron is therefore smaller for the carboxyl carbon and the binding energy of 1s electrons becomes larger, as can be seen in the electron spectrum. More detailed information on the molecular structure can be obtained from a

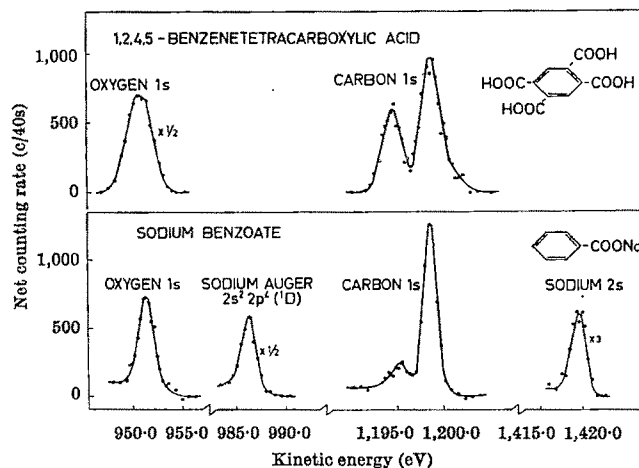


Fig. 1. ESCA spectra of 1,2,4,5-benzenetetracarboxylic acid and sodium benzoate. The electron spectra are obtained using aluminium K $\alpha$  radiation and contain both photo electron and Auger lines from the constituent elements of the compounds. In the upper part of the figure the carbon 1s level is shown to split up into two well separated components, with an energy difference of 3.8 eV. The line to the left corresponds to the 1s level in the carboxyl carbon. The relative intensities of the two lines are 4:6, in agreement with the structural formula. In the lower part of the figure a double carbon line can be seen. The intensity ratio between the two components is in this case 1:6, as expected from the structural formula. A comparison of the line shapes and line strengths of the oxygen lines in the spectra shows that the oxygen line in the carboxyl group is actually composed of two components each with the same intensity. The separation between the components is 1.1 eV.

discussion of the absolute line positions in the electron spectrum and a closer analysis of the benzene carbon line.

Comparison of the oxygen lines from the two compounds reveals a structure effect similar to that observed for carbon. The oxygen line widths in the two compounds are 3.1 eV and 1.8 eV. The broader of these lines can be resolved graphically in two components of equal intensity and half width 1.8 eV, and with a separation of 1.1 eV. The intensity ratios between carbon and oxygen in the two compounds, obtained from the areas under the lines, are consistent with the ratios calculated from the empirical formulae of the compounds.

We have recently observed large chemical structure effects in other organic compounds, in which the 1s electron line from nitrogen is observed to be split in components that differ by as much as 6.2 eV. This is an appreciable shift which is of the same order of magnitude as, for instance, the energy difference between the 2s and 2p atomic sub-shells in nitrogen.

There seems to be good reason to believe that the chemical structure effects in the electron spectrum of carbon and other elements which we have observed can be used in solving many structural problems in organic molecules.

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## X-ray Evidence of Regularly Distributed Lysine in $\alpha$ -Keratin

INVESTIGATIONS of the low angle X-ray diffraction patterns of various  $\alpha$ -keratins have established the presence of structural regularities with an axial pseudo-period of 198 Å (ref. 1). Evidence of chemical reactivity associated with longitudinal periodicity was first obtained by Kratky *et al.*<sup>2</sup>, who noted the appearance of intense low-angle meridional reflexions in human and horse hair which had been treated with nitric acid. Apart from a partial decomposition of the keratin, the main chemical effect of this treatment is nitration of the tyrosine residues.

In Lincoln wool an isomorphous replacement of tyrosine residues by 3,5-diiodo-tyrosine residues<sup>3-5</sup> intensifies the sixth order of the 198 Å repeat. Another axial periodicity of amino-acid residues in  $\alpha$ -keratin has been found<sup>7</sup> by specific staining of cystine in the tips of porcupine quills. A sample in which the more reactive fraction of the cystine was reduced and cross-linked with ethylene dibromide and the least reactive fraction was reduced and reacted with methyl mercuric iodide gave considerable intensification of the third order of 198 Å. These results suggest a regularity of tyrosine and cystine residues in the microfibrils.

In the structural units there is probably a periodic arrangement of lysine residues, for the basic amino-acids tend to occur in clusters rather than evenly distributed

along the peptide chains<sup>8</sup>. It was therefore interesting to see whether there is X-ray evidence for a regular distribution of lysine in the microfibrils. According to Zahn *et al.*<sup>9-11</sup>, the nitrophenylesters of carboxylic acids react with the side-chains of lysine only. This reaction introducing N-acetyl groups includes the possibility of labelling lysine residues specifically. Mohair gives the best X-ray pattern of all keratin fibres examined and so this material was chosen for chemical modification with the *p*-nitrophenyl esters of *o*-iodobenzoic, *p*-iodobenzoic, and 3,4,5-triiodobenzoic acid, which were prepared according to methods described by O'Donnell *et al.*<sup>12</sup> and by Zahn and Schade<sup>13</sup>. Degreased mohair was washed twice with a non-ionic detergent in a buffered solution of pH 5.04 (sodium acetate and acetic acid) at 40° C, and 0.3 g of dry fibres were allowed to react at 40° C with a solution of 0.15 g of ester or a saturated solution of the ester in 25 ml. of dimethylsulphoxide, with gentle stirring, for 15–24 h. The solution was later sucked off and the specimen was washed twice with dimethylsulphoxide followed by ether extraction and isoionic washing.

A partial iodination of mohair, carried out by the method of Richards and Speakman<sup>6</sup>, ensured complete dehydration. This was carried out because the tyrosine content of mohair is, especially in the  $\alpha$ -keratose component, smaller than in wool<sup>14</sup> and in order to see whether the fine structure remains unchanged after iodination treatment. The dry fibres were soaked for 8 days at 25° C in a saturated solution of iodine in absolute ethanol, and excess iodine was removed and the sample was washed isoionically.

The lysine and tyrosine content of mohair, determined by automatic amino-acid analysis, is 249 and 254  $\mu$ moles/g, respectively. A determination of the free side-chains of histidine, serine, cysteine, lysine and tyrosine by the dinitrophenyl technique<sup>15</sup> shows that nearly all of the lysine side-chains of the differently treated samples are acylated by the iodinated benzoic acids, whereas the concentrations of the other free side-chains remain unchanged within the accuracy of the dinitrophenyl analysis. The iodine contents were determined by quantitative elementary analysis and are summarized in Table 1. The labelled lysine of samples I to III was calculated, assuming that the *p*-nitrophenyl esters had reacted with the lysine side-chains only. The calculated quantity of labelled lysine of sample III shows, however, that some of the iodobenzoyl groups may be differently incorporated.

The low-angle X-ray diffraction patterns were obtained on films with nickel-filtered copper K $\alpha$ -radiation in an evacuated Kiessig-type camera<sup>16</sup>. The collimating system of a commercial pinhole camera was modified so that the patterns were absolutely free of pinhole scattering down to Bragg-angles equalling about 100 Å. Molybdenum disulphide powder was used as an internal standard (6.15 Å).

The pattern of the untreated mohair samples shows a series of sharp meridional and near meridional reflexions (Figs. 1a and 2). In comparison with this fibre diagram there is a relative intensification and broadening of the 39 Å-meridional reflexion in the fibre pattern of a mohair sample stained with triiodobenzoyl groups (Figs. 1b and 2). The same effect, although to a smaller extent, is observed in the patterns of the samples I and II, the lysine  $\epsilon$ -amino groups of which are acylated with monoiodobenzoic acids.

Table 1. IODINE CONTENT OF THE DIFFERENTLY TREATED MOHAIR SAMPLES AND ITS CALCULATED AMOUNTS OF LABELLED LYSINE OR TYROSINE

Sample	Treatment	Weight of iodine (per cent)	Amino-acid which reacted, calculated from the iodine content ( $\mu$ moles/g)
I	<i>o</i> -iodobenzoic acid ester	2.15	176 lysine
II	<i>p</i> -iodobenzoic acid ester	2.66	220 lysine
III	3,4,5-triiodobenzoic acid ester	10.53	309 lysine
IV	Iodination in ethanol	1.65	66 tyrosine*

\* Calculated assuming that iodine reacts only with tyrosine yielding 3,5-diiodotyrosine.

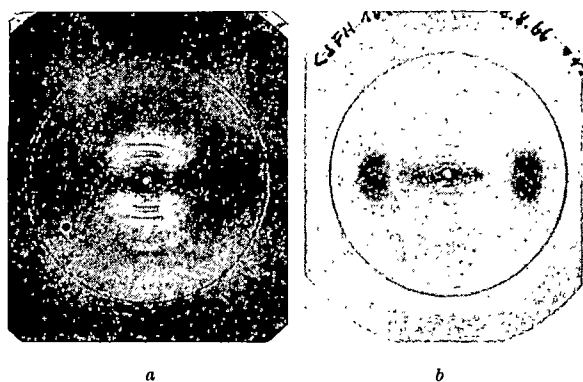


Fig. 1. X-ray patterns of fibres of mohair (axis of fibre vertical). *a*, Untreated; *b*, stained with triiodobenzoyl groups.

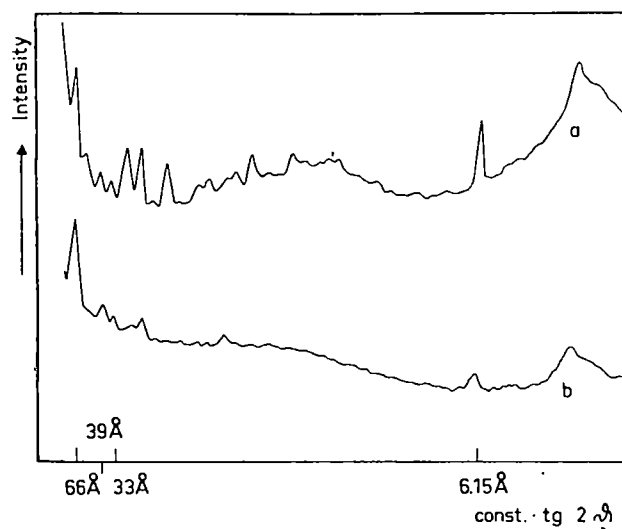


Fig. 2. Curves of X-ray patterns of fibres measured with a meridional photometer. *a*, Untreated mohair; *b*, mohair stained with triiodobenzoyl groups.

Lysine residues, therefore, seem to have an axial periodicity of 39 Å. The reason for the broad extension of this reflexion on its layer line is a statistical distribution of the strong scattering triiodobenzoylic residues which are situated relatively far from the polypeptide backbone. The rather less meridional broadening of this reflexion may have the same cause, but this unequal broadening can be explained by a statistical mobility of the amino-acid side-chains in the axial and lateral direction, which is probably different.

The 66 Å-reflexion is also intensified, and remains the prominent reflexion on the meridian. This is observed also in the patterns of partial iodinated mohair as well as of mohair which has reacted with unlabelled *p*-nitrophenyl esters. This effect, therefore, could depend on an axial repetition of structural sub-units which are more ordered after chemical treatment in a medium which causes swelling. This interpretation seems probable if it is remembered that cysting is located in this axial periodicity<sup>7</sup>, which could be confirmed in the case of mohair, and if it is accepted that the protofibrils are cross-linked with the matrix by cystine.

The fibre pattern of a mohair sample, the tyrosine residues of which are partially iodinated (sample IV), shows no alteration of the basic structure, but besides the well known intensification of the sixth order of the 198 Å repeat at 33 Å there is a somewhat smaller intensification of the ninth order at 22 Å. Contrary to the "lysine reflexion" at 39 Å, the "tyrosine reflexion" at 33 Å is not essentially broadened. This observation supports the

concept that the feature of intensified meridional reflexions depends on the geometry of the incorporated labelled residues. In this connexion it must be remembered that the results of our chemical variations are quasi-isomorphous replacements only.

All diagrams of fibres of mohair show discrete equatorial reflexions at 80 Å, 42 Å, 27 Å and 9.3 Å. The 27 Å reflexion, however, is intensified in patterns of the samples with the lysine content stained. We consider that the lysine residues are distributed on the periphery of the protofibrils. On the other hand, the 42 Å reflexions and the 27 Å reflexions are intensified in the pattern of the samples with lysine stained. If the 42 Å equatorial reflexion corresponds to the order of microfibrillar packing the tyrosine residues are situated on the periphery of the protofibrils as well. The lateral arrangement of the microfibrils is more ordered in the sample stained in the tyrosine (IV) than in the samples stained in the lysine and treated at elevated temperatures.

We have shown that specific staining of lysine is possible. We found an axial periodicity of lysine residues in the structural units of mohair and a distribution of these residues on the periphery of the protofibrils. Results could be reproduced for Lincoln wool but will be discussed elsewhere.

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## BIOPHYSICS

### Enzyme Activity in High Magnetic Fields

THE change of the catalytic enzyme activity under the influence of an external magnetic field was demonstrated for carboxydismutase<sup>1</sup>, for glutamate dehydrogenase<sup>2</sup> and for trypsin<sup>3</sup>. Dorfman<sup>4</sup> was the first to predict these magneto-catalytic effects and more recent theoretical discussions are due to Neurath<sup>5</sup>, Valentinuzzi<sup>6</sup> and Gross<sup>7</sup>. The following summary is based on Dorfman's work.

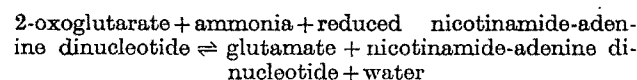
In a non-uniform magnetic field the force acting on a large molecule in solution tends to produce a concentration gradient. The distribution of diamagnetic macromolecules as a function of the distance  $x$  from the point of highest

magnetic field is given by Boltzmann's law  $dN = \text{constant} \exp(-\alpha x dx)$  with  $\alpha = \frac{M\chi_m H dH/dx}{2RT}$ . Here  $\chi$  is the diamagnetic susceptibility/g ( $\sim 10^{-6}$ ),  $M$  the molecular weight of the macromolecule and  $H$  the magnetic field. With  $H = 10^5$  G,  $dH/dx = 10^4$  G cm $^{-1}$  and  $M = 250,000$ , one finds that  $\alpha = 0.005$ , that is, the concentration is practically uniform. For macromolecules of larger molecular weights one can expect noticeable concentration gradients which could influence the velocity of enzyme reactions.

Effects in a uniform field could occur for magnetically anisotropic macromolecules, for example, fibrous protein molecules or nucleic acids made up of anisotropic building blocks. The magnetic field tends to align the molecules; the degree of orientation, or departure from an isotropic distribution of the axes of the molecules, is determined by the Boltzmann factor  $\exp(-\Delta\chi_m H^2/2RT)$ , where  $\Delta\chi_m$  is the anisotropy of the molar susceptibility. Here again the calculation shows that for strongly anisotropic diamagnetic macromolecules of molecular weights of the order of  $10^6$  the degree of alignment should be almost complete in fields of  $\sim 10^5$  G.

Earlier measurements<sup>1</sup> were carried out in a magnetic field of 15 kG. These have now been extended to 80 kG with the help of the high field facilities of the Clarendon Laboratory, Oxford. One of the standard watercooled solenoids of 5 cm inner diameter was used for these experiments.

For the study of the magnetocatalytic effect in L-glutamic dehydrogenase (GDH) the following reaction catalysed by GDH was used:



The enzyme activity is expressed in enzyme-units (E). This is defined as the amount of enzyme which, when dissolved in 1 ml, produces in 100 sec a change of 0.100 in the extinction of light of 3660 Å by a 1 cm layer of reduced nicotinamide-adenine dinucleotide (NADH), at a temperature of 25°C. In the experimental procedure 3 ml. of 0.05 molar triethanolamine buffer (pH = 8.0), 0.045 ml. of 0.01 molar ethylenediaminetetraacetate solution, and amounts ranging between 0.02 and 0.1 ml. of a solution of GDH (about  $10^{-7}$  moles/l.) were introduced into an absorption cell of 1 cm thickness. After adding 0.06 ml. of 0.4 molar Na 2-oxoglutarate solution the extinction was measured at 1–2 min intervals for a period of 5–10 min. The cell was then placed in the field for periods of 17–60 min and after removal from the field the rate of change of the extinction was measured again. Control measurements in zero magnetic field were done simultaneously on another identical sample kept as far as possible at the same temperature. The importance of good temperature control and the need to correct for small temperature variations have already been emphasized<sup>1</sup>.

In the case of catalase the influence of the magnetic field on the activity was determined by the catalytic decomposition of hydrogen peroxide. The catalytic activity is defined as  $\text{Cat.f} = k/c$  (ref. 8), where  $k$  is the reaction velocity constant of the decomposition of hydrogen peroxide (in min $^{-1}$ ) and  $c$  the concentration of the enzyme solution in g/50 ml. The method of Warburg and Krippahl<sup>9</sup> was used to determine  $k$ : the reaction was stopped after a given time by the addition of hydrocyanic acid and the remaining hydrogen peroxide determined spectrophotometrically with vanadic acid.

The experiments were carried out both in a uniform magnetic field (variation of 3 per cent over the sample length of 4 cm) and in a non-uniform field (variation of 30 per cent over 4 cm).

The results for GDH are given in Table 1. The last column shows that, while in a uniform field the decrease in activity fluctuates between about 5 per cent and 12 per

Table 1. EXPERIMENTS WITH GDH

Run No.	H (kG)	T (°C)	t (min)	$\frac{E}{E_0}$	$\Delta E$	$\frac{\Delta E}{E_0}$ (per cent)
1	50 u	23.2	40	8.24	-0.85	-9.4
	0	22.8		9.09		
2	70 u	21.7	17	11.30	-1.59	-12.3
	0	21.7		12.89		
3	60 u	24.5	40	27.65	-3.15	-10.2
	0	24.5		30.80		
4	70 u	25.9	40	48.50	-2.50	-4.9
	0	25.9		51.00		
5	70 n	25.5	42	4.36	-24.87	-85.0
	0	25.5		29.23		
6	70 n	21.5	60	0.93	-11.63	-92.7
	0	21.1		12.56		
7	60 n	20.1	41	39.55	-5.35	-11.9
	0	20.0		44.90		
8	70 n	20.5	35	4.46	-32.95	-88.0
	0	20.5		37.41		
9	78 n	22.0	41	24.00	-5.00	-17.2
	0	22.0		29.00		

H = Highest field over the sample (u = uniform field; n = non-uniform field).

T = Temperature.

t = Duration of the action of the field.

$E$ ,  $E_0$  = Enzyme units with and without field respectively. Both  $E$  and  $E_0$  refer to the temperature of the control experiment without field. The corrections to  $E$  were made with the help of the temperature coefficient measured in ref. 1.

Table 2. EXPERIMENTS WITH CATALASE

Run No.	H (kG)	T (°C)	t (min)	$\frac{10^{-3} \text{ Cat } f}{10^{-3} \text{ Cat } f_0}$	$10^{-3} \Delta \text{ Cat } f$	$\frac{\Delta \text{ Cat } f}{\text{Cat } f_0}$ (%)
1	60 n	21.6	10	18.90	5.00	36.0
	0	21.6		13.90		
2	60 n	22.0	15	13.89	1.89	15.8
	0	22.0		12.00		
3	60 n	22.1	15	13.21	3.95	42.6
	0	22.1		9.26		
4	60 n	21.8	15	8.25	2.82	52.0
	0	21.8		5.43		
5	60 n	22.0	15	6.18	2.03	48.5
	0	22.0		4.15		
6	60 n	21.9	15	5.39	1.53	39.7
	0	21.9		3.86		
7	60 u	21.8	15	8.75	0.73	9.1
	0	21.8		8.02		
8	60 u	22.2	15	5.35	0.25	4.9
	0	22.2		5.10		

cent, in a non-uniform field it reaches very high values. The fact that, as shown by runs Nos. 5, 6 and 8, it is possible to bring the reaction almost to a standstill reversibly by a physical method may lead to the development of new techniques for the study of enzyme reactions.

Table 2 lists the results for catalase. In contrast to the measurements by Haberditzl and Muller in 15 kG (ref. 1), which showed no effect, the present experiments indicate appreciable increases in the activity with field, especially for non-uniform fields.

A detailed discussion of these results will follow later. It may be mentioned, however, that in the case of catalase the unexpected increase in activity with field could be explained by a secondary effect, namely, the accelerated removal of the paramagnetic oxygen from the site of the reaction.

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## Nuclear Magnetic Resonance Spectroscopic Studies of Erythrocyte Membranes

THE nature of molecular interactions in the lipoprotein complexes of biological membranes has been a subject under active enquiry for many years. The classical Davson-Danielli paucimolecular model<sup>1</sup> postulates apolar mutual interaction between the lipid molecules and electrostatic binding of the lipid with the structural protein. Although this model has received support from biophysical studies, especially on myelin<sup>2,3</sup>, there is other experimental evidence which suggests that extensive hydrophobic binding may occur between lipids and proteins, for example, from studies on mitochondria<sup>4,5</sup>, halobacterium halobium<sup>6</sup>, erythrocyte ghosts<sup>7</sup>, plasma membrane fragments of Ehrlich ascites cells (D. F. H. Wallach and P. H. Zahler, personal communication) and chloroplast lamellae<sup>8</sup>.

A number of physical methods have now been used to study the structure of membranes. These include polarization microscopy, electron microscopy, X-ray diffraction, infra-red spectroscopy, ultra-violet optical rotatory dispersion and fluorescence emission spectroscopy. These methods have yielded valuable information regarding membrane thickness, fine structure of lipoprotein layers and conformational states of structural protein of the membrane. Little or no work has been reported on membranes utilizing nuclear magnetic resonance spectroscopy. In a previous communication, Chapman and Penkett<sup>9</sup> demonstrated the usefulness of nuclear magnetic resonance to investigate the interaction of phospholipids such as egg yolk lecithin with cholesterol. In order to examine the feasibility of using high resolution nuclear magnetic resonance spectroscopy as a structural probe for lipoprotein systems, this type of study is now being extended directly to a study of natural membrane systems. Preliminary results obtained with erythrocyte membranes using this spectroscopic technique are reported in this communication.

Haemoglobin-free ghosts were prepared in essential accordance with the method of Dodge *et al.*<sup>10</sup> from 2 week old human erythrocytes preserved in acid-citrate dextrose at 0°–4° C. For nuclear magnetic resonance study, the membrane material was treated as follows. (a) Freshly prepared ghosts derived from 3–5 c.c. packed erythrocytes were equilibrated by ultracentrifugation with deuterated 20 milliosmolar phosphate buffer pH 7.4, and resuspended in 1 ml. of the deuterated buffer. (b) Washed ghosts were desalted by dialysis against distilled and deionized water and freeze-dried. Between thirty and fifty milligrams of dried material was suspended in 1 ml. of either deuterated 20 milliosmolar phosphate buffer pH 7.4 or in deuterium oxide (99.7 per cent) alone. (c) 30–50 mg dried material was suspended in 1 ml. of 1 per cent sodium deoxycholate in 99.7 per cent deuterium oxide.

These materials were examined either as suspensions or as dispersions formed by ultrasonic irradiation. High resolution nuclear magnetic resonance spectra were obtained with a computer averaging technique attachment in a spectrometer at 34° C.

Fig. 1a shows the spectrum of freeze-dried membrane material resuspended in deuterated phosphate buffer pH 7.4. An identical spectrum was obtained with either freshly prepared ghosts in deuterated buffer or freeze-dried material in unbuffered deuterium oxide, and with or without sonication. The reproducibility of this spectrum was checked on three different preparations.

The high resolution signals are expected to originate as a result of the molecular motion of non-protein moieties in the membrane. This is because proteins in general have been shown not to give well defined nuclear magnetic resonance signals<sup>11</sup>.

The spectrum shows distinct signals which we assign to the probable functional groups corresponding to  $N^+(CH_3)_3$  groups of choline at 6.7  $\tau$  and  $N$ -acetyl groups at

7.88  $\tau$  from sialic acid. Sialic acid is considered to be a principal charge determinant of the erythrocyte membrane surface<sup>12</sup>. The identity of the latter signal was tentatively decided after examination of the nuclear resonance spectrum of pure sialic acid. It can be seen that the signals in the region which are characteristic of protons in the  $(CH_2)_n$  groups of alkyl chains near 8.8  $\tau$  are low and not discernible. A possible explanation for this could be that the hydrocarbon chains are restricted in their mobility because of the presence of hydrophobic interaction between sterol and lipid and/or lipid and protein. The sharpness of the signal at 6.65 p.p.m. indicates, however, that at least some of the methyl protons of the choline groups of the membrane lipids are free to move.

Sodium deoxycholate is widely used to solubilize biological membranes and so we have examined the nuclear magnetic resonance spectrum of a membrane treated with this material. The spectrum of freeze-dried material in 1 per cent sodium deoxycholate is shown in Fig. 1b (the nuclear magnetic resonance spectrum of sodium deoxycholate alone is shown as a dotted line). The membrane spectrum shows a fairly narrow signal near 8.8 p.p.m. which we associate with the protons of the hydrocarbon chains of the lipid material. The chains of the lipid apparently have more freedom to move as a result of the treatment. The detailed mechanism by which the deoxycholate brings about this change is uncertain. It is possible that it reduces hydrophobic interaction between the lipid and sterol or lipid and protein in the membrane. The modification of the nuclear resonance signals which arises from sodium deoxycholate in the presence of the membrane may be caused by the formation of a complex.

We have now obtained many other nuclear magnetic resonance spectra of membranes treated in various ways and are continuing to investigate in more detail the

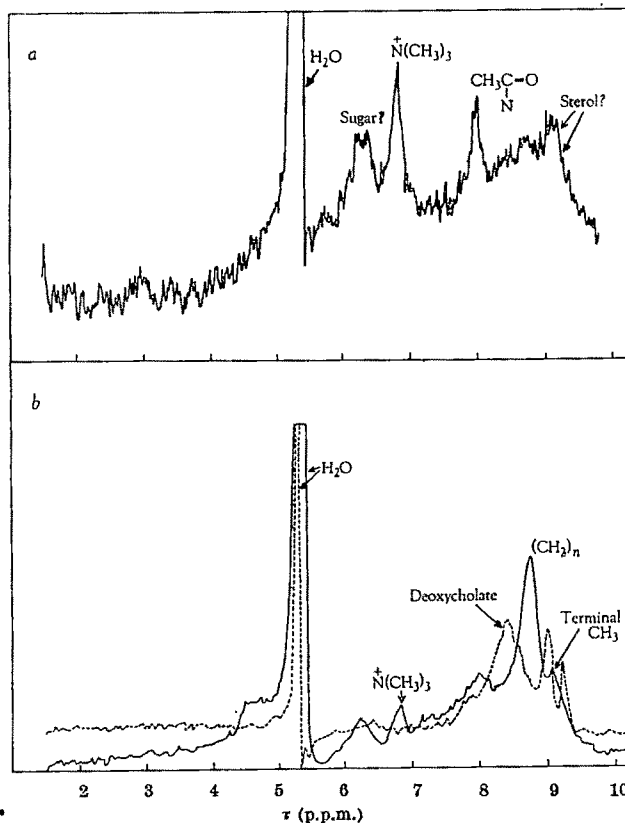


Fig. 1. High resolution proton magnetic resonance spectra (60 Mc/s) with probable assignments. a. Freeze-dried erythrocyte membrane resuspended in deuterated buffer pH 7.4 (62 scans). b. (i) Full line is of freeze-dried erythrocyte membrane in 1 per cent sodium deoxycholate in deuterium oxide 99.7 per cent (65 scans). (ii) Dotted line is of sodium deoxycholate solution alone.

significance of these observations as well as studying the effect of other variables such as temperature, pH, ionic strength, divalent metals and organic solvents.

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## BIOCHEMISTRY

### Characteristics of Adult and Foetal Myoglobin in the Visible Light Spectrum

A MYOGLOBIN which differed from adult myoglobin (MbA) was found in the urine<sup>1</sup> of a 28-year-old woman during an episode of idiopathic rhabdomyolysis with myoglobinuria. The same haem protein was later demonstrated in the patient's skeletal muscle<sup>2</sup> where it comprised the bulk of the benzidine positive protein after haemoglobin and cytochrome *c* had been separated from the myoglobin by column chromatography. This distinct chromoprotein was similar in its solubility in ammonium sulphate, spectroscopic characteristics and on cellulose acetate electrophoresis to foetal skeletal myoglobin (MbF) prepared in the same manner from the psoas muscle of infants stillborn at term. It was concluded that myoglobin, like haemoglobin, can present molecular variations which express themselves as disease syndromes, for example, rhabdomyolysis, under certain conditions. Although these clinical and biochemical findings have been supported by one investigator<sup>3</sup>, other reports do not regard MbF as an entity but rather as an artefact produced by the method used in preparing the myoglobin for analysis. The evidence against MbF has recently been summarized<sup>4</sup>. The present report examines the spectroscopic characteristics of MbA and MbF, adult and foetal haemoglobin (HbA and HbF, respectively) derived from muscle and blood of the human, dog and pig. The methods were designed to study the various pigments under optimum and standard conditions to minimize the effects of changing pH, salt concentrations, temperature, state of haem oxidation, crystallizations and other manipulations.

Cardiac and skeletal muscle was obtained fresh from adult and newly born dogs and pigs. The samples were quickly frozen and then ground in a meat grinder, titrated with small portions of cold distilled water and spun at 9,000g for 30 min to remove the particulate matter. Instead of grinding them before centrifugation, some of the samples were homogenized in a blender. The supernatant muscle liquor was then treated in one of two ways—either immediately freeze-dried for later analysis or added directly in 1–2 ml. amounts to a 2.5- by 30-cm 'Sephadex G-50' fine (200–400 mesh) gel column<sup>5</sup> which was developed in 0.05 molar phosphate buffer (pH 7.40)

with 0.05 molar sodium chloride. The spectroscopic results were the same regardless of the method at this step, that is, either freeze drying first or direct application to the 'Sephadex' column. The oxy forms were slowly converted, however, to the corresponding met compound in the freeze-dried state. Animal HbA and HbF were obtained from appropriate heparinized whole blood samples by aspirating the plasma and buffy coat and washing the residual cells three times with isotonic saline. The red cells were then haemolysed with two volumes of distilled water and the ghosts and other particles removed by centrifugation and filtration through acid-washed glass wool. The red cell liquor was applied to the 'Sephadex' column in 0.5–1.0 ml. amounts and similarly eluted with the developing solution. The total time taken for the elution was less than 2 h. Human muscle samples—both adult and foetal—were obtained *post mortem* and were treated in the same manner. Human HbA was prepared fresh from the blood of normal adult volunteers whereas HbF was prepared from foetal cord blood of healthy newborn infants. The 'Sephadex' column effectively separated myoglobin and haemoglobin either from the muscle homogenates or when haemoglobin and myoglobin were deliberately mixed together. All isolation procedures were carried out at 6° C and a Carey 15 automatic recording spectrophotometer was used for the final spectral readings. It should be noted that there were no differences in the rates of migration in the columns or effective separation when the eluted samples were applied directly to CMC columns<sup>6</sup>. The muscle homogenates of all adult species, including both skeletal and heart muscle, clearly separated on the 'Sephadex' column into a "fast" moving haemoglobin and a slower myoglobin band. Foetal muscle liquor, except that made from the heart muscle, was characteristically very opalescent and less coloured than the adult liquor or the haemoglobin preparations. This contributed to the high background in the visible light spectrum characteristic of skeletal MbF. Attempts to remove the opalescent colloidal-like background from the visible pigment by the use of 'Millipore' filters of graded pore size and centrifugal separation were not successful. It was thought that the opalescent quality was inherent to and characteristic of the foetal haem pigment. When the foetal skeletal muscle supernate was applied to the columns it yielded only a single band similar in this respect to the haemoglobins. The foetal pigment tended to show more column spread, although it remained homogeneously pink and opalescent in the oxy form. Two bands were formed on the column when MbF from pig heart was applied, but the distinction was not always present when MbF from human hearts was similarly chromatographed.

Table 1. SPECTROPHOTOMETRIC MAXIMA\* OF ADULT AND FOETAL HAEM PROTEINS

	Source	Oxy		Met-nitrite		Reduction-dithionite	
		$\alpha$	$\beta$				
Adult and foetal haemoglobin	Human	576	540	540	630	576	544
	Dog	577	540	542	630	578	540
	Pig	576	540	540	628	574	542
Adult myoglobin from skeletal muscle	Human	583	545	502	630	585	543
	Dog	582	543	500	628	583	544
	Pig	582	543	504	632	584	543
Foetal myoglobin from skeletal muscle	Human	578	540	540	630	578	542
	Dog	578	541	538	628	576	541
	Pig	577	540	538	630	577	542

\* Each sample was adjusted in the oxy form to read 0.200 optical density units at 580 m $\mu$ .

The results of all the spectroscopic maxima for the skeletal muscle are summarized in Table 1. Oxy HbA and HbF had the same maxima ( $\alpha$  576 m $\mu$  and  $\beta$  540 m $\mu$ ) and no species differences were noted. Oxy MbA had maxima at 583 m $\mu$  and 543 m $\mu$ . The spectrum of oxy MbF from skeletal muscle resembled that of oxy HbA and F in the human, dog and pig. In contrast, foetal heart muscle myoglobin from the pig had the same column separation pattern as the adult skeletal and heart muscle preparations and spectroscopically the second or slower



elution band had the characteristic peaks of MbA in the oxy form. As indicated, this finding was less consistent in the human foetal heart. Some specimens behaved exactly as those described for the foetal pig and others failed to separate into the two moieties. In the latter instance the single band had a spectral pattern indistinguishable from the haemoglobins in the oxy form. Conversion of the myoglobin liquors and the haemoglobins to the corresponding met forms by oxidation with small constant amounts of crystalline sodium nitrite yielded a maximum at 500 m $\mu$  only with MbA and maxima of 630 m $\mu$  for MbA, MbF, HbA and HbF in all species regardless of whether the muscle was cardiac or skeletal. The haemoglobins retained minimal peaks at 540 and occasionally a small peak at 576 m $\mu$  and these maxima were more resistant to met conversion than the  $\alpha$  and  $\beta$  peaks of MbA which completely disappeared. Thus only MbA exhibited a characteristic plateau between 560 m $\mu$  and 610 m $\mu$  (Fig. 1). Foetal skeletal muscle myoglobin resembled HbA and HbF in both the oxy and met forms, and thus the only distinct spectroscopic pattern was that of MbA. Foetal cardiac Mb, when indistinguishable from MbA (see above), was not different in the oxy spectral maxima but always differed in the met type by not developing either the 560–610 m $\mu$  plateau or the maximum at 500 m $\mu$ . Reduction with dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) of either auto-oxidized or sodium nitrite oxidized haemoglobin or myoglobin of each type and from each species returned the met spectra toward their previous oxy pattern. This proved to be a valuable analytical tool enabling the  $\alpha$  and  $\beta$  maxima to be analysed to identify properly the origin of the haem protein. The spectra of met HbA and F and skeletal muscle MbF after reduction with dithionite returned to about 576 m $\mu$  and 542 m $\mu$ , and MbA and cardiac myoglobin from the foetuses to 580 m $\mu$  and 545 m $\mu$ . Exceptions were noted with regard to the cardiac myoglobin derived from foetal human hearts where the oxy pattern was initially indistinguishable from that of the haemoglobins. Dithionite in this instance also returned the spectroscopic peaks to their original pattern. The contour of peaks after chemical reduction was somewhat less sharp in terms of the maxima; however, the  $\alpha$  peak clearly distinguished adult myoglobin from the haemoglobins and foetal skeletal myoglobin. The 630 m $\mu$  peak after conversion of either myoglobins or haemoglobins also disappeared after dithionite reduction. The Soret bands were not significantly different between haem protein types either in the oxy or met state or after dithionite reduction. Cytochrome *c* and catalase which have molecular weights similar to myoglobin could potentially on the basis of column separation alone interfere with the spectral analyses.

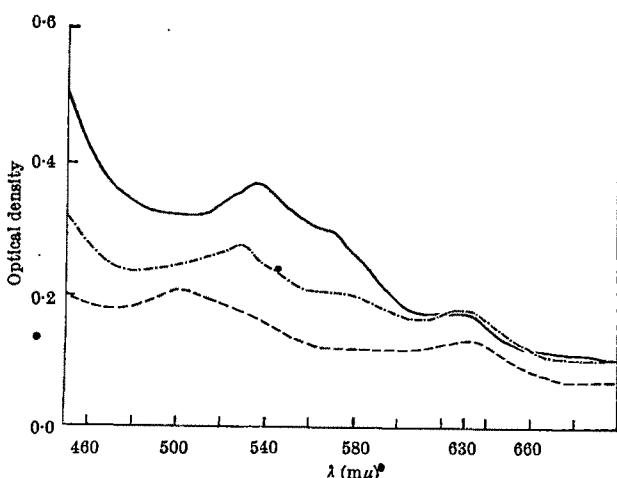


Fig. 1. Spectroscopic maxima of neutral (pH 7.4) haem proteins. The wavelengths in m $\mu$  are indicated on the abscissa and the optical density on the ordinate. —, Foetal methaemoglobin; ---, foetal met-myoglobin; - · - ·, adult met-myoglobin.

Under the conditions described, however, ferro- and ferri-cytochrome *c* have maxima at 550 m $\mu$  and 522 m $\mu$ , and 565 m $\mu$  and 530 m $\mu$ , respectively, while catalase at the neutral pH has a characteristic maximum at 624 m $\mu$ . In addition, dithionite reduction produces maxima for catalase at 594 m $\mu$  and 596 m $\mu$ . These peaks were not observed in our experiments.

These observations emphasize a further difference between MbA and MbF in skeletal muscle and support the impression<sup>2,7</sup> that MbF more closely resembles the haemoglobins than it does MbA. That MbF derived from cardiac muscle behaved in some experiments like MbA may indicate that the characteristics of adult myoglobin evolve first in heart muscle which more closely approximates the functional or physical activities of adult skeletal muscle *in utero* than does foetal skeletal muscle. The spectral evolution of oxy MbF begins in the  $\alpha$  and  $\beta$  peaks with the slight shift of the maxima toward the longer wavelengths. The last characteristics of MbA to appear are found in the met state with an increase in light transmittance between 5600 and 6100 Å and the appearance of the peak at 5000 Å. These changes are coincident with the progressive decrease in the quantity of rapidly migrating haem protein on electrophoresis<sup>1-3</sup>.

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### Reversible Inactivation of *Helix (pomatia)* Agglutinin by 2-Mercapto-ethanol

THAT certain snails contain an agglutinin with human anti-A specificity was first observed by Prokop *et al.*<sup>1</sup> and then independently by Boyd and Brown<sup>2</sup>. Since then, however, we have discovered that this agglutinin (which can be obtained from the protein gland of the sexual organ of *Helix pomatia*) also reacts with human O and B cells treated with neuraminidase, with the respective erythrocyte mucoids, and with many animal cells, especially after treatment with proteolytic enzymes or neuraminidase<sup>3</sup>. We have also found that some glycoproteins and glycolipids react with this agglutinin<sup>4</sup>, and have come to the conclusion that for the reaction with the *Helix* agglutinin only the presence of terminal non-reducing bound *N*-acetyl-D-galactosamine, often blocked by neuraminic acid, is necessary irrespective of whether it is in  $\alpha$ - or  $\beta$ -linkage.

In order to obtain further information on the nature of this agglutinin, we treated it with 2-mercapto-ethanol (1 molar, 0.5 molar and 0.1 molar) at room temperature. This procedure led to a complete inactivation of the agglutination power, even when the clumped cells were exposed to this reducing agent. Dialysis or standing the test-tubes overnight resulted in a restoration of the agglutinating power, obviously as a result of re-oxidation. These experiments suggest that the structures (shown in Fig. 1a and b) may be taken into consideration for the

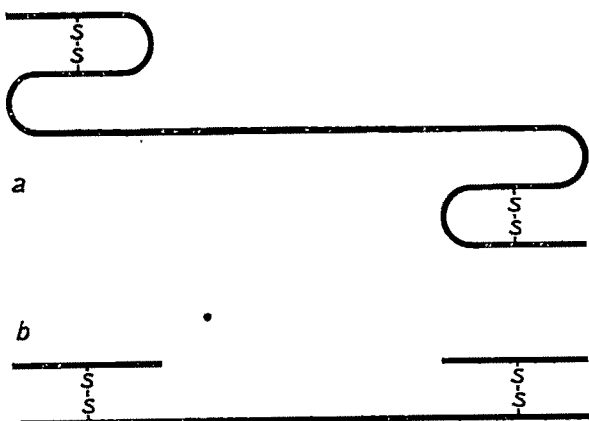


Fig. 1. Proposed structural models for the agglutinin from *Helix pomatia*. Protein chain(s).

*Helix* agglutinin, provided that the mode of action of 2-mercapto-ethanol causes a reversible reduction of the disulphide bonds which are involved in building up the combining site of the agglutinin.

By "reversed" immunoelectrophoresis using secreted A substance and neuraminidase-treated human erythrocyte mucoids as indicator, we were able to demonstrate the presence of *Helix* agglutinin in the region of the gamma-globulins. It is possible, therefore, that it plays some part in establishing an "immune" barrier towards the micro-organism; moreover, we have seen bacterial agglutination by this agglutinin. Preliminary experiments have revealed that the *Helix* agglutinin is remarkably resistant towards the destructive action of proteolytic enzymes.

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### Restoration of Enzyme Activity of Heat-denatured Acetylcholinesterase by Antibodies to the Native Enzyme

THE enzyme acetylcholinesterase (AChE), which catalyses the hydrolysis of acetylcholine to choline and acetic acid, has been the subject of a great deal of interest not only in connexion with its structure and mode of action but also because of the role usually attributed to acetylcholine in the transmission of nerve stimuli<sup>1</sup>. We have carried out experiments to investigate the effect of heat on the activity and antigenic properties of this enzyme, and these have revealed that the activity of the enzyme can be restored after heat denaturation by adding antibodies to the native enzyme. What follows is a report on these experiments.

A stock solution of 20 mg/ml. bovine red blood cell AChE preparation was made up in *tris*-buffered saline (0.01 molar *tris* buffer pH 7.9 containing 0.15 molar sodium chloride). Tubes containing 0.70 ml. of *tris*-buffered saline were heated to the desired temperatures in a thermo-

statically controlled water bath equipped with a shaker; 0.05 ml. of the enzyme stock solution was then added to each tube. 0.05 ml. portions were withdrawn at intervals from each tube and assayed for activity according to the colorimetric method of Ellman *et al.*<sup>2</sup>. Reaction rates were obtained by spectrophotometry at 412 mμ for 5 min, at 1 min intervals; results are expressed in terms either of optical density units per hour, or the same units per min. Fig. 1 shows that the critical denaturation temperature for this enzyme preparation is 58° C. Heating at temperatures below 58° C over a period of 10 min caused a gradual loss of enzyme activity, but heating at 59° C and at 60° C for only 3–5 min resulted in a sharp decline in enzyme activity.

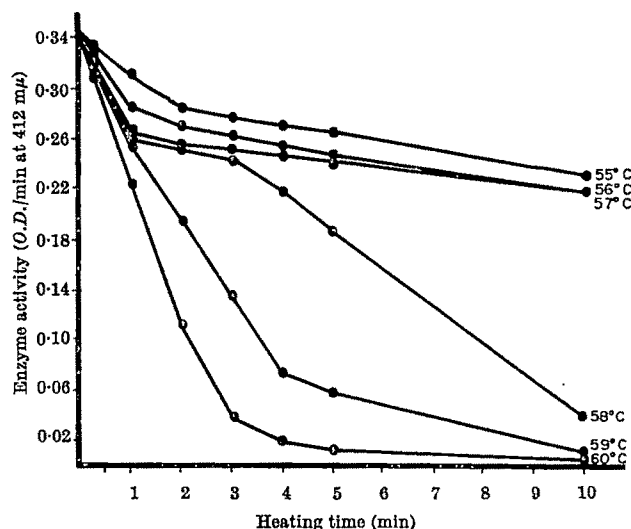


Fig. 1. Effect of heat on the enzyme activity of AChE.

In order to investigate the effect of antibodies to acetylcholinesterase\* on the denatured enzyme, 0.05 ml. portions of stock AChE solution were added to 0.70 ml. *tris*-buffered saline at 60° C in a water bath. Each tube was then heated for a specified time as indicated in Table 1, removed from the water bath and allowed to cool to room temperature. After cooling, 0.5 ml. portions of *tris*-buffered saline containing anti-AChE globulins (derived from 0.5 ml. antiserum) were added to each tube. The tubes were left overnight in the cold and the immune precipitates were removed by centrifugation. The precipitates were washed twice with *tris*-buffered saline and were suspended in 1.25 ml. of *tris*-buffered saline. 0.05 ml. portions of the precipitates and supernatants were assayed for AChE activity.

The results are given in Table 1. They show that virtually all enzyme activity (99–100 per cent) was destroyed when the enzyme was heated at 60° C for the given periods. On addition of anti-AChE globulins to the denatured enzyme, however, about 7.5 per cent of the initial activity was found in the supernatant. No significant activity could be detected in the immune precipitate. The results in Table 1 further show that antibody to native AChE is essential for the renaturation of heat denatured AChE; without addition of the anti-AChE globulins or on the addition of control globulins, the denatured enzyme failed to regain activity within a period of 24 h.

The appearance of enzyme activity in the supernatant after addition of anti-AChE globulins to denatured

\* The antibodies were obtained by repeated intramuscular injections of rabbits with 10 mg of the AChE preparation in 1 ml. saline emulsified with an equal volume of Freund's complete adjuvant. Anti-AChE globulins and control globulins were prepared by three precipitations of sera at 33 per cent saturation of ammonium sulphate. These globulin preparations lacked esterase activity.

Table 1. EFFECT OF ANTI-ACETYLCHOLINESTERASE GLOBULINS ON HEAT DENATURED ENZYME

Preparation	Time of heating at 60° C (min)	Enzyme activity* after heating	Enzyme activity* on addition of globulins Anti-acetylcholinesterase Activity in supernatant precipitate	Control globulins Total activity
AChE in tris-buffered saline, pH 7.9	0	19.740	11.844	
	5	0.187	0.030	
	10	0.197	0.045	
	15	0.122	0.045	
	30	0.094	0.045	
	45	0.000	0.045	
	60	0.000	0.045	
	5†	0.234	1.485	
	60	0.000	1.500	0.000
Tris-buffered saline, pH 7.9	0	0.000		

\* Optical density per hour (at 412 mμ).

† Antibodies were added after storage at 4° C for 18 h.

AChE indicates the possibility that as a result of the interaction between the denatured enzyme and antibodies to the native AChE, a partial renaturation of the enzyme occurred. The presence of a soluble complex resulting from this interaction was shown by adding goat anti-rabbit serum to the supernatant described in Table 1. This resulted in the quantitative precipitation of the renatured enzyme.

We attempted to investigate the kinetics of the renaturation process by assaying the restoration of enzyme activity at different intervals after the addition of anti-AChE globulins. The results are summarized in Table 2. Table 2 clearly indicates that the renaturation process is complete within 1 min of addition of antibody. Essentially the same amount of activity was restored when antibodies were added either immediately or 24 h after heat treatment of the enzyme.

Table 2. TIME INVESTIGATIONS ON THE RENATURATION OF ACETYLCHOLINESTERASE FOLLOWING ADDITION OF ANTI-ACETYLCHOLINESTERASE GLOBULINS

Time of addition of globulins after heating	Time interval between addition of globulins and assay of enzyme activity	Enzyme activity*
Immediate	1 min	1.560
	15 min	1.650
	30 min	1.620
	90 min	1.635
	18 h	1.575
24 h	1 min	1.515

\* Optical density per hour (at 412 mμ).

The mechanism by which the enzyme activity of heat denatured enzyme is restored by antibodies to the native enzyme preparation is still unknown. The combination of antigen antibody may have resulted in a partial refolding of the denatured enzyme sufficient to restore part of its enzyme activity. It is also possible that the formation of the enzyme antibody complex may have resulted in the contribution of a structural component by the antibody, necessary for the catalytic activity of the enzyme. These possibilities are at present under investigation.

We wish to thank Dr. Ben F. Feingold for his interest and encouragement throughout this work.

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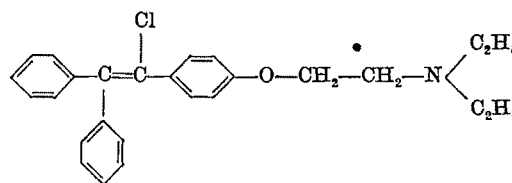
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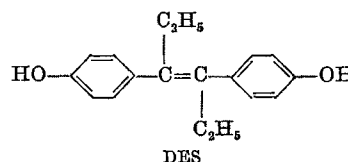
## Inhibition of Glutamic Dehydrogenase by Clomiphene

GLUTAMIC dehydrogenase (GDH) has been used as a model enzyme in the study of the interaction between steroid hormones and enzymes<sup>1</sup>. It was found that in experiments *in vitro* 17β-oestradiol, oestriol, oestrone and diethylstilboestrol (DES) inhibited the action of GDH, DES being the strongest inhibitor. During the past few years, clomiphene, that is, 1-[p-(β-diethylaminoethoxy)-phenyl]-1,2-diphenyl-2-chloroethylene,



Clomiphene

has been applied to induce ovulation in anovulatory patients<sup>2</sup>, although the mechanism of its action is not yet fully understood. Comparison of the structures of DES (that is, α,α'-diethylenstilbestenediol)



DES

and clomiphene reveals some similarities: both compounds are derivatives of ethylene and both contain at least two phenyl groups.

GDH catalyses the reaction: α-ketoglutarate + ammonium ion (NH<sub>4</sub><sup>+</sup>) + reduced nicotinamide-adenine dinucleotide (NADH) ⇌ L(+)-glutamate + nicotinamide-adenine dinucleotide (NAD) + water. The reaction can be studied by determining the decrease of extinction at 366 nm, caused by the oxidation of NADH (ref. 3).

The activity of GDH and the degree of inhibition were measured by the "Boehringer GLDH test". The inhibitors (clomiphene as citrate and DES) were dissolved in a 1 : 1 mixture of propylene glycol and water.

The results are shown in Fig. 1. For comparison, the experiment of Yielding and Tomkins<sup>1</sup> with DES was repeated. We found in our experiments that DES caused a 50 per cent inhibition at a concentration of 2 × 10<sup>-6</sup> mole/l., which is in agreement with previous results<sup>1</sup>. Clomiphene caused the same inhibition at about the same concentration, 1.9 × 10<sup>-6</sup> mole/l. The maximum amount of inhibition (about 65 per cent) by clomiphene was at a concentration of 4 × 10<sup>-6</sup> mole/l.; at the same concentration, DES caused an inhibition of 85 per cent.

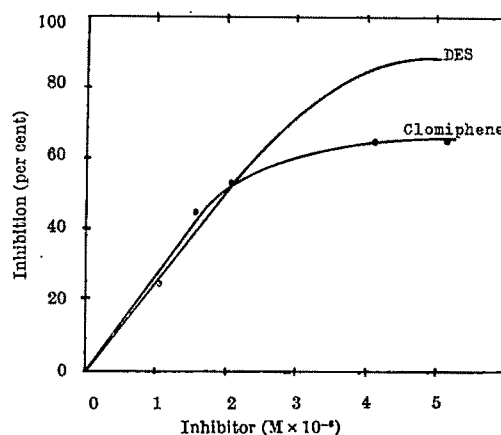


Fig. 1. Effect of DES and clomiphene on the GDH reaction.

Yielding and Tomkins<sup>1</sup> suggested that the inhibition of the GDH activity by steroids might be caused by the splitting of the GDH molecule into smaller units, while Hofmann<sup>4</sup> has directed attention to the aromatic character of steroids which are  $\pi$  shell compounds. In this connexion, we note that only DES—the most powerful inhibitor—and clomiphene have as a common part in their structure a  $—C=C—$  bond. It represents a special type of reactive  $\pi$  electron bond. This might be the key point of the inhibiting property of these two compounds. Being very reactive, this bond may attack the hydrophobic points of GDH molecule and so split it into smaller units which has been suggested to be the cause of inactivation<sup>1</sup>.

We thank Messrs. Star, Tampere, Finland, for their gift of clomiphene citrate.

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### Nucleotide Pyrophosphatase of *Tetrahymena pyriformis*

IN an investigation of the synthesis of nicotinamide adenine dinucleotide (NAD) by *Tetrahymena pyriformis* we examined the enzyme cleavage of NAD by this ciliate. There seems to be a lack of information about the cleavage of this coenzyme by Protozoa<sup>1,2</sup>. In preliminary experiments we incubated cell-free homogenates with NAD and saw that its activity, measured with alcohol dehydrogenase, disappeared rapidly, and detailed investigation to identify the enzyme responsible for the cleavage of NAD seemed to be necessary.

In the case of NADase, nicotinamide is liberated and the complex with cyanide is lost<sup>1</sup>. When the cleavage is in the pyrophosphate bond, the activity of the coenzyme is destroyed, but the capacity to form a complex with cyanide is maintained. NADase is also inhibited by nicotinamide, while pyrophosphatase is not. Phosphorus is released as in the case of sweet almonds and intestinal pyrophosphatases<sup>3</sup>.

*Tetrahymena pyriformis*, variety I, mating type II, cultured in 2 per cent peptone 'Difco' and 0.5 per cent glucose, was used for these experiments. In some cases, 0.1 per cent 'Difco' yeast extract was also added to the medium. After 3 days of incubation at 25° C, without shaking, the cells were collected and washed with distilled water and centrifuged at 2,000 r.p.m.

The cells were suspended in 0.02 molar phosphate buffer, pH 7.0, and submitted to ultrasonic treatment in an MSE apparatus for 3 min in an ice bath (until no intact cells could be detected microscopically). The sonicate was centrifuged at 6,000g and 4° C and the supernatant was used in the experiments. Coenzyme activity of the extracts prepared with 0.02 molar phosphate buffer, pH 7.0, was determined with alcohol dehydrogenase<sup>4</sup>. Crystalline alcohol dehydrogenase diluted to contain 500 u/ml. and NAD (97.5 per cent pure) were used. Phosphorus was estimated by Fiske and Subbarow's method modified by Gomori<sup>5</sup> and proteins by the method of Warburg and Christian, using the formula of Kalkar<sup>6</sup>. The cyanide complex was measured according to Colowick, Ciotti and Kaplan<sup>4</sup>. The extracts for phosphorus were prepared with 0.1 molar *tris* buffer, pH 7.0.

The experiments summarized in Table 1 showed that the coenzyme activity decreased rapidly but that the

Table 1. ACTIVITY OF THE NUCLEOTIDE PYROPHOSPHATASE OF *Tetrahymena pyriformis*

Time of incubation (min)	NAD ( $\mu$ -moles/mg of protein)	Phosphorus split ( $\mu$ g/mg of protein)	Potassium cyanide complex. Optical density at 340 m $\mu$ †
7	2.12	0.70	0.25
10	0.98	0.30	—
30	0.11	0.03	—
60	0.03	0.01	—
120	0.0	0.0	0.20
180	0.0	0.0	0.25
Heated at 70° C§	0.0	0.0	—
Incubated for 30 min with 3 mg of nicotinamide	0.12	0.0	—

\* Cell free sonicate (1 ml.) incubated with 7  $\mu$ moles of NAD and the activity measured with 0.1 ml. of 10 per cent ethanol, 1.8 ml. of 8.7 per cent sodium pyrophosphate, pH 9.5, and 0.1 ml. of alcohol dehydrogenase solution. Optical density measured in a spectrophotometer at 340 m $\mu$ .

† Phosphorus (acid soluble) was split by incubating 1 ml. of the sonicate extract, adjusted to contain 3 mg protein and 0.6 mg of NAD. Deproteinization with 12 per cent perchloric acid with 'Celite' as a filter aid. The centrifugate was assayed by Gomori's method and the colour was measured using a red filter in a photocolourimeter<sup>5</sup>.

‡ Cell free sonicate (0.2 ml.) was incubated with 0.2  $\mu$ moles of NAD and cooled; added with 1 molar potassium cyanide solution to make 3 ml. and the optical density was read at 340 m $\mu$  after 1 min<sup>4</sup>.

§ The extract (2 ml.) was heated in a water bath at 70° C for 2 min and cooled in an ice bath for the enzyme assay and determination of phosphorus. All these results represent mean values based on five separate experiments.

cyanide complex was unchanged even after 120 min of incubation. Acid soluble phosphorus increased with the time of incubation.

It is known that nicotinamide inhibits NADase and not pyrophosphatase; the enzyme from *Tetrahymena*, as expected, was not sensitive to nicotinamide (Table 1). Heating at 70° C for 2 min inactivated the enzyme, showing that a protein inhibitor is not present as in the case described for *Proteus vulgaris*<sup>7</sup>.

I concluded that the enzyme cleavage of NAD by sonicated extracts of *Tetrahymena pyriformis* is caused by a nucleotide pyrophosphatase and not NADase.

I thank the Conselho Nacional de Pesquisas for a fellowship, and Dr. S. H. Hutner, Haskins Laboratories, New York, for supplying the culture of *Tetrahymena pyriformis*.

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### Electrophoresis of Glucose-6-phosphate Dehydrogenase: a New Technique

WE wish to report a new technique for the investigation of the electrophoretic behaviour of glucose-6-phosphate dehydrogenase (G6PD), which offers, in our opinion, some advantages over the starch gel technique used at present<sup>1,2</sup>.

The medium on which the electrophoresis is performed is cellulose acetate in a gel form ('Cellologel', manufactured by Chemetron, via G. Modena 24, Milano, Italy); this has already been used in investigations of the lactic dehydrogenase isozymes<sup>3</sup>. We use strips 4 cm wide, 17 cm long and 0.25 mm thick. It is advisable to make sure that the same batch of strips is used throughout each series of experiments. The electrophoresis buffer consists of 1 l. of 0.075 molar *tris* (containing 0.004 moles EDTA), to which is added 250 ml. of 0.075 molar citric acid solution; this gives a pH of 7.5.

The 'Cellogel' strips, which can be stored in 50 per cent methanol, are blotted with filter paper and soaked in buffer for at least 1 h. The operation is repeated with fresh buffer once or twice to ensure that the strips are completely free from methanol. They are then blotted again and placed in the electrophoresis tank (Shandon Universal Electrophoretic Tank MKII), well stretched, with the porous surface upwards. The plastic supports in the tank are adjusted to a distance of 9 cm, so that the terminal parts of the strips are immersed in the buffer and thus paper wicks are not required. The strips are equilibrated for about 10 min, with the current on (4 m.amp per strip). The current is then stopped and the samples are applied with a micro-pipette, in the form of thin streaks 12–13 mm long, at about 5 mm from the plastic support (cathodic end). The best results are obtained when only two samples are applied on each strip, leaving a free margin of about 5 mm on either side. Each sample consists of about 1.5  $\mu$ l. of haemolysate with an approximate concentration of 15 g per cent of haemoglobin. It is advisable to add some TPN to the haemolysate (0.10  $\mu$ moles/l. or less) before the run<sup>4</sup>.

Within about 1 min the samples are adsorbed on the gel, and the current is switched on. The run usually takes 3 h, at 4 m.amp per strip (constant amperage) and about 190 V.

A few minutes before the end of the run, the developing solution (ref. 5, modified) is prepared as follows:

Tris-HCl 1 mole/l. pH 8.6 (0.004 moles/l. in EDTA)	1.25 ml.
TPN (10 mg/ml.)	0.10 ml.
G6P (25 mg/ml.)	0.15 ml.
MTT tetrazolium (2 mg/ml.)	0.25 ml.
Phenazine methosulphate (2 mg/ml.)	0.25 ml.
Cobaltous chloride (0.5 moles/l.)	0.10 ml.

The total amount (2.1 ml.) is sufficient for the development of five strips. Tetrazolium and phenazine solutions are freshly prepared on each occasion. Cobaltous chloride forms an insoluble precipitate with the formazan originating from the tetrazolium<sup>6</sup>.

On completion of the run, the strips are cut free of the parts hanging in the buffer and marked. The developing reagent is poured on to a clean glass plate, and the strips are dipped into it one by one for about 5 sec, and gently blotted to remove any excess reagent.

The strips are now placed in a moist chamber, and the reaction is allowed to take place at 37° C for about 20 min. If the enzyme activity of the samples is high, the blue bands will appear almost immediately. The reaction is stopped by dipping the strips into a 10 per cent solution of formol. If the developed strips are kept in the dark in the formol solution at room temperature they can be preserved for about 2 weeks, but there is some fading of the colour. No fading of the colour will occur, however, if they are stored at -20° C.

Fig. 1 shows a typical electrophoresis pattern after the development of the enzyme bands. On each strip an artificial mixture of G6PD type A<sup>+</sup> and B<sup>+</sup> has been run together with a sample of type B<sup>+</sup>. The clear separation of the enzyme bands and the good reproducibility of the pattern can be observed. In the A<sup>+</sup>B<sup>+</sup> mixture, the A<sup>+</sup> enzyme has a lower activity due to long storage.

With fresh blood samples of type B<sup>+</sup> with a high enzyme activity, an "isozyme" band is often seen at about 6 mm behind the main B-band.

When this technique is compared with those utilizing starch gel<sup>1,2</sup>, the following advantages become evident: (a) only 3.5 h is required for the run and the development, as compared with 16–20 h when the starch gel technique is used; (b) it is not necessary to add TPN to the electrophoretic system because of the very small amount of heat generated in the strip and because of the short duration of the electrophoresis; (c) the run is performed at room temperature; (d) the amount of costly reagents (TPN,

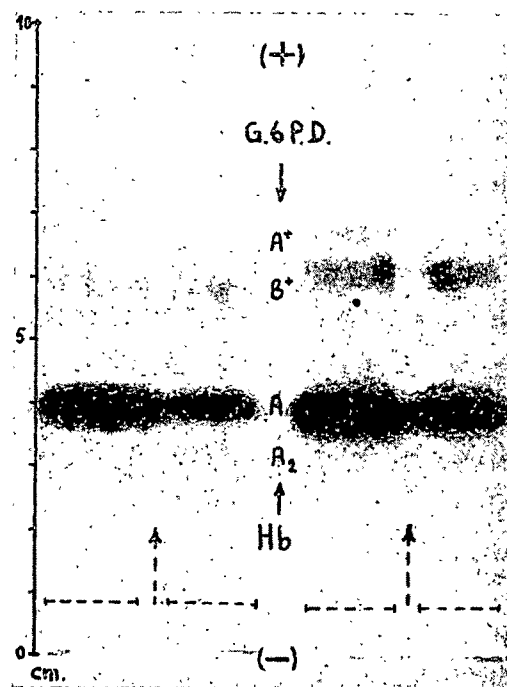


Fig. 1. Electrophoretic patterns of G6PD A<sup>+</sup> and B<sup>+</sup> enzymes. On each strip is placed a mixture of A<sup>+</sup> and B<sup>+</sup> (left), and B<sup>+</sup> (right). The origin is at the cathode (bottom). The thin band behind the dark HbA band is HbA<sub>2</sub>.

G6P) required for the development is greatly reduced; (e) it is not necessary to prepare gel.

We think that this technique may prove to be a useful tool for population studies on G6PD, particularly because it is easy to perform under "field work" conditions.

From preliminary observations on samples kindly provided by Dr. Kirkman and on a series of haemolysates obtained from Indian tribal populations, we think that this technique is suitable not only for the discrimination of the "A" and "B" electrophoretic types of G6PD, but also for the detection of at least the most obvious electrophoretic variants.

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### Effects of Pyruvate on the Formation of Urea in Rat Liver Slices

THE presence of the L-ornithine-pyruvate transaminase system in tissues has been well established<sup>1,2</sup>. In view of the possible presence of large concentrations of pyruvate (instead of the plasma lactate)<sup>3,4</sup> in the livers of tumour bearing rats, we have attempted to demonstrate the effect of this on the formation of urea.

Liver slices were prepared using a novel many-bladed slicer<sup>5</sup> and incubated for 100 min at 37.5° C in Krebs-

Ringer bicarbonate (KRB) pH 7.4 and the appropriate substrates with gas phase of 95 per cent O<sub>2</sub>: 5 per cent CO<sub>2</sub>. Substrates used were 10 millimolar pyruvate (P), 20 millimolar L-glutamine (G) and 0.3 millimolar L-ornithine (O). Liver with a wet weight of 50–100 mg was incubated in 3.0 ml. of medium in vessels such that adequate diffusion was possible for the liver slices, which were 0.4–0.6 mm thick. Samples of 2.0 ml. of incubation media were deproteinized<sup>6</sup> with barium hydroxide and zinc sulphate, and urea estimated by the Archibald method<sup>7</sup>. Amino-acids were separated electrophoretically (pyridine-acetic acid buffer pH 4.7 and acetic acid pH 2) and ornithine was estimated by the Chinard method<sup>8</sup>.

Table 1. EFFECT OF HIGH CONCENTRATIONS OF PYRUVATE ON UREA PRODUCTION AND ORNITHINE CONCENTRATION IN RAT LIVER SLICES

a			b		
Urea			Ornithine		
KRB	GO	GOP	KRB	GO	GOP
0.048	0.443	0.176	0.007	0.066	0.032
0.096	0.554	0.212	0.007	0.066	0.033
0.056	0.379	0.156	0.013	0.047	0.041
0.022	0.463	—	0.008	0.056	0.040
0.001	0.427	0.239	0.008	0.066	0.010
0.045	0.453 ± 0.064	0.196 ± 0.037	0.008	0.060 ± 0.0085	0.031 ± 0.0125
	1.96 < 0.453	(P < 0.005)		0.031 < 0.060	(P < 0.005)

KRB = Krebs-Ringer bicarbonate buffer; GO = KRB containing glutamine and ornithine; GOP = KRB containing glutamine, ornithine and pyruvate.

Each value given is a mean ± standard deviation of the results from 10 liver slices. Values are expressed in  $\mu$ moles of the substance changed in 100 min/ $\mu$ g dry weight tissue.

The results shown in Table 1 (a) indicate a decrease of about 50 per cent in the amount of urea formed from L-glutamine in the presence of high concentrations of pyruvate. There is also a decrease of about 50 per cent in the concentration of L-ornithine when it is present in excess—Table 1 (b). These results indicate that, contrary to the views of Bach *et al.*<sup>9</sup>, L-glutamine can be converted to urea via the Krebs ornithine cycle<sup>10</sup> (urea cycle). From these data it must be concluded that, under these experimental conditions, L-ornithine was channelled out of the urea cycle, and thus there is reduced urea production.

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### Preferential *in vitro* Binding of Radioactive Vitamin B<sub>12</sub> by an Abnormal Serum Protein in Chronic Myeloid Leukaemia

ALTHOUGH considerable work has been done in the past decade on *in vivo* and *in vitro* binding of vitamin B<sub>12</sub> (refs. 1–4), the precise mechanisms of binding and the physiological significance of the binding patterns obtained remain uncertain.

*In vitro* binding of radioactive vitamin B<sub>12</sub> to normal serum proteins takes place principally in the  $\alpha_2$  and  $\beta$  fractions, while in chronic myeloid leukaemia the majority of radioactive vitamin B<sub>12</sub> is bound to a protein moving with the  $\alpha_1$  globulin fraction, possibly the seromucoid fraction<sup>5,6</sup>. Some authors hold that the increased binding capacity of myeloid leukaemia serum is caused by an increased amount of seromucoid fraction<sup>6</sup>; others dis-

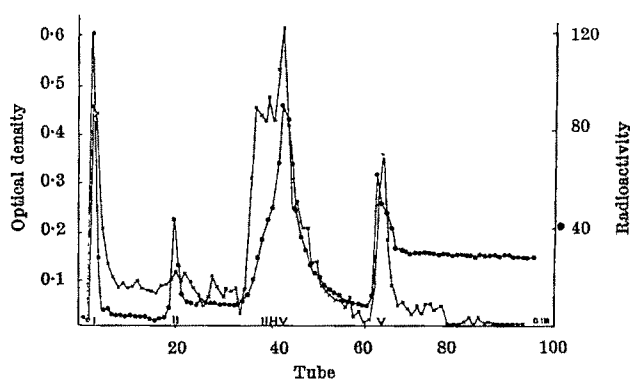


Fig. 1. Column chromatogram of dialysed labelled normal serum. ●, Optical density at 280 Å; ×, radioactivity in counts per min above background. Changes in eluent were at tubes 17, 33 and 83.

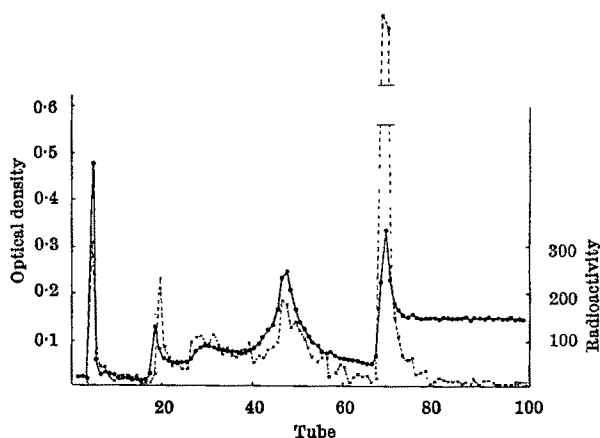


Fig. 2. Column chromatogram of dialysed labelled serum from a patient with acute myeloid leukaemia, white cell count 360,000/mm<sup>3</sup>. ●, Optical density at 280 Å; ×, radioactivity in counts per min above background. The two highest points of radioactivity in peak V were 2,810 c.p.m. and 2,598 c.p.m. above background respectively. Changes in eluent were at tubes 16, 33 and 65.

agree<sup>7,8</sup>. It has also been suggested that the increase in binding to the  $\alpha_1$  fraction is a result of saturation of the "specific" B<sub>12</sub> binding proteins, and that the binding of added vitamin B<sub>12</sub> to other proteins is produced by an "overflow" effect<sup>8,9</sup>.

Recent studies have indicated that the binding of added cobalt-58 labelled vitamin B<sub>12</sub> (<sup>58</sup>Co-B<sub>12</sub>) to the serum proteins in chronic myeloid leukaemia is a preferential binding, and not an "overflow" phenomenon. Dialysed serum labelled with <sup>58</sup>Co-B<sub>12</sub> was obtained by a method previously described<sup>9</sup>, and column chromatography of the dialysed labelled serum was then carried out<sup>10</sup>.

A column of 'Sephadex DEAE-40' 15 cm × 1 cm was obtained by suspending approximately 1.8 g of DEAE in a solution of twice molar sodium chloride in 0.01 molar *tris* phosphate buffer pH 6.5; packing took place by gravity. Following equilibration with the buffer used for dialysis, a sample of dialysed labelled serum was placed on the column and a step-wise system of elution was used, starting with 0.01 molar *tris* phosphate buffer pH 6.5 and continuing to 0.20 molar *tris* phosphate buffer, pH 4.0. Each sample was counted in a well type scintillation counter, and its optical density was measured in an ultra-violet spectrophotometer at 280 Å. A chromatogram typical of normal labelled serum is shown in Fig. 1. Four major peaks are apparent, and are designated I, II, III-IV and V; complex III-IV is the most radioactive. The chromatogram shown in Fig. 2 is of dialysed labelled serum from a patient with myeloid leukaemia (white cell count 360,000/mm<sup>3</sup>); radioactivity in peak V was



Table 1

	Haemo- globin (g/100 ml.)	White cell count ( $\times 10^3/\text{mm}^3$ )	Platelet count ( $\times 10^3/\text{mm}^3$ )	Serum B <sub>12</sub> ( $\mu\text{g}/\text{ml.}$ )	
				Total	Free
July 1961	7.9	161	1,452	2,864	80
Jan. 1963	10.6	27	2,200	1,530	0
April 1965	12.9	37.5	715	0	0
May 1965*	—	—	—	1,440	Most

\* After 2,000  $\mu\text{g}$  vitamin B<sub>12</sub> intramuscular injection.

in excess of 5,600 c.p.m. above background. Similar patterns have been obtained with sera from other patients with acute and chronic myeloid leukaemia. Purified labelled  $\alpha_1$  acid glycoprotein appears entirely at peak V when run on this type of chromatogram, indicating that the abnormal binding protein has mobility properties of an  $\alpha_1$  globulin.

Because of an unusual combination of clinical events, an opportunity was provided to study a patient with chronic myeloid leukaemia on several different occasions over a four-year period when the serum level of vitamin B<sub>12</sub> was successively elevated, low and then corrected therapeutically. The patient, who was diagnosed as having chronic myeloid leukaemia in 1961, had had a partial gastrectomy performed several years before. The binding capacity of her serum for radioactive B<sub>12</sub> was measured in 1963 and was found to be 7.36  $\mu\text{g}/\text{ml.}$  at the 10  $\mu\text{g}/\text{ml.}$  level. A sample of serum taken in 1961 was studied at the same time and showed similar results. In early 1965 the patient was found to have no vitamin B<sub>12</sub> detectable in her serum (see Table 1 for haematological data); presumably her deficiency had arisen because of her partial gastrectomy<sup>11</sup>. Column chromatography of dialysed labelled serum, collected at the time when the patient was deficient in vitamin B<sub>12</sub>, shows that all detectable vitamin B<sub>12</sub> is present in peak V; a 96 per cent recovery was obtained (Fig. 3). An identical pattern with 98 per cent recovery was obtained from dialysed labelled serum obtained after therapeutic vitamin B<sub>12</sub> had been given, at a time when her serum vitamin B<sub>12</sub> level was 1,440  $\mu\text{g}/\text{ml.}$ , mostly in the free form.

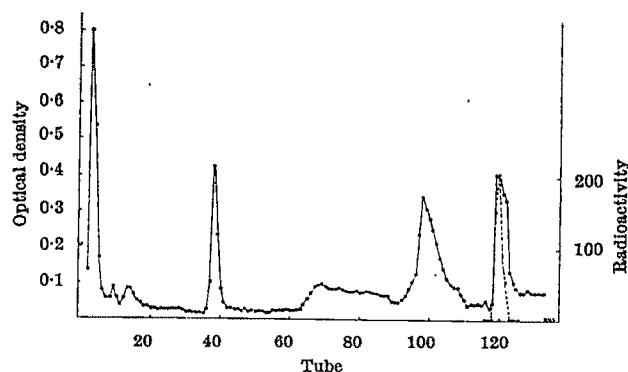


Fig. 3. Column chromatography of dialysed labelled serum from a patient with chronic myeloid leukaemia and no detectable vitamin B<sub>12</sub> in serum. ●, Optical density at 280 Å; ×, radioactivity in counts per min above background. No radioactivity was detected in fractions other than those shown. The radioactivity present accounts for 96 per cent of that placed on the column. Changes in eluent were at tubes 34, 85 and 116.

These results indicate that the abnormal protein present in the serum of myeloid leukaemia patients, possibly an  $\alpha_1$  acid glycoprotein, binds all added vitamin B<sub>12</sub> when vitamin B<sub>12</sub> deficiency is present. Thus the phenomenon of increased avidity for the vitamin appears to be specific and preferential, rather than an overflow phenomenon resulting from saturation of other binding sites.

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### Evidence for a New Mechanism of Respiratory Stimulation and Proton Ejection in Ehrlich Ascites Tumour Cells dependent on Potassium Ions

OVER the past decade, evidence has accumulated that fragments of cellular membranes exhibit ATPase activity which is dependent on sodium ions and inhibited by ouabain, and which is probably involved in the active transport of sodium and potassium ions<sup>1</sup>. Another type of cation transport, characterized by an insensitivity to ouabain and coupled directly to the energy transfer system of the respiratory chain, has been demonstrated in isolated mitochondria. Interest in this system has been concerned primarily with the transport of divalent cations, and their exchange with hydrogen ions<sup>2</sup>, but it is now obvious that a similar mechanism can operate for the transport of monovalent cations, provided that the mitochondria are treated with certain polypeptide antibiotics. Valinomycin specifically promoted the uptake of potassium, rubidium and caesium ions<sup>3,4</sup>, whereas gramicidin also activated uptake of sodium and lithium ions by isolated mitochondria<sup>5</sup>.

A cell membrane mechanism dependent on sodium and potassium ions and sensitive to ouabain clearly exists in the intact cell, but the other mechanism for monovalent cation transport has only been demonstrated with isolated mitochondria. The results reported here suggest that valinomycin stimulated a mitochondrial exchange of hydrogen for potassium ions in the intact cell. The data also provide information about the quantitative importance of each of these two transport mechanisms and their interrelationship at the level of the intact cell. Investigations of the respiration and the concomitant ejection of protons by Ehrlich ascites tumour cells depleted of potassium provided evidence which is consistent with the operation of a dual mechanism for the active uptake of potassium ions in these cells. One mechanism which needs ATP is sensitive to ouabain and independent of valinomycin. The other mechanism is linked directly to the respiratory chain, is insensitive to ouabain, and is dependent on valinomycin. The data also indicate that each of the mechanisms contributes about a half of the total respiration which is dependent on potassium ions and about half of the proton release. Furthermore, the two mechanisms can operate independently.

Ehrlich ascites tumour cells collected from the peritoneal cavity of white mice 8–12 days after inoculation were placed in one of four different media buffered at pH 7.4 (the ionic composition of the media appears in figure and table legends). The cell suspensions were maintained at 4° C throughout all subsequent operations. The cells were separated from the ascitic fluid by centrifugation at 40g for 5 min, resuspended in the medium in which

they were collected, and then sedimented at 110g for 5 min. This procedure was repeated six times at 30 min intervals. The cells were finally sedimented at 250g for 5 min and resuspended in 2 volumes of the same medium. Suspended cells (1 ml.) were incubated with 4 ml. of the medium in which the cells were collected, but without the phosphate or *tris*-buffer. Incubations were carried out at 37° C. Oxygen uptake and release of hydrogen ions were detected and recorded continuously and simultaneously with an oxygen electrode and a glass electrode, respectively. The protein content of the cells was determined by the biuret method<sup>6</sup>. The concentration of potassium in the cells depleted of potassium ions, as determined with the flame spectrophotometer on an aliquot of supernatant solution from cells that had been treated overnight with 0.1 normal nitric acid, varied between 2 and 4 mequiv./l. of cells, which confirmed previous observations<sup>7</sup>. The volume of cells in suspension was determined by a micro haematocrit technique. Valinomycin was prepared and added to the incubation medium in alcoholic solution. Oligomycin, rotenone and antimycin A were added to the incubation medium in alcoholic solution; ethanol, in the concentration used, was without effect on the parameters investigated. The ouabain was dissolved in the medium and added to the incubation mixture in this form.

Addition of valinomycin to ascites tumour cells which were oxidizing endogenous substrate produced changes which were similar to those observed with mitochondria. An appreciable increase in respiration and in proton release (Fig. 1) was observed almost immediately after the addition of valinomycin. There was release of inhibition of respiration induced as oligomycin and extrusion of hydrogen ions was also apparent in the presence of valinomycin.

In order to decide whether the effect of valinomycin on oxygen uptake and the extrusion of protons was affected by potassium ions, it was necessary to deplete the cells of potassium ions. The response to valinomycin of cells depleted of potassium ions in a medium containing sodium ions, and in media in which the sodium was replaced by lithium or choline, was compared with cells prepared in the complete ("normal") medium (Table 1). Addition of valinomycin to cells depleted of potassium ions resulted in either an absence or a slight increase in oxygen uptake and release of hydrogen ions, in contrast to the marked stimulation observed with the normal cells. Further addition of potassium ions to the cells depleted of these ions resulted in a significant effect on both parameters; for the cells depleted in the media containing

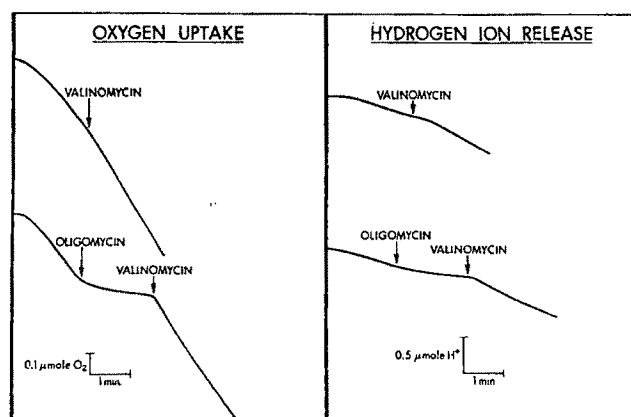


Fig. 1. The effect of valinomycin on uptake of oxygen and release of hydrogen ions. The cells were collected and washed in the "normal" medium: 178 moles/l. of sodium; 6 moles/l. of potassium; 1.5 moles/l. of magnesium; 180 moles/l. of chloride; 1.5 moles/l. of sulphate, and 12 moles/l. of phosphate. Valinomycin was added to a final concentration of  $10^{-7}$  moles/l. In this and in all subsequent experiments. Oligomycin was present at a concentration of  $2 \mu\text{g/ml}$ . The protein content of the cells/5 ml. of incubation medium was 74.4 mg. \*

Table 1. RESPONSE OF NORMAL CELLS AND CELLS DEPLETED OF POTASSIUM IONS TO VALINOMYCIN

	Normal	Depleted of potassium ions		
		Sodium medium	Lithium medium	Choline medium
Uptake of oxygen ( $\mu\text{moles/mg protein/min}$ )				
Control	2.99	1.56	1.08	1.28
plus valinomycin	4.03	1.75	1.08	1.34
plus potassium ions	3.90	3.18	1.23	1.42
Release of hydrogen ( $\mu\text{moles/mg protein/min}$ )				
Control	2.2	1.3	0.8	0.7
plus valinomycin	3.5	1.5	1.0	1.0
plus potassium ions	3.7	3.0	1.7	0.7

The composition of the normal medium is detailed in the legend of Fig. 1. The composition of media for depleting cells of potassium ions was as follows: the sodium medium had 178 mmoles/l. of sodium; 1.5 mmoles/l. of magnesium; 154 mmoles/l. of chloride; 1.5 mmoles/l. of sulphate; 12 mmoles/l. of phosphate. The lithium medium had 178 mmoles/l. of lithium; 1.5 mmoles/l. of magnesium; 12 mmoles/l. of *tris* buffer; 166 mmoles/l. of chloride; 1.5 mmoles/l. of sulphate. Potassium ions were added to a final concentration of  $6 \times 10^{-3}$  molar and valinomycin to  $10^{-7}$  molar in this and all subsequent experiments. Cells (1 ml.) were added to 4 ml. of the appropriate medium. The protein content of the cells in each 5 ml. of incubation mixture was 76.2 mg of normal cells; cells depleted of potassium ions in sodium medium: 74.4 mg; in lithium medium: 74.4 mg; and in choline medium: 89.0 mg.

either sodium or lithium ions, the effect of valinomycin plus potassium ions in depleted cells in the choline medium was inconsistent and smaller. The changes observed after the addition of potassium ions to normal cells and to cells depleted of potassium ions are recorded in Table 2. A significant increase in respiration and release of hydrogen ions was observed with cells depleted of potassium ions in the sodium medium, but not in the lithium or choline media; addition of potassium to normal cells was without effect. Valinomycin caused a further augmentation of respiration and release of hydrogen in the depleted cells in the sodium medium and in normal cells; depleted cells in the lithium or choline medium responded to valinomycin with an initial increase in respiration and release of hydrogen. These observations suggested that the increase in oxygen uptake and hydrogen release on addition of potassium ions depleted cells in the sodium medium could be caused by the exchange of potassium ions for sodium ions and hydrogen ions across the plasma membrane. To evaluate this possibility further, transport of sodium was blocked by inhibiting ATPase of the membrane with ouabain (Table 3). Under these conditions, addition of potassium ions to cells which were depleted in the sodium medium was without effect on respiration

Table 2. RESPONSE TO POTASSIUM IONS OF NORMAL CELLS AND CELLS DEPLETED OF POTASSIUM IONS

	Normal	Sodium medium	Depleted Lithium medium	Choline medium
Uptake of oxygen ( $\mu\text{moles/mg of protein/min}$ )				
Control	2.88	1.63	1.12	1.03
plus potassium ions	2.93	2.31	1.08	1.01
plus valinomycin	4.14	3.38	1.35	1.44
Release of hydrogen ions ( $\mu\text{moles/mg of protein/min}$ )				
Control	2.4	1.1	1.0	0.6
plus potassium ions	2.6	1.9	0.7	0.7
plus valinomycin	3.9	3.2	1.7	1.4

The composition of the media and the protein content of the cells are indicated in the legend of Table 1.

Table 3. INFLUENCE OF OUABAIN ON RESPIRATION AND RELEASE OF HYDROGEN IONS

Additions	Uptake of oxygen ( $\mu\text{moles/mg of protein/min}$ )		Release of hydrogen ( $\mu\text{moles/mg of protein/min}$ )	
	without ouabain	with ouabain	without ouabain	with ouabain
Experiment 1				
Control	1.63	1.50	1.1	0.9
plus potassium ions	2.31	1.50	1.9	0.8
plus valinomycin	3.38	2.69	3.2	2.3
Experiment 2				
Control	1.56	1.30	1.3	0.9
plus valinomycin	1.75	1.50	1.5	1.1
plus potassium ions	3.19	2.26	3.0	1.9

Cells depleted of potassium ions (1 ml.) in the sodium medium (containing 74.4 mg of protein) were added to 4 ml. of the sodium medium without phosphate. The ouabain concentration was  $10^{-3}$  molar.

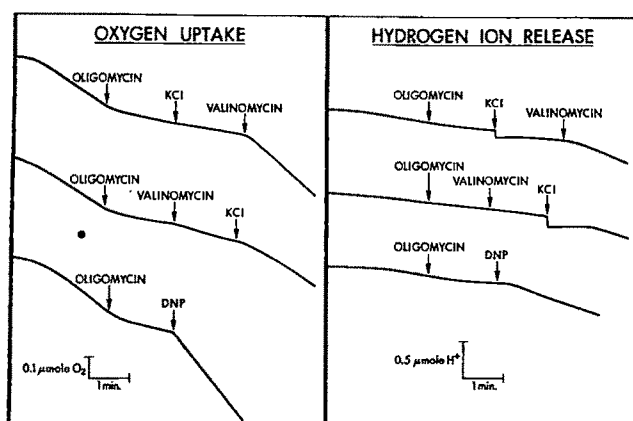


Fig. 2. Release of respiration by inhibited oligomycin and proton extrusion in cells depleted of potassium ions. The protein content of the cells/5 ml. of incubation medium was 74.4 mg. The final concentration of the additions was: 2  $\mu$ g/ml. of oligomycin,  $10^{-4}$  moles/l. of dinitrophenol.

and release of hydrogen. The expected effect of valinomycin was observed in every instance as long as potassium ions were present.

Addition of oligomycin to depleted cells in a medium containing sodium resulted in almost complete inhibition of respiration in a manner which had previously been observed with cells prepared in a normal medium without repeated washing<sup>8</sup>. Under these conditions, ATP production was inhibited and addition of potassium ions had little or no effect on either uptake of oxygen or release of hydrogen (Fig. 2). In agreement with results from isolated mitochondria<sup>9</sup>, further addition of valinomycin resulted in complete release of inhibition of respiration and release of protons. Thus, the effect of valinomycin on respiration and release of hydrogen in the presence of potassium ions was independent of ATP production; as shown before (Table 1), this effect of valinomycin was observed only when potassium was present. It has previously been suggested, on the basis of experiments with liver slices of which the activity is inhibited by oligomycin, that high energy intermediates other than ATP could provide energy for cation movements<sup>10</sup>. The release of respiratory inhibition induced by oligomycin by potassium ions plus valinomycin was similar to the release by dinitrophenol of respiration induced by oligomycin, except that the release by dinitrophenol was not dependent on the presence of potassium ions (bottom curve, Fig. 2).

The data of Table 1 indicated that neither sodium ions, lithium ions nor choline could effectively substitute for potassium ions in the intact cell system. Addition of either rubidium or caesium ions to the cells depleted of potassium ions in either the sodium, lithium or choline medium could replace potassium for demonstration of the valinomycin effect (Table 4), in agreement with results obtained with isolated mitochondria<sup>4</sup>. In other work with cells depleted of potassium ions and inhibited by oligomycin, either rubidium or caesium ions could support the valinomycin effect. The energy dependency of the two transport mechanisms for potassium ions in cells depleted of these ions was demonstrated by the data shown in Table 5. Ejection of protons by the cell preparations used in these experiments was undetectable in control conditions. Inhibition of the respiratory chain with rotenone (Experiment 1) or antimycin A (Experiment 2) blocked both the respiration dependent on potassium ions and the respiration stimulated by valinomycin and proton release. In contrast, inhibition of ATP formation with oligomycin (Experiment 3) markedly depressed respiration dependent on potassium ions, but not respiration stimulated by valinomycin and proton release. In Experiment 4, rotenone was seen to inhibit the mitochondrial exchange

of protons and potassium ions in the system when plasma membrane ATPase was blocked with ouabain.

The data presented here are consistent with the conclusion that Ehrlich ascites tumour cells which are depleted of potassium ions possess two mechanisms for active uptake of these ions from the medium: a plasma membrane component and a mitochondrial component (Table 6). Both are energy dependent, which was revealed by their sensitivity to respiratory inhibitors. The plasma membrane mechanism is inhibited by low concentrations of oligomycin, which indicates that it is dependent on ATP as the source of energy. It is dependent on the presence of sodium ions in the cells, for it is not observed when cells are prepared in the absence of sodium ions. It is independent of valinomycin and inhibited by ouabain. These are identical to the conditions necessary to identify the membrane ATPase activity which is thought to be associated with active transport of sodium and potassium ions<sup>1</sup>. ATP generated by glycolysis can support cell membrane transport of monovalent cations<sup>11</sup>. The mitochondrial mechanism is insensitive to oligomycin, which indicates that it can derive energy directly from high energy intermediates generated by the respiratory chain. It is dependent on valinomycin and insensitive to ouabain. It requires the presence of potassium, rubidium or caesium ions. These features are identical with those found for the cation-proton exchange induced by valinomycin in

Table 4. VALINOMYCIN EFFECT ON CELLS DEPLETED OF POTASSIUM IONS IN THE PRESENCE OF RUBIDIUM AND CAESIUM IONS

Additions	Uptake of oxygen ( $\mu$ moles/mg of protein/min)		
	Sodium medium	Lithium medium	Choline medium
Control	1.08	1.08	1.11
plus rubidium ions	1.35	1.08	1.11
plus valinomycin	1.74	1.23	1.37
Control	1.15	1.23	1.20
plus caesium ions	1.24	1.08	1.13
plus valinomycin	1.78	1.16	1.25
Additions	Release of hydrogen ( $\mu$ moles/mg of protein/min)		
	Sodium medium	Lithium medium	Choline medium
Control	0.8	1.2	0.5
plus rubidium ions	0.9	0.3	0.4
plus valinomycin	2.3	1.5	1.2
Control	0.9	1.0	0.2
plus caesium ions	1.0	0.3	0.2
plus valinomycin	2.2	1.3	0.6

Cells (1 ml.) were added to 4 ml. of the appropriate phosphate-free medium. The protein content of the cells in each 5 ml. of incubation medium was: cells depleted of potassium ions, 66.0 mg in sodium medium, 49.0 mg in lithium medium, and 77.0 mg in choline medium. Rubidium chloride and caesium chloride (40  $\mu$ l. of 0.75 molar solution) were added as indicated.

Table 5. RESPONSE OF CELLS DEPLETED OF POTASSIUM IONS TO INHIBITORS OF MITOCHONDRIAL METABOLISM

Additions	$\mu$ moles of oxygen/mg of protein/min	$\mu$ moles of hydrogen/mg of protein/min
Experiment (1) Control	2.45	0.0
plus potassium ions	3.50	2.8
plus valinomycin	4.52	4.6
plus rotenone	0.00	1.1
Experiment (2) Control	2.45	0.0
plus potassium ions	3.32	2.1
plus valinomycin	5.63	5.6
plus antimycin A	0.00	0.0
Experiment (3) Control	2.85	0.0
plus potassium ions	4.13	2.4
plus oligomycin	0.24	0.7
plus valinomycin	5.63	3.5
plus rotenone	0.00	0.0
Experiment (4) Control	2.22	0.0
plus potassium	3.17	2.1
plus ouabain	1.01	1.1
plus valinomycin	4.76	3.2
plus rotenone	0.00	0.0

The protein content of the cells/5 ml. of incubation medium was 58.6 mg. The final concentration of the additions were  $2 \times 10^{-4}$  molar rotenone; 2  $\mu$ g/ml. of oligomycin; 0.6  $\mu$ g/ml. of antimycin A;  $10^{-6}$  molar ouabain.

Table 6. CHARACTERISTICS OF RESPIRATION WHICH IS DEPENDENT ON POTASSIUM IONS AND RELEASE OF HYDROGEN

	Site	
	Plasma membrane	Mitochondrial membrane
Energy dependent	+	+
Sensitive to oligomycin	+	-
Dependent on sodium	+	-
Sensitive to ouabain	+	-
Sensitive to valinomycin	-	+

isolated mitochondria<sup>4</sup>. Advantage has been taken of the different characteristics of these two types of transport mechanism to demonstrate the independent operation of each in the intact cell.

In the presence of valinomycin, the mitochondrial mechanism can contribute as much as half of the total respiration and release of hydrogen ions by the cells, which is dependent on potassium ions. Whether, and to what extent, this represents a physiological reality will depend on the demonstration of the existence and the efficiency of naturally occurring agents which act like valinomycin. It is interesting that parathyroid hormone, which has been shown to enhance the uptake of magnesium ions by isolated liver mitochondria, has also been reported to stimulate the uptake of potassium ions by mitochondria<sup>12</sup>.

The results of this investigation indicate that activation of the cellular membrane transport system by the addition of potassium ions to cells which are loaded with sodium ions and depleted of potassium ions leads to stimulation of respiration and release of hydrogen ions. The increased respiratory response is a consequence of the activation of the pump for sodium ions, in agreement with the interpretation of Maizels *et al.*<sup>7</sup>. Evidence which supports the relationship between sodium transport and respiration has recently been summarized<sup>13</sup>. The release of protons is in some manner coupled with the sodium pump, for it is also blocked when transport of sodium and potassium ions are inhibited at the plasma membrane. The origin of the protons (whether mitochondrial or extramitochondrial) is an important question, but cannot be decided from the results of these experiments.

An intriguing feature revealed by the present work concerns the ability of the mitochondrial mechanism for uptake of potassium ions to operate even when the extramitochondrial mechanism is blocked by ouabain or oligomycin. This finding indicates that mitochondria might accumulate potassium ions from the extracellular medium when active transport of these ions across the cellular membrane is blocked. This interpretation implies that there exists a direct, functional communication between the extracellular fluid and the mitochondria. Demonstration of the absence of passive or facilitated transport of potassium ions across the cell membrane is, however, necessary before such an implication can be accepted with certainty.

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## Effect of Growth Hormone on Tubular Reabsorption of Glucose and Phosphate

It is now well established that growth hormone has some renotropic properties. Besides its enhancing effect on renal plasma flow and glomerular filtration rate (GFR) in dog<sup>1,2</sup> and man<sup>3-5</sup>, growth hormone has been shown to raise tubular reabsorption of phosphate in man<sup>3-5</sup> and in normal and parathyroidectomized dogs<sup>6</sup>. In some respects, the mechanism of tubular transport of glucose is similar to that of phosphate; some common metabolic pathways are probably involved in their tubular transport since glucose has been shown to compete with phosphate for reabsorption<sup>7</sup>. It was therefore of interest to investigate the effect of growth hormone on tubular transport of glucose. For this purpose maximal tubular reabsorption of glucose (TmG) was measured before and after administration of bovine growth hormone (BGH) in the dog.

Unanaesthetized female mongrel dogs, weighing 15 to 20 kg, were used for these experiments and were fed freely.

TmG studies were done in five dogs. A priming injection of glucose and creatinine was given, then a sustaining solution containing glucose and creatinine was infused at a rate designed to maintain blood glucose and creatinine at a level adequate for GFR and TmG measurement. Glucose was measured in blood and urine by the method of Schaffer and Hartman<sup>8</sup>. Three days after the control experiment, administration of BGH (1.5 mg/kg/day) was begun. On the eighth and last day of BGH treatment, TmG was again measured in the same manner as before.

In a second group of dogs, parallel studies of the effect of BGH on maximal tubular reabsorption of phosphate (TmPO<sub>4</sub>) were performed, using the same pattern as for TmG. Methods were as previously described<sup>9</sup>.

The results are shown in Tables 1 and 2. TmG increased after growth hormone in all cases, although slightly in some of them. GFR also increased. The ratio TmG/GFR or mean renal threshold for glucose decreased in all experiments indicating a relatively smaller effect on glucose Tm than on filtration rate.

Table 1. EFFECT OF BOVINE GROWTH HORMONE ON MAXIMAL TUBULAR REABSORPTION OF GLUCOSE (TmG) AND GLOMERULAR FILTRATION RATE (GFR)

Dog	GFR (ml/min)		TmG (mg/min)		TmG/GFR × 100	
	A	B	A	B	A	B
IK	45.8	60.6	204	229	445	378
DO	49.9	61.5	225	238	452	388
TA	69.4	95.7	230	288	332	300
FR	41.0	58.3	179	230	436	398
LO	80.9	89.2	294	297	363	333

A: Control values.

B: After administration of bovine growth hormone (BGH).

Table 2. EFFECT OF BOVINE GROWTH HORMONE ON MAXIMAL TUBULAR REABSORPTION OF PHOSPHATE (TmPO<sub>4</sub>) AND GLOMERULAR FILTRATION RATE (GFR)

Dog	GFR (ml/min)		TmPO <sub>4</sub> (mg/min)		TmPO <sub>4</sub> /GFR × 100	
	A	B	A	B	A	B
TR	81.5	47.1	1.22	2.44	3.88	5.19
TA 1	81.4	120.3	3.19	7.01	3.82	5.83
2	93.3	123.6	3.78	5.81	4.06	4.69
FR	54.7	69.4	1.87	3.86	3.42	5.57
LO	91.7	107.7	4.07	5.34	4.44	4.96

A: Control values.

B: After bovine growth hormone administration.

In the experiments with TmPO<sub>4</sub> tubular reabsorption of phosphate increased much more than that of glucose, although the relative increase in GFR was no different from that in the TmG experiment. The ratio TmPO<sub>4</sub>/GFR increased in all experiments.

The action of growth hormone on the kidney for glucose excretion therefore appears to be different from phosphate excretion. Growth hormone tends to favour glucose excretion and to induce phosphate retention.

In physiological states the effect of growth hormone on glucose excretion is not apparent as blood glucose remains far below the renal threshold. In diabetes induced

by growth hormone, however, the action of growth hormone on the renal threshold could be a factor contributing to the glycosuria.

It is highly probable that growth hormone secretion plays a part in the physiological control of blood phosphorus and phosphaturia. The high concentration of phosphorus in the blood, as seen in active acromegaly, may be a result of the tubular effect of growth hormone. It has been shown that the maximum tubular reabsorption of phosphorus per unit of filtrate is increased in active acromegaly<sup>9</sup>.

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### Synthesis of Zeatin, a Factor inducing Cell Division

COMPOUNDS which induce cell division in certain cultures of plant tissue have been termed cytokinins<sup>1</sup>. Zeatin, the first substance of this type to be isolated from a plant extract, has been shown to be 6-(4-hydroxy-3-methylbut-*trans*-2-enyl)aminopurine<sup>2</sup>. Shaw and Wilson<sup>3</sup> prepared 4-amino-2-methylbut-*trans*-2-en-1-ol from methyl tiglate and condensed it with 6-methylmercaptapurine to yield zeatin. An alternative synthesis for zeatin is now described. Values of the melting and boiling points are recorded for new compounds only. These all gave satisfactory elemental analyses.

Acetone was condensed with cyanoacetic acid in cyclohexylamine (compare ref. 4 where piperidine, a much more expensive solvent, is used) to give 3-methylcrotononitrile which was then brominated with *N*-bromosuccinimide, using benzoyl peroxide as catalyst, in boiling carbon tetrachloride to yield 4-bromo-3-methylcrotononitrile (I), boiling point 58°/0.9 mm. Since zeatin has a *trans* configuration, (I) and other intermediates are probably also *trans* structures. This was confirmed as follows: hydrolysis of (I) with 2 normal sodium hydroxide at 70° yielded an acid (isolated as the *S*-benzyl-*iso*-thiuronium salt, melting point 139°) identical with the 4-hydroxy-3-methylcrotonic acid which is prepared by alkaline hydrolysis of methyl 4-bromo-3-methylcrotonate<sup>5</sup> and which appears to possess a *trans* configuration<sup>6</sup>.

A mixture of (I), potassium acetate and acetic acid when heated under reflux yielded 4-acetoxy-3-methylcrotononitrile (II), boiling point 60°/0.08 mm. This was also prepared in much lower yield by the oxidation of 3-methylcrotononitrile with selenium dioxide in acetic anhydride. 4-Hydroxy-3-methylcrotononitrile (III), boiling point 74–75°/0.05 mm, was prepared by hydrolysis of (II) with dilute potassium hydroxide and then refluxed with 2,3-dihydropyran containing *p*-toluenesulphonic acid to yield the acetal 3-methyl-4-(tetrahydropyran-2-yloxy)crotononitrile (IV), boiling point 84°/0.08 mm. Reduction of (IV) with aluminium lithium hydride in ether gave the corresponding amine (tetraphenylborate, melting point 68–70°) which was hydrolysed at pH 0.6

at 20° to yield 4-amino-2-methylbut-2-en-1-ol (V). Attempts to prepare (V) by aluminium lithium hydride reduction of (II) yielded the saturated amine 4-amino-2-methylbutan-1-ol. (V) was condensed with 6-chloropurine in boiling butan-1-ol and the product (VI) recrystallized from ethanol.

Chromatography, spectroscopy (infra-red, ultra-violet, and nuclear magnetic resonance), mixed melting points, cell-division-promoting activity in carrot phloem cultures and oxidative degradation established that recrystallized (VI) and zeatin were identical. The structure already assigned to zeatin is thus confirmed. The reactions outlined above may be useful for preparing other unsaturated hydroxyamines.

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### Fatty Acids of Fruiting Bodies of Basidiomycetes

ANALYSIS of the fatty acids of the mycelial lipids of a range of fungi, including basidiomycetes, grown in submerged culture on a defined medium has been described<sup>1</sup>. The fatty acids of some basidiomycete fruiting bodies, collected in the wild, have now been analysed.

A cluster of mushrooms (*Agaricus campestris*) and a group of toadstools (probably species of *Collybia*), each apparently representing one colony and homospecific, were gathered from pasture used by cows. A lobe of a bracket fungus (species of *Fomes*) was taken from the hawthorn on which it was growing. (*Agaricus* and *Collybia* are both in the family Agaricaceae, while *Fomes* is of the Polyporaceae.) The fructifications were washed, dried, macerated and extracted; the extract was saponified and the fatty acids were recovered, methylated and analysed by gas liquid chromatography, as before<sup>1</sup>. Table 1 shows the gross fatty acid compositions of the lipids extracted.

The stipe can be separated cleanly from the pileus in the toadstool and so these tissues were extracted separately for analysis of their fatty acids. The results are shown in Table 2. In the saponified lipids from each of the three types of fruiting body, there was a high proportion of linoleic acid, but little oleic acid. Previous results indicated that the fatty acids of mature basidiomycete mycelia characteristically included roughly equal parts of oleic and linoleic acid as major components<sup>1,2</sup>.

Lipids have been extracted from basidiomycete mycelia<sup>2</sup>, fruiting bodies<sup>3</sup> and certain pigmented surfaces<sup>4</sup>.

Table 1. FATTY ACIDS OF BASIDIOMYCETE FRUITING BODIES

	<i>Agaricus campestris</i>	Probably species of <i>Collybia</i>	Probably species of <i>Fomes</i>
Dry weight taken (g)	7.0	17.11	24.50
Saponifiable lipid (g)	0.35	0.47	0.51
Fatty acids (moles per cent)			
12:0	2.2	2.5	1.2
14:0	1.0	1.8	1.2
14:1	1.8	1.4	1.0
16:0	15.0	13.1	12.4
18:1	4.0	1.9	2.1
18:0	3.0	1.7	3.1
18:1	5.0	6.3	4.6
18:2	65.5	54.3	70.3
18:3	—	17.0	4.1
20:2	1.2	—	—
20:3	1.3	—	—

They have been divided into neutral and polar or phospholipid fractions and analysed with respect to the fatty acids produced by saponification. In each case, the neutral lipids contained a high proportion of oleic acid (31 per cent to 56 per cent) plus some linoleic acid (24 per cent to 32 per cent), while the polar or phospholipid fractions contained 67 per cent to 70 per cent linoleic acid, but very little oleic acid.

These results suggest that, apart from certain fluctuations which may occur in early growth<sup>2</sup>, the overall fatty acid composition of the lipids of a basidiomycete depends on the relative proportions of neutral and of polar lipids present; each gross fraction has its own characteristic fatty acid composition which does not vary markedly from species to species, or between vegetative and reproductive tissue in a given species<sup>2</sup>.

Table 2. FATTY ACIDS FROM PILEUS AND STIPE OF TOADSTOOL

	Pileus	Stipe
Dry weight taken (g)	8.56	8.61
Saponifiable lipid (g)	0.38	0.27
Fatty acids (moles per cent)		
12:0	1.9	3.7
14:0	1.5	2.6
14:1	1.3	1.4
16:0	14.4	10.4
16:1	1.5	2.8
18:0	1.5	2.0
18:1	3.9	11.1
18:2	62.0	39.0
18:3	12.0	27.0

The toadstool used was probably a species of *Collybia*.

The polar lipid fractions of basidiomycetes are evidently rich in linoleic acid, and so the high contents of linoleic acid reported here could be ascribed to the presence of a large polar lipid fraction, which is known to be the case in other basidiomycete fruiting bodies. The proportions of neutral and of polar lipid present in the fruiting bodies of *Clitocybe illudens* were found to be in the ratio 1:2 (ref. 3). In *Amanita muscaria* pigmented surfaces the ratio was 2:1 (ref. 4). Much of the lipid from the mycelium of a basidiomycete (*Tricholoma nudum*), especially in older cultures, is, however, neutral, with little polar lipid present<sup>2</sup>. It is evidently as a consequence of this that the lipids of basidiomycete mycelia are generally low in linoleic acid<sup>1,5</sup>, for in the mycelium of *T. nudum* any linoleic acid which is present is concentrated in a small phospholipid fraction<sup>2</sup>.

Table 2 shows that the composition of the fatty acids from the stipe is intermediate between that of the pileus and that reported for the mycelia of other basidiomycetes<sup>1,2</sup>.

The lower proportion of linoleic acid relative to other fatty acids found in vegetative as opposed to reproductive tissue of basidiomycetes suggests that linoleic acid may have a specific role in the reproductive mechanism of these fungi. The probability that this linoleic acid is largely combined in a polar lipid fraction makes a specific function more likely, by analogy with animals and plants the polar lipids of which are rich in certain polyunsaturated fatty acids which are believed to have specific functions related to their structure.

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## Neuraminidase-like Enzyme present in *Mycoplasma gallisepticum*

*Mycoplasma gallisepticum* is the agent responsible for chronic respiratory disease in chickens. It will agglutinate mammalian and avian erythrocytes, and this phenomenon has been used to detect *M. gallisepticum* haemagglutinating inhibition antibodies. Two antigens have been used in the haemagglutination-inhibition (HI) test. The first will here be referred to as the "prepared antigen".\* This was obtained by growing the organism in broth, centrifuging and resuspending the organism in equal parts of glycerol and phosphate buffered saline (pH 7.0), freezing and storing until required for use, when it is further diluted in phosphate buffered saline. The other antigen is a rapidly growing 24 h broth culture grown in PPLO broth, 10 per cent horse serum, 1 per cent yeast autolysate, and 0.1 per cent dextrose, with the pH adjusted to 7.8 (ref. 1).

When the prepared antigen was used in the HI test, haemagglutination was often inhibited by normal chicken serum<sup>2</sup>, and the titres ranged from 1/5 to 1/20 when titrated against four haemagglutination (HA) units. We have found that these non-specific HI titres obtained with the prepared antigen were removed when the sera were first treated with neuraminidase or sodium metaperiodate. Non-specific HI titres were not obtained when the broth antigen was used<sup>1,3</sup>.

It is well known that  $\alpha$ -inhibitors of influenza virus haemagglutination have a similar chemical structure to erythrocyte receptors for myxoviruses. These inhibitors exist in chicken serum<sup>4</sup> and it seems likely that the same  $\alpha$ -inhibitors caused the non-specific inhibition when the prepared antigen of *M. gallisepticum* was titrated against normal serum in the HI test. A neuraminidase-like enzyme was present in *M. gallisepticum*; it was active in the broth antigen but inactive or absent in the prepared antigen. The non-specific inhibition, seen with the prepared antigen, may have resulted from the effect of phosphate buffered saline on *M. gallisepticum*. Alternatively, it may have occurred because the neuraminidase-like enzyme is extracellular, as with certain clostridia, and was removed when the prepared antigen was made up. When *M. gallisepticum* was suspended in phosphate buffered saline, 81 per cent of the organisms were dead within 4 h. It seems probable that the prepared antigen diluted in phosphate buffered saline behaves in much the same way as indicator influenza virus when they are titrated against normal chicken serum.

The effect of neuraminidase treatment on the agglutination of chicken erythrocytes by *M. gallisepticum* was investigated. Neuraminidase diluted in calcium saline was added to a 1 per cent suspension of chicken erythrocytes; the mixture was then incubated at 37° C. Aliquots were removed at intervals, centrifuged and washed three times in sodium citrate saline, and resuspended in physiological saline to a concentration of 1 per cent. Chicken erythrocytes suspended in calcium saline without neuraminidase were treated similarly and served as controls. The haemagglutination titres of treated and control cells by *M. gallisepticum* were then assessed, using 4 HA units of prepared antigen. Receptors for *M. gallisepticum* were not destroyed after incubation for 8 h at 37° C.

The failure of neuraminidase to remove receptors to *M. gallisepticum* was in marked contrast to the successful removal of receptors to the WR1 strain of avian mycoplasma; the receptors to WR1 were removed after the erythrocytes had been treated for 1 h with neuraminidase<sup>5</sup>. When the WR1 strain was used in the HI test, non-specific inhibition of agglutination was seen with both the prepared and broth antigens when these were titrated against normal chicken serum. This neuraminidase-like enzyme thus appears to be absent in the WR1 strain.



Neuraminidase activity of myxoviruses has been attributed a role in cell penetration<sup>6,7</sup>. The pathogenicity of *M. gallisepticum* may be attributed to the neuraminidase-like enzyme; this enzyme is absent in the WR1 strain and this organism is non-pathogenic for chickens<sup>8</sup>. The ease with which cell receptors to avian mycoplasma are removed by neuraminidase may also be an indication of pathogenicity. It is interesting to note that an antibody response to *M. anatis* in ducks was not stimulated without the presence of an influenza A virus<sup>9</sup>.

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### Stability of Dextran during Prolonged Storage

MEASUREMENTS made on clinical dextran solutions after storage at 4° C for 5 years revealed no significant deterioration<sup>1</sup>. These measurements have now been repeated after 10 years. Dextran solutions A and B were made in the United States and C and D in Great Britain. Where applicable the methods of testing described in the *British Pharmacopoeia* were used and, as before, the specific optical rotation of each dextran was used in calculating the concentrations of solutions for the viscosity measurements. Table 1 records the intrinsic viscosity measurements. Dextran A, B and C showed relatively small changes even after 10 years; there was certainly no sign of progressive change. Dextran D had given some indication of change at 5 years. Two bottles of this dextran were examined after 10 years. The intrinsic viscosities were 0.352 and 0.356; their mean is entered in Table 1.

The renal excretion of these dextrans in rabbits is shown in Table 2. Two rabbits were used for each determination (1965) and the daily results averaged; Table 2 shows totals for 3 days after injection. After 5 years storage there appeared to have been an increase in the renal excretion of dextran A, but this was not corroborated by other measurements made at the time or more recently.

Table 1

Dextran	Intrinsic viscosity at 25° C		
	1954	1959	1965
A	0.249	0.248	0.248
B	0.254	0.251	0.252
C	0.304	0.383	0.394
D	0.362	0.326	0.354

Table 2

Dextran	Percentages of injected dose excreted		
	1954	1959	1965
A	37.2	47.6	43.8
B	43.1	39.1	46.7
C	13.5	14.6	15.0
D	15.9	15.4	21.1

Table 3. PLASMA CONCENTRATION OF DEXTRAN AS A PERCENTAGE OF CONCENTRATION 10 MIN AFTER INJECTION

Dextran		Day			
		1	2	3	4
A	1954	23.8	11.7	0.4	0.0
	1959	29.6	15.2	2.25	0.8
	1965	23.5	11.3	2.1	0.1
B	1954	29.2	13.8	3.6	0.0
	1959	30.0	14.1	2.0	0.6
	1965	29.4	12.4	2.9	0.1
C	1954	56.8	34.6	12.3	3.8
	1959	61.0	40.9	18.3	10.0
	1965	51.7	33.5	9.2	6.9
D	1954	60.5	40.2	22.8	12.8
	1959	56.6	38.7	22.0	—
	1965	42.0	30.4	20.1	10.3

The retention of each dextran in the plasma was determined by taking the average of daily estimations in the two rabbits. Table 3 shows that little if any change had occurred in the dextrans, apart possibly from dextran solution D, affecting their retention in the circulation.

The original and subsequent measurements of intrinsic viscosity, renal excretion, and retention in the plasma of dextran solution D may indicate that some hydrolysis occurred.

From our observations we conclude that during the 10 year period there was little, if any, change in the molecular composition, with the possible exception of dextran solution D. The change observed in this solution, however, would not be noticeable in clinical use. These results were obtained with 6 per cent dextran in 0.9 per cent sodium chloride in glass bottles with rubber closures and are not necessarily valid for other containers or other solvents, for example, dextran in 5 per cent glucose solution. Probably a dextran of molecular weight intermediate between those tested would be equally stable; measurements have not been made on dextrans of lower molecular weight. Little or no deposit was seen in any of the bottles.

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## HAEMATOLOGY

### Inheritance and Sub-unit Composition of Haemoglobin in the Horse, Donkey and their Hybrids

INVESTIGATIONS of mammalian haemoglobins have given much biological information<sup>1-3</sup>, but although notable advances, particularly in human haemoglobins, have been made, many aspects of the subject remain obscure or hypothetical. The genetics of haemoglobin is particularly in need of clarification. Valuable genetic information has been acquired from the use of model hybrid systems<sup>4,5</sup>, and the findings complement information laboriously obtained from investigation of occasionally suitable human families. We have investigated the pattern of inheritance of haemoglobins in the Equidae and their interspecific hybrids, in which the karyotypes of the individual species have been well defined<sup>6</sup>, by electrophoresis, peptide mapping, and separation of globin sub-units.

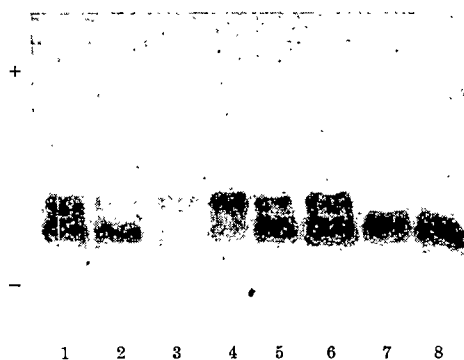


Fig. 1. Electrophoretic patterns on cellulose acetate of whole haemoglobins from the horse, donkey and their hybrids. (1) Female hinny; (2) male hinny; (3) female horse; (4) male horse; (5) female mule; (6) male mule; (7) female donkey; (8) male donkey.

Nine horses, five donkeys, two hinnies and eleven mules were used. Haemoglobin solutions were prepared according to Ingram, with minor modifications<sup>7</sup>, and stored at  $-20^{\circ}\text{C}$  until used. Electrophoresis of these solutions was carried out on cellulose acetate using a *tris*-hydrochloric acid buffer<sup>8</sup>, ethylene diamine tetraacetic acid-borate-*tris* (EBT) buffer at pH 8.8 (ref. 9). Cellulose acetate (Fig. 1) and starch gel electrophoresis (Fig. 2) of haemoglobin of horse, mule and hinny showed two bands, the rates of migration of which were identical in the three species. The donkey showed a single band only, corresponding to the cathodic band of the horse, mule and hinny. Sex differences were not noted.

Peptide mapping was carried out by a modification of Ingram's procedure<sup>7</sup>, that is, heat denaturation and tryptic digestion, chymotrypsin in the enzyme preparation being inhibited by 0.5 per cent 1-1-tosyl amido-2-phenylethyl chloromethyl ketone (TPCK)<sup>10</sup> at  $37^{\circ}\text{C}$  for 48 h, followed by electrophoresis on Whatman No. 3 paper (18 in.  $\times$  22 in.) in pyridine, acetic acid and water (10:0.4:90), followed after drying by descending chromatography for 15 h with pyridine, iso-amyl alcohol and water (30:30:35) as solvent. The colour was developed with ninhydrin after drying. Peptide maps yielded some differences: the donkey, mule and hinny were almost identical, but the horse differed from these in several peptides.

Globin was prepared from the dialysed haemolysate and separated electrophoretically on starch gel using a urea-veronal buffer at pH 8.0, according to Chernoff and Pettit<sup>11</sup>, to determine the number and the electrophoretic properties of the haemoglobin sub-units. Similarly, globin was prepared from the various haemoglobin solutions after electrophoresis on starch gel using EBT buffer<sup>9</sup>, by eluting each haemoglobin separately. Globin prepared from the eluate of each band was separated electrophoretically on urea-veronal starch gel as mentioned previously. The sub-units of each haemoglobin band could then be identified by staining with 1 per cent amido black B as described by Fine<sup>12</sup>.

The separation of sub-units of globin prepared from whole haemoglobins showed that the horse, mule and hinny globins separated into three bands—two cathodic and one anodic (Fig. 3). The globin of the donkey differed in having only two bands. When compared with human haemoglobins A and S as reference standards, the faster cathodic band of all four species migrated at the same rate as the  $\alpha$ -chains of human haemoglobin A. The anodic bands in all cases moved more rapidly than the  $\beta$ -bands of haemoglobin A.

The globin recovered from the faster haemoglobin bands of all four animal types on EBT-starch gel consisted primarily of the  $\beta$  sub-units and the slow  $\alpha$  sub-units. The globin prepared from the slower bands contained  $\beta$  sub-units and the faster  $\alpha$  sub-units. These results were consistent in horse, mule and hinny. Globin derived from the single haemoglobin species of the donkey behaves identically with that of the slow bands of the other animals (Fig. 4).

The data presented demonstrate that the two haemoglobins of horse, mule and hinny are composed of three polypeptide sub-units the  $\beta$ -band of which is common to both. This is clearly shown by the results of urea-starch gel electrophoresis (Fig. 3), in which the three sub-

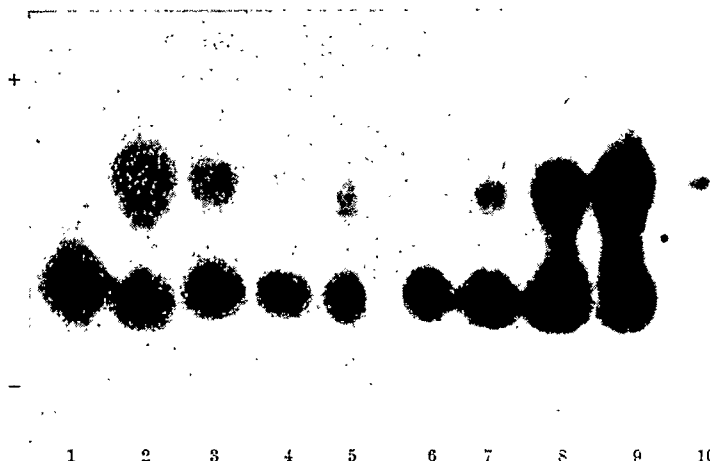


Fig. 2. Electrophoretic patterns on starch gel of whole haemoglobins of horse, donkey, and their hybrids, and artificial mixtures of horse and donkey haemoglobins in varying proportions. (1) Male donkey; (2) male horse; (3) male hinny; (4) male mule; (5) horse: donkey 50:50; (6) horse: donkey 20:80; (7) horse: donkey 40:60; (8) horse: donkey 60:40; (9) horse: donkey 80:20; (10) horse: donkey 80:20.

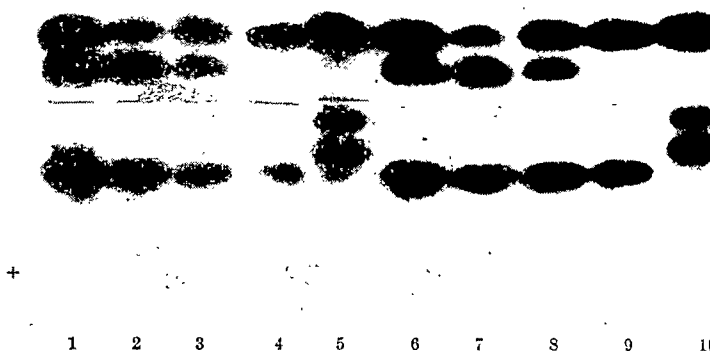


Fig. 3. Separation on urea-starch gel of globin sub-units derived from human A and S, donkey, horse, mule and hinny haemoglobins. Globin sub-units prepared from: (1 and 6) mule; (2 and 7) horse; (3 and 8) hinny; (4 and 9) donkey; (5 and 10) human A and S.

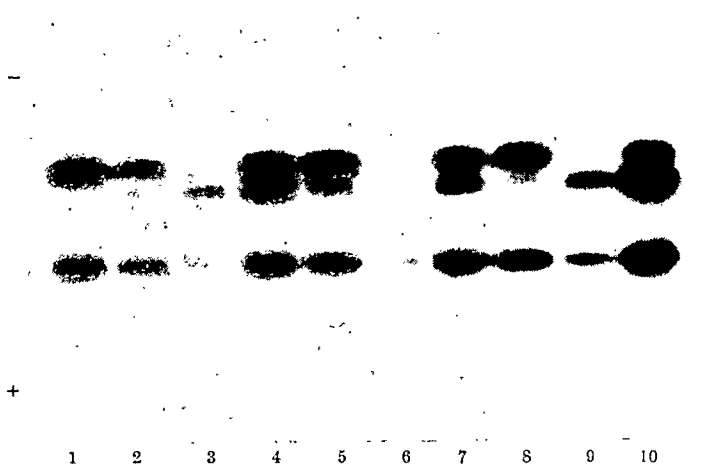


Fig. 4. Identification on urea-starch gel of the globin sub-units derived from the fast and slow haemoglobin bands of the mule and hinny. (1) Donkey globin-whole haemoglobin; (2) slow band, male mule; (3) fast band, male mule; (4) male mule globin, whole haemoglobin; (5) slow band, female mule; (6) fast band, female mule; (7) female hinny globin, whole haemoglobin; (8) slow band, female hinny; (9) fast band, female hinny; (10) horse globin, whole haemoglobin.

units are illustrated. If globin from individual haemoglobin bands is similarly treated, each separates into an anodic and a cathodic band. The cathodic bands are electrophoretically identical, while the anodic bands migrate at different rates (Fig. 4).

Whole haemoglobins separated on paper electrophoresis have given results very similar to those found on EBT-starch gel, and suggest that in the mule and hinny three haemoglobins could exist—two from the horse and one from the donkey<sup>13</sup>. This matter could not be resolved satisfactorily by electrophoresis of mixtures of horse and donkey haemoglobin in varied proportions (Fig. 2).

Electrophoretic study of the globin sub-units derived from donkey haemoglobin yields two sub-unit fractions identical in mobility to the fractions of the comparable haemoglobin band of the horse, but this does not eliminate the possibility of electrophoretically silent differences. That human and horse  $\alpha$ -chains, which are known to be chemically different, migrate identically in this system supports the probability of biological differences. Results of peptide mapping, though not conclusive, also lend support.

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## Interaction of Plasma Kinins and Granulocytes

KININS are polypeptides derived from naturally occurring plasma protein substrates (kininogen). The known kinins have been synthesized and are nonapeptides, decapeptides or undecapeptides<sup>1</sup>. Five principal pharmacological actions have been attributed to plasma kinins: stimulation of some smooth muscle, increase in vascular permeability, vasodilation, leucotaxis and induction of pain<sup>2</sup>. These actions are the cardinal manifestations of inflammation. It is not surprising, therefore, that kinins have been implicated among the mediators of the response of tissue to injury. The nonapeptide (bradykinin) can evoke an inflammatory response when applied to subcutaneous tissues<sup>3</sup>. Kinin levels increase at the site of inflammation caused by burns<sup>4</sup>, and during acute arthritis<sup>5</sup>.

We investigated the interaction between kinins and leucocytes, the principal cellular component of inflammation, and found that granulocytes caused a rapid but transient liberation of kinins from whole plasma and purified plasma substrate. The kinins formed in this

way were destroyed slowly by intact granulocytes or granulocyte fragments. Neither granulocyte metabolism nor phagocytosis was affected by bradykinin. Using these observations, we have formulated a model of the interaction of granulocytes and the plasma kinins in inflammation.

Leucocytes were obtained from the heparinized venous blood of normal subjects, patients with polycythaemia vera and patients with arteriosclerosis. The preparation of the samples has been described previously<sup>6,7</sup>. The separation of lymphocytes and the *in vitro* incubation conditions have also been described in detail<sup>6,7</sup> and were modified only by the use of Eagle's minimal essential medium<sup>8</sup> containing 25 mg/ml. human albumin as the suspending medium. The methods used to measure the conversion of glucose-1-<sup>14</sup>C to <sup>14</sup>CO<sub>2</sub>, incorporation of <sup>14</sup>C-amino-acids into protein, and of tritiated uridine into ribonucleic acid, lactate production, granulocyte acid-phosphatase activity, and the quantitation of the phagocytosis of polystyrene particles by granulocytes have all been reported<sup>6</sup>.

Various cells or cell fractions were incubated with (1) plasma; (2) purified  $\alpha_2$  globulin substrate (kininogen) prepared by a minor modification of the method of Webster and Pierce<sup>9</sup> and free of kininase and kinin generating activities; or (3) with synthetic bradykinin. The concentration of biologically active peptides was measured by bioassay<sup>10</sup> using synthetic bradykinin as the standard. The peptides were characterized by their insensitivity to trypsin, their inactivation by chymotrypsin and by their hypotensive effect on rats. The concentration of kininogen in plasma or substrate was calculated from the difference between kinin levels before (spontaneously formed kinin) and after the trypsin reaction described by Diniz and Carvalho<sup>11</sup>. Because kinins were simultaneously released from the substrate and destroyed by the activity of plasma and granulocyte kininase, their concentration at any time was a function of these two opposing processes. Consequently, the fall in substrate concentration was used as a measure of the total generation of kinin.

The addition of leucocytes (60–80 per cent granulocytes and 20–40 per cent lymphocytes) to cell-free plasma or serum at pH 7.4–7.6 increased the plasma kinin concentration two to three times and decreased the substrate about the same amount (Fig. 1 and Table 1, Series 1). The changes occurred within 5 min of addition of the cells, both at 20° and 37° C. Only white cell populations containing at least  $0.5 \times 10^6$  granulocytes/ml. of incubate produced kinin. Normal and neoplastic lymphocytes (94–98 per cent lymphocytes and 1–2 per cent neutrophils) were inactive (Fig. 1) at concentrations up to  $3 \times 10^7$  cells/ml. The generation of kinin was proportional to the concentration of granulocytes and occurred at physiological granulocyte levels (Table 1, Series I). Thus, neutrophils and myelocytes, at concentrations of  $3 \times 10^6$ /ml., converted an amount of substrate corresponding to

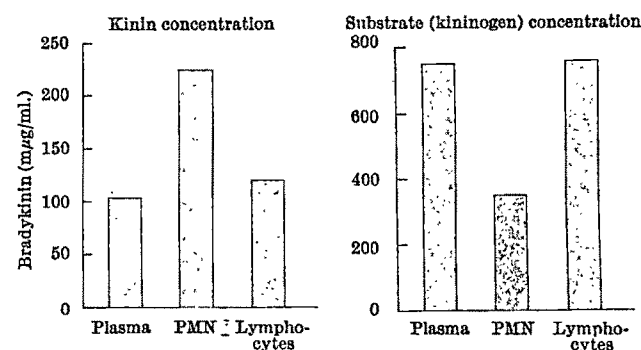


Fig. 1. A representative experiment depicting the effects of addition of various leucocytes to plasma. The kinin concentration rose and kininogen concentration fell after granulocytes were added to plasma (left). • Lymphocytes did not alter either concentration.

approximately 50 per cent of the initial plasma concentration to kinin within minutes.

To determine whether granulocytes were capable of generating kinin directly from substrate, well washed cells ( $3 \times 10^6$ /ml.) were added to purified kininogen (1,000  $\mu\text{g}/\text{ml}.$ ) suspended in saline. Within 30 min, the cells had converted 750  $\mu\text{g}/\text{ml}.$  of substrate to active peptide (Table 1, Series II).

Generation of kinin by granulocytes appeared to require protein and RNA synthesis and an intact glycolytic pathway as this activity was partially inhibited by  $2 \times 10^{-2}$  molar sodium fluoride, by  $4 \times 10^{-5}$  molar puromycin and by 5  $\mu\text{g}/\text{ml}.$  actinomycin D (Table 1, Series III). These levels of puromycin and actinomycin inhibit 90–95 per cent of protein and RNA synthesis, respectively, in granulocytes within 10 min. Kinin generation by white cells was completely inhibited by  $2.6 \times 10^{-6}$  molar cortisol but not by corresponding concentrations of deoxycorticosterone (Table 1, Series III).

The kinin formed when granulocytes were added to plasma disappeared within 60–120 min. The peptides could have been destroyed or inactivated by either plasma kininase or leucocyte kininase. When we incubated 2  $\mu\text{g}/\text{ml}.$  of synthetic bradykinin with  $3 \times 10^6$ /ml. well washed leucocytes at 37° C, the following observations were made (Table 2). Intact granulocytes but not lymphocytes had kininase activity and destroyed approximately 0.5–0.9  $\mu\text{g}$  of bradykinin/h/ $10^6$  cells. Kininase activity was not affected by incubating intact leucocytes with  $4 \times 10^{-5}$  molar puromycin or  $6 \times 10^{-5}$  molar cortisol, but was partially inhibited by  $2 \times 10^{-2}$  molar sodium fluoride. When granulocytes were disrupted by sonication in 0.1 molar *tris* buffer, pH 7.4, the specific kininase activity was greatest in that fraction which did not sediment at 100,000g (60 min). The pH optimum of soluble granulocyte kininase activity was between 7.0 and 7.4 (Fig. 2).

Table 1. KININ FORMATION AFTER ADDITION OF HUMAN LEUCOCYTES TO NORMAL PLASMA OR PURIFIED KININOGEN\*

Cell type (concentration $\times 10^6$ /ml.)	No. of experi- ments	Compound added (concentration)	Kinin con- centration (% of control)	Kininogen remaining (%)
Series I				
Substrate: plasma (2,810–4,670 $\mu\text{g}$ kininogen/ml.)				
Granulocytes (3)	11		259 $\pm$ 55	50 $\pm$ 10
Granulocyte con- centration effect				
(0)	3		65 $\pm$ 30	98 $\pm$ 2
(0.65)			87 $\pm$ 57	81 $\pm$ 4
(2.5)			98 $\pm$ 20	61 $\pm$ 7
(5.0)			183 $\pm$ 42	52 $\pm$ 20
(25)			237 $\pm$ 50	37 $\pm$ 9
Lymphocytes (3–100)	4		78 $\pm$ 14	97 $\pm$ 4
Series II				
Substrate: purified kininogen (1,000 $\mu\text{g}/\text{ml}.$ )				
Granulocytes (3)	4		138 $\pm$ 18	29 $\pm$ 10
Series III				
Substrate: plasma (2,810–4,670 $\mu\text{g}$ kininogen/ml.)				
Granulocytes (3)	5	None	290 $\pm$ 50	48 $\pm$ 4
Granulocytes (3)	5	Sodium fluoride ( $2 \times 10^{-2}$ mmoles/ml.)	176 $\pm$ 39	71 $\pm$ 6
Granulocytes (3)	3	Puromycin ( $4 \times 10^{-5}$ mmoles/ml.)	155 $\pm$ 32	77 $\pm$ 11
Granulocytes (3)	3	Actinomycin (5 $\mu\text{g}/\text{ml}.$ )	155 $\pm$ 49	64 $\pm$ 3
Granulocytes (3)	4	Cortisol ( $2.5 \times 10^{-6}$ mmoles/ml.)	114 $\pm$ 8	96 $\pm$ 3
Granulocytes (3)	3	Deoxycorticosterone ( $4 \times 10^{-6}$ mmoles/ml.)	350 $\pm$ 104	37 $\pm$ 9

\* Granulocyte preparations contained an average of 83 per cent neutrophils and 13 per cent lymphocytes. Leucocytes or cell-free incubation medium were added to plasma or purified kininogen and the concentrations of kinins and kininogen were determined after 10 min. The control samples contained no added cells. All values for kinin and kininogen concentration are expressed as the per cent of control and are the mean values  $\pm$  S.E.

Table 2. KININ DESTRUCTION BY HUMAN LEUCOCYTES (KININASE ACTIVITY)\*

Cells added	No. of experiments	Drugs added (mmoles/ml.)	Kinin remain- ing (per cent)
None	6		90 $\pm$ 3
Granulocytes	6		31 $\pm$ 6
Granulocytes	3	Sodium fluoride ( $2 \times 10^{-2}$ )	45 $\pm$ 8
Granulocytes	2	Cortisol ( $2.5 \times 10^{-6}$ )	46 $\pm$ 10
Granulocytes	4	Puromycin ( $4 \times 10^{-5}$ )	33 $\pm$ 9
Lymphocytes	4		84 $\pm$ 6

\* Synthetic bradykinin (1,000–7,980  $\mu\text{g}/\text{ml}.$ ) was added to leucocytes or cell-free medium and incubated for 60–120 min. All values were expressed as the per cent of the bradykinin concentration present at the initiation of incubation and are given as the mean  $\pm$  one S.E. of the mean. In all experiments the cell concentration was  $3 \times 10^6$ /ml.

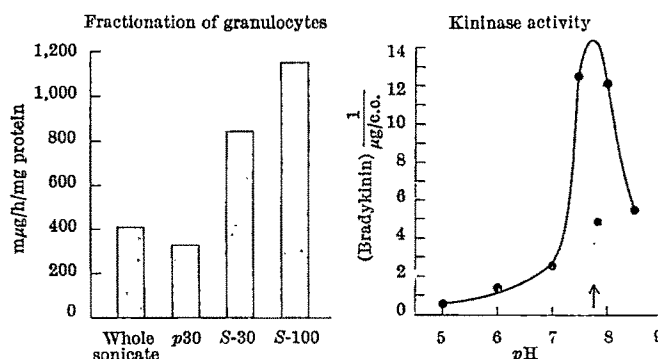


Fig. 2. Study of kininase activity of granulocytes. The S-100 supernatant fraction of sonicated granulocytes contained greatest kininase activity (left). The pH optimum of the kininase was near physiological levels.

Kinins are leucotactic and are concentrated at the sites of inflammation. If phagocytic leucocytes are to function in such an environment, they should be able to withstand exposure to high concentrations of kinins. We determined the incorporation of tritiated uridine into RNA and  $^{14}\text{C}$ -amino-acids into protein, the conversion of glucose-1- $^{14}\text{C}$  to  $^{14}\text{CO}_2$ , the production of lactic acid, acid phosphatase activity, and the phagocytosis of polystyrene particles by granulocytes. Synthetic bradykinin had no significant effect on these functions (Table 3).

Table 3. EFFECT OF BRADYKININ ON GRANULOCYTE METABOLISM\*

Function	No. of experiments	Without bradykinin	With bradykinin
RNA synthesis (c.p.m./ $\mu\text{g}/\text{h}$ )	7	1,184 $\pm$ 69	1,139 $\pm$ 25
Protein synthesis (c.p.m./ $10^6$ cells/h)	6	971 $\pm$ 34	954 $\pm$ 53
Lactate synthesis ( $\mu\text{g}/10^6$ cells/h)	4	134 $\pm$ 7	135 $\pm$ 7
Conversion glucose-1- $^{14}\text{C}$ to $^{14}\text{CO}_2$ (c.p.m./h/ $10^6$ cells)	3	1,630 $\pm$ 112	1,433 $\pm$ 378
Acid phosphatase ( $\mu\text{g}$ phosphorus/3 $\times 10^6$ cells/h)	6	71.2 $\pm$ 4.9	70.3 $\pm$ 1.6
Polystyrene ingestion (per cent of cells containing more than six par- ticles per cell)	3	60	70

\* All incubations were for 60 min after the addition of 50–100  $\mu\text{g}$  synthetic bradykinin/ml. of solution.

The results of our studies indicate that human granulocytes can generate and can destroy kinins, but normal or neoplastic lymphocytes cannot. High concentrations of bradykinin have no effect on any of the observed parameters of the metabolism of granulocytes. Kininase activity is present in disrupted as well as intact granulocytes and is most pronounced near physiological pH values. Puromycin, sodium fluoride and cortisol inhibit kinin production but have relatively little effect on kininase activity. According to a preliminary report<sup>13</sup>, many of these observations appear to be equally true for leucocytes of rabbits obtained in peritoneal exudates.

The mechanism of the release of kinin from substrate by intact granulocytes is not immediately apparent. One might reasonably ask why granulocytes are not continuously generating kinin *in vivo* as they circulate in the bloodstream. A possible answer may be that the manipulations involved in the isolation and incubation of leucocytes alter the cells in some way so that leucocyte kallikrein activity is "unmasked" without altering other granulocyte functions such as phagocytosis. If leucocytes are intentionally damaged by repeated cycles of washing and incubation, however, they lose their kallikrein activity. Therefore, a certain amount of damage to granulocytes may be necessary to unmask kallikrein activity.

We propose the following hypothesis of the interaction of granulocytes and kinins in inflammation. Injured tissue activates Hageman factor which releases kinin<sup>12</sup>; kinins thus released contribute to the signs of inflammation, including capillary and arteriolar dilatation with increased blood flow, and increased capillary permeability, and oedema. The kinins attract intact phagocytes which migrate to the walls of the blood vessels at the site of

injury<sup>3</sup>. The granulocytes, in turn, release more kinin from the available substrate and accelerate the inflammatory process. As the granulocytes accumulate and disintegrate, more kinins are destroyed than are formed and (as in our *in vitro* system) kinin levels fall. The character of a local inflammatory reaction would thus reflect complex interrelationships between kinins and granulocytes and undergo a gradual transition from the manifestations of kinin action to those of leucocyte accumulation.

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## PHARMACOLOGY

### Blockade by Propranolol of the Effect of Isoprenaline on Heart Rate and its Reversal by Methamphetamine

THE adrenergic receptors have been broadly classified into two groups: alpha and beta<sup>1</sup>. Adrenergic receptors present in the mammalian cardiac muscle are now thought to belong to the beta type, and to initiate stimulation when occupied. Isoprenaline<sup>2</sup> is the catecholamine which best fits the beta receptor. The recently introduced drug propranolol hydrochloride (I.C.I. 45520; 'Inderal') is a

electrocardiograph. All solutions were made up in distilled water.

In eight dogs, isoprenaline sulphate 1 µg/kg, injected intravenously over a period of 10 sec (10 µg/ml. solution), produced a marked increase in heart rate (mean  $32.29 \pm S.D.$  7.92 per cent). There was a marked fall in blood pressure (mean 52, range 42–68 mm of mercury). Both the heart rate and the blood pressure returned to normal in 5–7 min. After recording two control responses to isoprenaline in each animal, a 0.1 per cent solution of propranolol hydrochloride 0.3 mg/kg was injected intravenously. This drug produced a distinct decrease in the heart rate. The maximal effect was seen in about 5 min. 10 min after administration of propranolol, the injection of isoprenaline was repeated. There was no increase in heart rate in six experiments, while in two experiments the rate was increased by 6 and 12 beats/min. The heart rate did not return to control levels in either of these experiments (Table 1). The mean reduction in the effect of isoprenaline produced by propranolol was 96 per cent. The fall in blood pressure was completely blocked, and a brief pressor response was seen with isoprenaline (10–15 mm mercury). Another dose of isoprenaline also produced similar results. Methamphetamine 0.3 mg/kg, injected intravenously (0.1 per cent solution), produced a sustained rise in blood pressure (28–46 mm mercury). The heart rate increased gradually in five experiments, and in one of these experiments it reached the control level before propranolol was administered. In the remaining three experiments there was no increase in heart rate after methamphetamine. Injection of isoprenaline was then repeated. This drug now again increased the heart rate (mean  $21.10 \pm S.D.$  5.26 per cent). This increase in rate was 34.64 per cent less than that produced in controls before propranolol injection. The results are summarized in Table 1. The blood pressure response was now biphasic; a brief pressor response (10–15 mm mercury) was followed by a brief depressor response (15–20 mm mercury). An increase in the dose of methamphetamine did not fully restore the effect of isoprenaline on heart rate. Similarly, a purely depressor response to isoprenaline could not be restored.

In the remaining four dogs, propranolol completely blocked the effect of isoprenaline on the heart rate. Repeated injections of adrenaline hydrochloride and noradrenaline bitartrate (5 µg/kg), ephedrine sulphate and methoxamine (0.3 mg/kg) failed to restore the isoprenaline response. The reversal of the blocking action of pro-

Table 1. EFFECT OF PROPRANOLOL AND METHAMPHETAMINE ON HEART RATE INCREASING EFFECT OF ISOPRENALINE

Experiment No.	Before propranolol			Heart rate After propranolol*			After methamphetamine†		
	Control heart rate	After isoprenaline ‡	Increase in rate (%)	Control heart rate	After isoprenaline ‡	Increase in rate (%)	Control heart rate	After isoprenaline ‡	Increase in rate (%)
1	178	252	41.52	152	158	3.94	178	240	28.40
2	200	276	38.00	186	186	0.00	186	230	23.65
3	184	240	30.43	150	150	0.00	188	204	21.43
4	200	256	28.00	168	168	0.00	180	214	19.44
5	176	230	30.68	156	156	0.00	160	186	16.25
6	188	214	27.38	150	150	0.00	160	180	20.00
7	150	200	33.33	136	136	0.00	144	178	23.61
8	214	276	28.97	188	200	6.38	200	236	18.00

\* Propranolol 0.3 mg/kg intravenously.

† Methamphetamine 0.3 mg/kg intravenously.

‡ Isoprenaline 1.0 µg/kg intravenously.

potent blocking agent for beta receptors, and it has been shown to block the action of isoprenaline in increasing the heart rate (J. Raventos, personal communication). In the present investigation, methamphetamine was found to antagonize this block, while adrenaline, noradrenaline, ephedrine and methoxamine were ineffective.

The experiments were performed in twelve mongrel dogs of either sex, weighing between 8 and 17 kg. The animals were anaesthetized with pentobarbitone sodium 35 mg/kg injected intravenously. Blood pressure was recorded from the right carotid artery with a mercury manometer. The right femoral vein was cannulated for injection of the drugs. The heart rate was recorded on an

pranolol by methamphetamine thus appears to be specific. The reversal was always incomplete. The mechanism of this reversal, and the effect of other pressor amines is being investigated.

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## PATHOLOGY

## Conjugated Plasma Bilirubin in Jaundice caused by Pigment Overload

WITH overproduction of bilirubin due to haemolytic anaemia, a minor though significant fraction of the plasma bilirubin may exhibit a direct diazo reaction in the absence of detectable liver dysfunction<sup>1</sup>. If the direct-reacting material is in fact conjugated bilirubin<sup>2</sup>, this would support the concept<sup>3</sup> that the secretion of conjugated pigment from the hepatic cell into the bile is a rate limiting step in bilirubin transport across the liver. Chemical identification of this direct-reacting pigment fraction as conjugated bilirubin is difficult, however, because the amounts available for analysis are very small, the extraction procedures are semi-quantitative<sup>4,5</sup> and part of the unconjugated pigment may react directly with the diazo reagent<sup>1,6</sup>.

The appearance of conjugated bilirubin in the plasma was studied in eight intact male Sprague-Dawley rats infused with unconjugated bilirubin at constant rates for 2–12 h. The infused pigment was bound to human albumin or dissolved in murine serum; similar results were obtained with both vehicles. The rates of infusion were selected so that they were below what has been considered the maximal excretory rate for bilirubin in this species<sup>3,7</sup>. Conjugated and unconjugated bilirubin were estimated by the method of Weber and Schalm<sup>8</sup> on blood samples obtained from the tail at hourly intervals. Biliary obstruction as a cause of retention of conjugated bilirubin was investigated with bromsulphophthalein (BSP); 2 h before the end of each experiment 5 mg BSP were injected intravenously and serum samples were analysed at 5, 10, 20, 30 and 45 min thereafter<sup>9</sup>.

Conjugated bilirubin appeared in the plasma within the first hour of infusion, and thereafter the levels of conjugated and total pigment in individual rats remained reasonably constant throughout the experiments (Table 1). In the eight animals the relationship between infused pigment load, total plasma bilirubin and conjugated fraction showed considerable variation (Table 1). In all instances, at least 95 per cent of the injected BSP had disappeared from the plasma in 45 min, indicating unimpaired excretion of the dye<sup>9</sup>.

Table 1. SERUM BILIRUBIN CONCENTRATIONS DURING CONTINUOUS INTRAVENOUS INFUSION OF UNCONJUGATED BILIRUBIN IN RATS

No.	Rate of infusion ( $\mu\text{g}/\text{min}/100\text{ g}$ )	Length of infusion (h)	Serum bilirubin concentrations (mg/100 ml.)		
			Total	Conjugated	Conjugated per cent
1	47	12	12.4 $\pm$ 1.8*	1.6 $\pm$ 0.2*	12.9
2	42	5	10.8 $\pm$ 2.3	1.1 $\pm$ 0.3	10.2
3	40	12	13.8 $\pm$ 1.5	1.2 $\pm$ 0.2	8.7
4	31	7	9.0 $\pm$ 0.8	0.7 $\pm$ 0.2	7.8
5	27	8	10.1 $\pm$ 1.0	0.7 $\pm$ 0.3	6.9
6	23	8	9.9 $\pm$ 0.9	1.3 $\pm$ 0.3	13.1
7	17	9	7.3 $\pm$ 0.7	0.7 $\pm$ 0.1	9.6
8	14	8	3.8 $\pm$ 0.6	0.3 $\pm$ 0.1	7.9

\* Results are expressed as the mean value plus or minus the maximal deviation from the mean for the duration of each experiment.

The polar pigment fraction separated by the method of Weber and Schalm<sup>8</sup> was identified as conjugated bilirubin in the following manner. A rat was infused with <sup>14</sup>C-bilirubin (ref. 10) at a constant rate of 26  $\mu\text{g}/\text{min}/100\text{ g}$  for 2 h and the animal was then exsanguinated. Concentrations of serum <sup>14</sup>C-bilirubin per 100 ml. were: total, 5.9 mg; conjugated<sup>8</sup>, 0.5 mg (that is, 8.4 per cent of total). An aliquot of this serum (2.5 ml.) was given intravenously to a congenitally icteric Gunn rat with an external bile fistula. Gunn rats lack the apparatus for conjugating bilirubin and therefore cannot transfer unconjugated pigment into the bile<sup>11</sup>, but they excrete injected conjugated bilirubin as efficiently as normal rats<sup>3</sup>. During the first hour after injection of the serum, 7.9 per cent of the administered radioactivity was excreted in the bile, and 82 per cent of this label was recovered in <sup>14</sup>C-bilirubin crystallized from this bile sample. By contrast, another

Gunn rat injected with a comparable dose of unconjugated <sup>14</sup>C-bilirubin excreted only 1.3 per cent of the radioactivity in the bile, and most of the label was present in metabolites other than bilirubin<sup>11</sup>.

These findings indicate that with chronic bilirubin overload, the appearance in the plasma of direct-reacting material indeed reflects retention of small amounts of conjugated bilirubin. They further suggest that secretion of conjugated pigment into the bile may become rate-limiting at infusion rates below the maximal excretory capacity of the liver<sup>3,7</sup>.

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## Bile Acids on the Skin of Patients with Pruritic Hepatobiliary Disease

CHOLIC and chenodeoxycholic acids are formed from cholesterol in the liver and are then excreted in the bile to the intestinal tract. There they are broken down to form, for example, deoxycholic and lithocholic acids during an enterohepatic circulation<sup>1</sup>.

Bile acids have been implicated in the genesis of pruritus associated with liver disease because: (1) external biliary drainage relieves pruritus in patients with biliary obstruction<sup>2</sup>; (2) the oral administration of ox bile or bile salts increased pruritus in patients with liver disease and caused pruritus to return in those with biliary fistulae<sup>2,3</sup>; (3) Serum bile acids (SBA) are low in normal individuals but are elevated in patients with pruritic hepatobiliary disease<sup>4,5</sup> and elevated in pregnant women with pruritus during the third trimester<sup>6</sup>. (4) The lowering of SBA by cholestyramine, an anion exchange resin which increases the faecal bile acid excretion<sup>7</sup>, is associated with the relief of pruritus in most patients with incomplete biliary obstruction<sup>8–11</sup>.

Bile acids have not been proved to cause pruritus because: (1) external biliary drainage could as well remove other biliary factors which may be responsible for the itching; (2) commercial preparations of bile salts administered have not been pure and feeding of pure cholic acid produced a high SBA without pruritus<sup>6</sup>; (3) all patients with increased SBA do not have pruritus<sup>6,12</sup>; furthermore, the relief of pruritus by norethandrolone could not be correlated with the level of SBA<sup>12</sup>; (4) cholestyramine might remove other factors which may be responsible for pruritus. Also, relief of itching by cholestyramine without a concomitant decrease in SBA has been reported<sup>9</sup>.

Itching is a subjective response to chemical, mechanical, thermal or electrical stimuli acting on nerve fibres in the subepidermal area<sup>13</sup>. If bile acids are related to pruritus they should be present in the area of these sensory nerve endings. There has been no previous report of bile acids on the skin. This communication presents the identifica-



tion by gas liquid chromatography and mass spectrometry and the quantitation by gas liquid chromatography<sup>14</sup> of bile acids on skin.

The skin of the back was swabbed twice over an area of about 25 × 30 cm with cotton wool moistened with distilled acetone. The cotton was then extracted with chloroform/methanol, 1 : 1 (v/v), and the solution filtered. The filtrate was dried *in vacuo* and submitted to a three stage countercurrent distribution between 50 ml. petroleum ether and 50 ml. 70 per cent ethanol. The 70 per cent ethanol extract was dried *in vacuo* and analysed for bile acids.

With or without previous alkaline hydrolysis, the aqueous solution of free bile acids was acidified, extracted with ethyl acetate and methylated with diazomethane. The methylated bile acids were purified on an aluminium oxide (activity grade IV) column, and partial trimethylsilyl ethers and trifluoroacetates were prepared as previously described<sup>14</sup>.

Table 1. GAS-LIQUID CHROMATOGRAPHY OF DERIVATIVES OF COMPOUNDS EXTRACTED FROM SKIN SURFACE

Reference and skin compound*	Retention times relative to methyl deoxycholate		
	Methyl ester	TMSI	TFA
Lithocholic (3a)†	0.55	0.29	0.45
Skin compound 1	0.52	0.31	0.47
Deoxycholic (3a, 12a)	1.00	0.55	0.65
Skin compound 2	1.00	0.57	0.66
Chenodeoxycholic (3a, 7a)	1.14	0.61	0.83
Skin compound 3	1.15	0.63	0.83
Ursodeoxycholic (3a, 7β)	1.24	0.86	0.92
Skin compound 4	1.20	0.37	0.93
Cholic (3a, 7a, 12a)	2.26	1.24	1.27
Skin compound 5	2.32	1.23	1.26

\* 3 per cent QF-1 on acid washed, silanized 'Gas-Chrom P', 230° C, argon inlet pressure 2.0 kg/cm<sup>2</sup>.

† Greek letters denote orientation of hydroxyl groups at carbons 3, 7 or 12 of cholanic acid. TMSI, partial trimethylsilyl ether. TFA, trifluoroacetate.

Table 2. QUANTITATIVE ANALYSIS OF SERUM AND SKIN BILE ACIDS

Subject	Hepatobiliary diagnosis	SBR mg/100 ml. <1.1	Total μg/ml. 0.69-1.20	Serum bile acids Percentage of total			Total μg 0.66-12.30	Skin bile acids Percentage of total		
				D 21-32	CD 36-63	C 15-40		D 24-49	CD 16-36	C 21-40
Controls (range, 4 subjects)	Normal									
I.G.	Sclerosing cholangitis*	12.0	97.0	1	27	72	42.0	43	23	34
N.L.	Carcinoma of pancreas	10.5	96.4	2	33	65	24.8	19	60	21
		1.8	2.69	4	79	17	0.77	100	—	—
V.M.	Cirrhosis of liver	3.1	65.0	2	71	27	2.15	35	37	28
J.W.	Metastasis to liver	32.1	39.2	1	31	68	7.8	33	30	37

\* Pruritus with excoriations (there were no excoriations on the back).

† 1 month after cholecystojejunostomy and relief of pruritus.

SBR, Serum bilirubin; D, deoxycholic; CD, chenodeoxycholic; and C, cholic acid.

Table 1 shows the retention times relative to methyl deoxycholate of reference standards (as used in previous investigations of this series) and of the peaks on chromatograms of derivatives of the compounds extracted from skin surface. The identifications of deoxycholic, chenodeoxycholic and cholic acids were confirmed by combined

gas liquid chromatography and mass spectrometry, but lithocholic and ursodeoxycholic acids were not positively identified. Bile acids were also found in swabbings of the extremities. In one normal subject and one with pruritus, about 85 per cent of the bile acids on the skin were free (serum bile acids are mostly conjugated)<sup>6,14</sup>.

Table 2 shows the results of the quantitative analyses of the three major bile acids extracted from the skin surface and from serum. In one normal subject, re-extraction of the skin surface immediately after a previous extraction containing 12.3 μg of bile acids yielded no bile acids, but an extraction several days later yielded 8.2 μg. The serum bile acids in normal subjects were as previously reported<sup>14</sup>. Bile acids were not detected on the skin of two additional normal subjects and only deoxycholic acid was seen on the chromatograms from two other controls. Individual bile acids would have been detected if about 0.1 μg were extracted from the skin surface. There were more total bile acids on the skin of the patients with pruritus than on that of normals or of the patients with hepatobiliary disease without pruritus, even when the latter had high SBA. The percentages of individual bile acids on the skin of all subjects were about the same as found in normal serum. Deoxycholic acid, however, was relatively more prominent on the skin than in the serum of patients with hepatobiliary disease (Fig. 1).

Information has not yet been obtained on the secretion and physiological significance of bile acids on the skin. The new finding of bile acids on the skin, however, places them close to the appropriate nerve endings but does not necessarily implicate them in the aetiology of pruritus.

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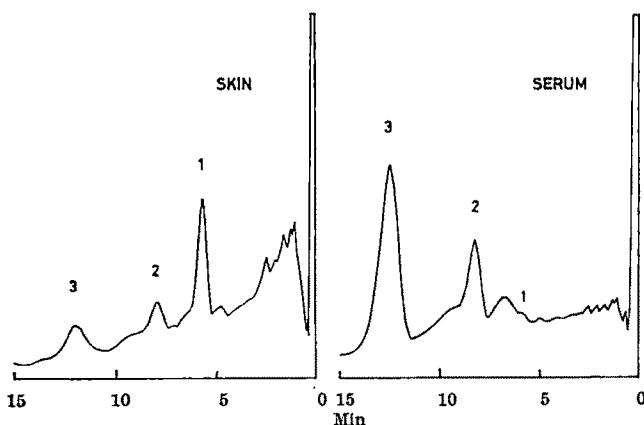


Fig. 1. Gas chromatograms of trifluoroacetates of methylated bile acids prepared from extracts of the skin and serum of subject I.G. (sclerosing cholangitis with pruritus). The derivatives are from (1) deoxycholic, (2) chenodeoxycholic and (3) cholic acids. Degradation products appear just after the peaks of the derivatives of deoxycholic and chenodeoxycholic acids. Conditions: 0.5 per cent CNSI<sup>14</sup> on acid washed, silanized 'Gas-Chrom P', column temperature 211°, argon inlet pressure 1.4 kg/cm<sup>2</sup>.

## PHYSIOLOGY

### Relation between the Airborne Diameters of Respiratory Droplets and the Diameter of the Stains left after Recovery

SEVERAL workers have investigated the numbers and sizes of droplets expelled during expiratory manoeuvres such as coughing or sneezing. When droplets are recovered by settling, by impaction or by some form of air sampler, it is necessary to calculate the airborne diameter of the original droplet from the diameter of the stain left after recovery. Duguid<sup>1</sup> repeated the experiments of Strauss<sup>2</sup> in which large droplets of saliva (1–3 mm in diameter) were measured with a micrometer eyepiece, first while they hung from fine glass capillaries and then again after they had fallen, flattened and evaporated on a slide. When a glass slide was used, the diameters of the original droplets were about half those of the stain marks. Buckland and Tyrrell<sup>3</sup>, using glass slides, calculated the airborne diameter of droplets as  $1/2.5$  of the diameter of the stain marks left after impaction. May<sup>4</sup> described the use of glass slides coated with magnesium oxide in estimating the airborne diameter of droplets; droplets landing on this surface will penetrate the magnesium oxide crust like stones falling into snow, and the diameter of the entry-hole is related to the airborne diameter of the droplet. He investigated this relationship for droplets with airborne diameters ranging from  $20\mu$  to  $250\mu$ , determining the airborne diameter by an "absolute method" which involved trapping the droplet in oil, and measuring it microscopically<sup>5</sup>. He concluded that the airborne diameter averaged 0.858 times the magnesium oxide entry-hole diameter, and that this relationship was independent of the speed of impaction and, within the range investigated, of the diameter of the droplet involved.

Slides coated with magnesium oxide are not suitable for use in air samplers, and have other disadvantages in the investigation of droplets originating in the respiratory tract. Investigations undertaken in our laboratories on expulsion of droplets have led us to use a variety of media on which droplets may be recovered by impaction, by settling, and by air sampling, and to investigate the relationship between airborne droplet diameter and the diameter of stains left after recovery.

The vibrating reed aerosol generator<sup>6</sup> produces a stream of droplets of remarkably uniform size. These droplets were allowed to fall on a series of surfaces which were pulled in succession under the stream (Fig. 1). Six types of recovery surface were used: glass slides, glass slides coated thinly with oil, 'Millipore' filter disks (type HA), 5 per cent chloride-free agar gel with 6 per cent silver nitrate, high-grade bond paper, and glass slides coated to a depth of one millimetre with magnesium oxide. The last was used as the reference surface, and was included in each run. The ratio between magnesium oxide entry-hole diameter and stain diameter was found to be the same whether filtered saliva or saline was used to form the aerosol, and saline solution was used for the majority of the experiments. Dye was added to the material used in the vibrating reed aerosol generator to aid in measurement of the stains; in the case of the silver nitrate-agar surface the stain is formed by the precipitate of silver chloride. Droplets with airborne diameters in the range from  $35\mu$  to  $280\mu$  were investigated, and diameters of stains or entry-holes were measured with a micrometer eyepiece, using  $450\times$  magnification.

The droplet stains on clean glass slides and on oiled glass slides were irregular in size and shape, and it was apparent that many of the droplets had shattered on landing or had dried irregularly.

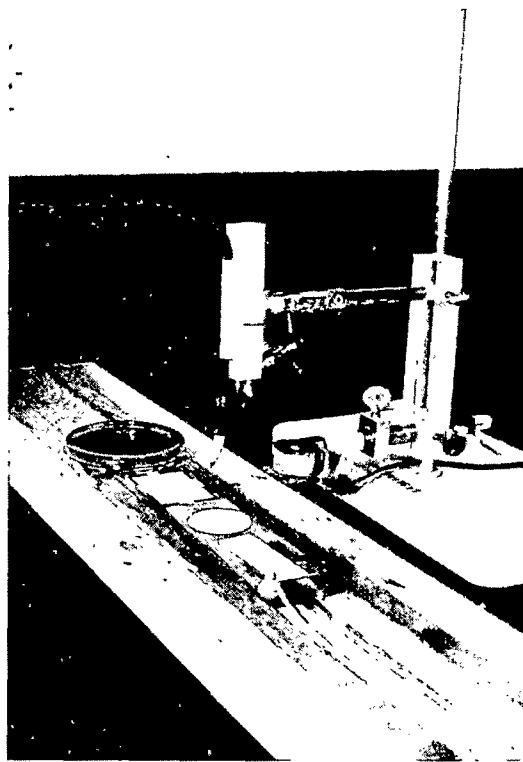


Fig. 1. Vibrating reed aerosol generator and apparatus used to pull various surfaces under stream of uniform droplets.

These surfaces were therefore not considered further. In no case did the diameters of the entry-holes on the magnesium oxide slides vary to a measurable extent during any one run. The diameters of the stains on the three other surfaces ('Millipore' filter disk, silver nitrate-agar gel, and bond paper) were strikingly uniform. Every third droplet stain diameter was measured to a total of twenty measurements; only very rarely did one measurement differ from the others, and then only by one or two microns. The appearances of the entry-holes, and stains are shown in Fig. 2. The relationships between the diameters of the magnesium oxide entry-holes and stain diameters for droplets from the same stream are shown in Table 1 and Fig. 3.



Fig. 2. Appearance of droplet entry-holes on magnesium oxide coated slide (upper left), and of droplet stains on 'Millipore' filter disk (upper right), silver nitrate agar (lower left), and bond paper (lower right). All droplets were from the same stream and measured  $170\mu$  in airborne diameter ( $\times 33$ ).

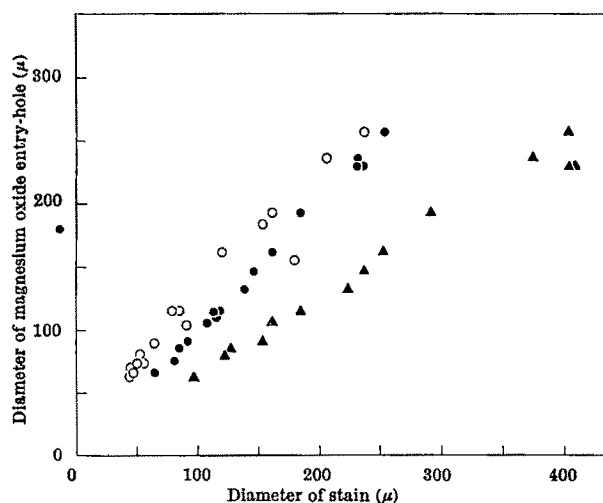


Fig. 3. Scatter diagram showing relationship between diameter of droplet stains (horizontal axis) and diameter of entry-holes on magnesium oxide coated slide (vertical axis).  $\Delta$ , 'Millipore' filter;  $\circ$ , silver nitrate agar;  $\bullet$ , bond paper.

The relationship between magnesium oxide entry-hole diameter and stain diameter appeared to be linear for these three surfaces over the range of droplet size investigated, and linear regression equations were calculated together with 95 per cent confidence intervals and 95 per cent prediction intervals. Analysis of variance showed that the regression equations were each significant at the 0.1 per cent level ( $P < 0.001$ ) by a considerable margin, and thus that the relationships were in fact linear over the range of diameters investigated. Intercepts on the  $y$  axis were significantly

Table 1. DIAMETERS OF STAINS LEFT AFTER SETTLING OF DROPLETS ON VARIOUS SURFACES, COMPARED WITH DIAMETERS OF ENTRY-HOLES OF DROPLETS FROM SAME STREAM ON MAGNESIUM OXIDE COATED SLIDES

'Millipore' filter disk	Silver nitrate-agar gel	Bond paper	Magnesium oxide
97	44	67	64
	48		67
	46		69
	51		74
	51		74
		76	76
122			81
127	53	85	81
			87
154	64	92	90
			92
161	92	108	104
			106
184		115	110
		115	110
		115	115
	81		115
	81		115
223		138	133
237		147	147
	179		156
253	120	161	161
	154		184
292	161	193	193
419		230	230
416		235	230
375	207	230	237
03	237	253	258

Table 2. 95 PER CENT CONFIDENCE INTERVALS AND 95 PER CENT PREDICTION INTERVALS FOR LOWEST, MEDIAN AND HIGHEST VALUES USED IN REGRESSION ANALYSES

	Predicted value	95 per cent confidence intervals lower	95 per cent confidence intervals upper	95 per cent prediction intervals lower	95 per cent prediction intervals upper
'Millipore' filter disk					
Lowest value	29.41	25.48	33.34	19.81	39.01
Median value	63.75	61.40	66.10	54.09	72.82
Highest value	108.23	103.94	112.52	93.48	117.98
Silver nitrate-agar					
Lowest value	30.09	26.16	34.01	18.20	41.98
Median value	45.50	42.50	48.51	33.89	57.12
Highest value	111.01	104.37	117.65	97.97	124.05
Bond paper					
Lowest value	28.28	27.30	29.26	25.75	30.81
Median value	49.64	48.97	50.30	47.21	52.07
Highest value	110.65	109.47	111.83	108.03	113.27

above the origin for the regression lines for 'Millipore' filter disks and for silver nitrate-agar gel.

The regression equations are:

Magnesium oxide entry-hole diameter = diameter of stain on 'Millipore' filter disk  $\times 0.563 + 13.26\mu$ .

Magnesium oxide entry-hole diameter = diameter of stain on silver nitrate-agar  $\times 0.963 + 27.10\mu$ .

Magnesium oxide entry-hole diameter = diameter of stain on bond paper  $\times 1.017 - 2.78\mu$ .

The 95 per cent confidence intervals and 95 per cent prediction intervals were calculated for the predicted values, and the upper and lower limits of these intervals are shown in Table 2 for the lowest, median and highest predicted values for each of the three recovery media.

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## Respiratory Currents of Flatfish

WE have observed specimens of the following species of flatfish (Pleuronectiformes) in an aquarium with a transparent bottom: *Scophthalmus maximus* (L.) (Bothidae); *Limanda limanda* (L.), *Pleuronectes platessa* L. and *Platichthys flesus* (L.) (Pleuronectidae); *Solea solea* (L.) and *Buglossidium luteum* (Risso) (Soleidae). When these fish are resting on the bottom, both opercula move in the manner usual in teleost respiration. When a suspension of carmine in sea water, or ink diluted with sea water, is released from a pipette such that it is drawn into the mouth with the respiratory current, however, it is normally expelled solely through the upper opercular opening (that is, the opercular opening of the ocular side). Hughes<sup>1</sup> thought that a greater volume of water issued from the upper opercular opening than from the lower one of *Pleuronectes* resting on the bottom, but did not find much difference in volume between the two sides. We, however, did not see carmine or ink expelled from the lower opercular opening except when the fish were behaving in an agitated manner.

Our observations suggest that flatfish resting on the bottom pump water over the gills of both sides, but expel it all from the upper opercular opening. This is possible because the opercular cavities communicate ventrally (Fig. 1).

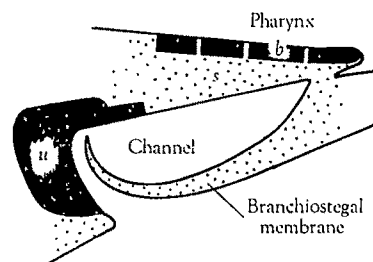


Fig. 1. Diagrammatic median section showing the structures surrounding the channel which connects the opercular cavities of a flatfish. The right side of the figure is ante-ior. b, Basibranchial; s, Inter-branchial septum; u, urohyal.

The urohyal bone is V-shaped, with the angle of the V posterior. The branchiostegal membranes either overlap (for example, *Psettodes*, most Bothidae) or fuse (Pleuronectidae, Soleidae) ventrally. Their posterior edges are folded inwards. They lie inside the V of the urohyal, and enclose a channel connecting the two opercular cavities. We suggest that water pumped through the gills of the blind side passes through this channel to escape through the opercular opening of the ocular side, when the fish is resting on the bottom. We have demonstrated that water can pass through the channel by putting ink in the opercular cavity of the blind side of *Scophthalmus*, *Pleuronectes* and *Solea*, and quickly replacing the fish in the aquarium. Much of the ink is expelled through the opercular opening of the ocular side.

Flatfish spend much of their time in aquaria, and presumably in their natural habitat, resting on the bottom with most of the body covered by sand (see, for instance, ref. 2). Water expelled through the opercular opening of the blind side would then have to percolate through the sand, which might be expected to offer an appreciable resistance to its flow. It would not be impossible to expel water from this side, for the gill openings of *Raia* (Selachii), which often buries itself in sand, are ventral, but it would seem to be disadvantageous.

We have tried to assess the importance of the resistance offered by the sand by experiments with a crude model. The model is a piece of galvanized iron cut to the outline of a *Platichthys* weighing 105 g. A copper tube passes through the model, to which it is soldered, and has a posteriorly directed opening in the position of the siphon of the blind side (the siphon is the small dorsal part of the opercular opening which is not blocked by the in-turned branchiostegal membrane, and through which water can therefore be expelled<sup>3,4</sup>). The internal diameter of the tube is 4 mm. The model is buried about 1 cm below the surface of sand in an aquarium and connected to an aspirator as shown in Fig. 2. Water is added to the aspirator to give a head,  $h$ , of 2.5 cm. The rate of flow of water through the model, as the head fell from 2.5 to 2.0 cm, was found in four experiments to be 30–130 ml./h. A resting fish which weighs 105 g is likely to use at least 2 ml. oxygen/h<sup>5</sup>; it could obtain this by extracting 70 per cent of the dissolved oxygen<sup>5</sup> from 400 ml. of air-saturated water every hour. If the opercular openings were used equally, at least 200 ml. of water should flow through each every hour. The opercula are open for about one third of each cycle<sup>1</sup> so the rate of flow during the part of each cycle when the opercula are open should be about 600 ml./h. Our experiments indicate that such a rate of flow from the lower operculum would require a positive pressure substantially greater than 2 cm water

in the opercular cavity. Hughes<sup>1</sup> found that the maximum positive pressure developed in the opercular cavities of *Pleuronectes* held clear of the bottom was less than 0.5 cm water. It seems likely that the evolution of the channel allowing water to flow from the lower to the upper opercular cavity has substantially reduced the pressure required in the lower cavity during expiration.

In some flatfish the opercular cavities are further connected by a fenestra in the interbranchial septum<sup>6</sup> (the interbranchial septum is shown in Fig. 1). This fenestra probably shares the function of the channel described in this paper. *Lepidorhombus whiffiagonis* (Walbaum) has a large fenestra and a rather small channel.

In *Psettodes belcheri* Bennett the channel described in this paper is poorly developed and may well not be functional. Only a very small ventral portion of each branchiostegal membrane is folded in, and the urohyal is not V-shaped but only slightly emarginate. *Psettodes* is generally agreed to be the most primitive living flatfish<sup>4,7</sup>.

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### Inhibition of Membrane Permeability to Chloride by Copper in Molluscan Neurones

Tauc and Gerschenfeld<sup>1</sup> showed that acetylcholine (ACh) iontophoretically injected on the soma of the so-called *H*-neurones of molluscs produces a transient hyperpolarization (ACh potential) and that the inhibitory post-synaptic potentials (IPSP) recorded in these cells were cholinergic.

Kerkut and Thomas<sup>2</sup> investigated the ionic mechanism of the cholinergic IPSP and the ACh-potential in *H*-neurones of *Helix aspersa*. They showed that these potentials are due to an increase in the permeability of the subsynaptic and somatic membranes to chloride and potassium ions. These changes in the ionic permeability result in an influx of chloride and an efflux of potassium. Chloride ions carry about 90 per cent of the current, while the participation of potassium is minimal. These findings were confirmed in *H*-neurones of *Cryptomphallus aspersa*<sup>3</sup>.

When 10<sup>-5</sup> molar copper sulphate is added to the Ringer solution bathing the outer face of the abdominal skin of frogs it produces a dramatic reduction in the permeability to chloride<sup>4,5</sup>. The change in the potential difference between the two faces of the skin is similar to that observed when chloride is replaced in the saline solution bathing the skin by the non-penetrating sulphate ion.

Frog skin has a very complex structure<sup>6</sup> and copper sulphate may act on many possible sites, so that it seemed interesting to see whether copper sulphate has the same effect in a simpler system such as a neuronal membrane. It has been found that copper sulphate practically abolishes the cholinergic IPSP and the ACh potential in snail *H*-neurones and that this effect is due to a reduction of the membrane permeability to chloride.

Neurones from the isolated perioesophageic ganglionic ring of *Cryptomphallus aspersa* were impaled with double-

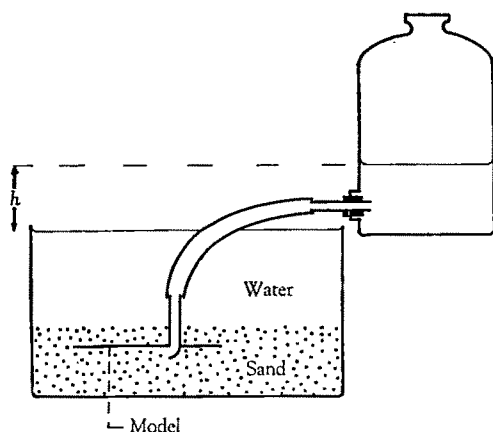


Fig. 2. The apparatus for the experiment with a model described in the text.

barrelled microelectrodes filled with 0.6 molar potassium sulphate. One of the barrels was connected to a standard direct current set and used to record intracellularly the electrical activity of the neurones, while the other was used, when necessary, to drive the membrane potential to desired levels. ACh was injected iontophoretically on the neuronal perikarya from micropipettes filled with molar ACh iodide, and braking currents were always used to avoid desensitization due to drug leakage<sup>7</sup>. The neurones were bathed with a suitable Ringer solution<sup>8</sup> to which copper sulphate was added to give a final concentration of  $10^{-4}$  moles/l. A total of eleven *H*-neurones were examined.

The changes observed in the cholinergic IPSP after adding copper sulphate to the Ringer solution are illustrated in Fig. 1A. A control IPSP is shown in A1 while those recorded 4, 8 and 15 min after the addition of copper sulphate are shown in A2, A3 and A4, respectively. It can be seen that the IPSP became reduced to 20 per cent of its original amplitude. On the other hand, Fig. 1B shows that copper sulphate fails to reduce (B2-4) the non-cholinergic IPSP present in other cells of the same nervous system<sup>9</sup>, which are solely due to an increase in the potassium permeability of the subsynaptic membrane<sup>10</sup>.

The *H*-neurones examined had resting potentials ranging from  $-40$  to  $-50$  mV and were depolarized by 4–5 mV on addition of copper sulphate. Only in one case was a transient hyperpolarization of 5 mV observed. In some cells an increase in the synaptic noise was recorded which coincided with the depolarization.

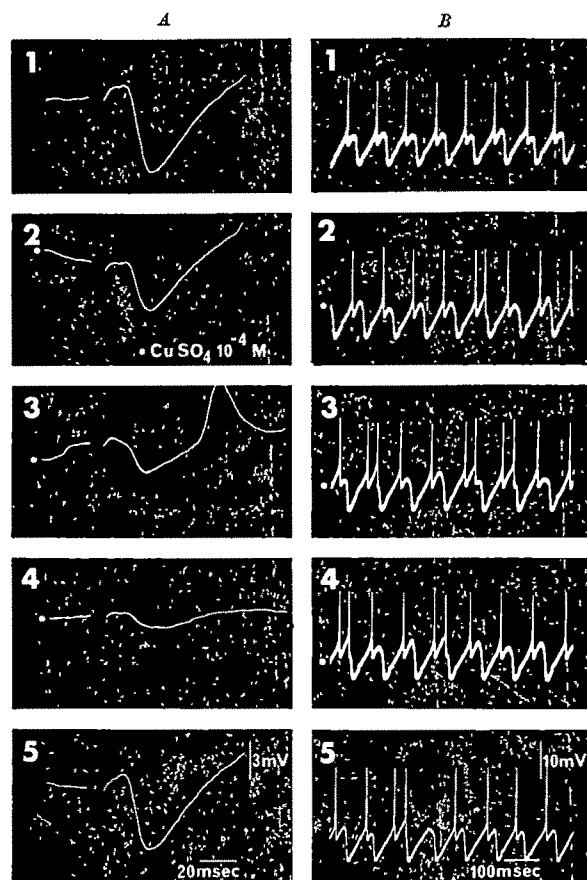


Fig. 1. A, Intracellular recording from an *H*-neurone showing the effect of  $10^{-4}$  molar copper sulphate on a cholinergic IPSP evoked by electrical stimulation. A1, Control cholinergic IPSP. A2–A4, The same IPSP 4, 8 and 15 min after the copper sulphate was added. A5, The cholinergic IPSP recovered after prolonged washing with snail Ringer solution. In B, recording from a neurone with potassium-dependent non-cholinergic IPSP (the upper part of the spikes is not shown in the records). B1, Spontaneous non-cholinergic IPSPs intermingled with spikes before addition of copper sulphate. B2–B4, The same activity 5, 10 and 20 min after adding copper sulphate; B5, wash.

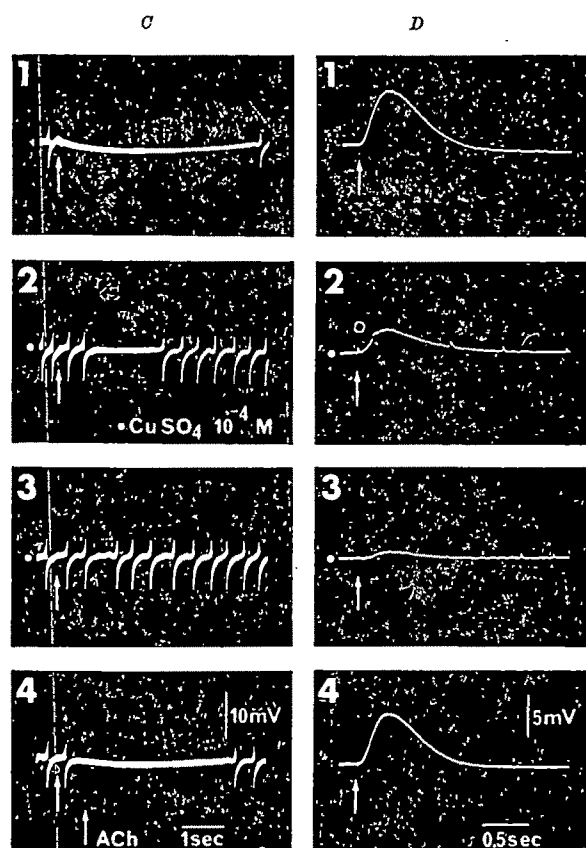


Fig. 2. Effect of  $10^{-4}$  molar copper sulphate on ACh potentials in *H*-neurones (injections at arrows). C, Effect on the hyperpolarizing response (the upper part of the spikes is not shown in records). C1, Control ACh potential. C2, 2 min, and C3, 6 min after copper sulphate was added. C4, Effect of ACh is recovered after washing the preparation. In D, reversed ACh potential by hyperpolarizing the neurone (see text). D1, Control injection. D2, 1 min, and D3, 4 min after adding copper sulphate. D4, Recovery of the depolarizing effect of ACh after washing.

Fig. 2C shows that copper sulphate is also active on the ACh potential: 6 min after adding copper sulphate to the Ringer solution the ACh potential is practically abolished (compare C3 with C1).

When the resting potential of an *H*-neurone is artificially increased beyond the inversion level of the ACh potential ( $E_{ACh}$ ), the latter is reversed and becomes depolarizing<sup>1</sup>. Fig. 2D corresponds with this situation in an *H*-neurone with a measured  $E_{ACh}$  of  $-55$  mV, in which the membrane potential was driven by passing inward current from the resting level of  $-48$  mV to  $-85$  mV. In these conditions copper sulphate also blocks the reversed ACh potential (D2 and D3). The effect of copper sulphate on ACh injections is faster than on the cholinergic IPSP, and the maximal effect on ACh potential is attained in 4–7 min. This agrees with the fact that the naked neuronal soma is in direct contact with the saline solution containing copper sulphate, while the synapses are more deeply located in the ganglion neuropile at a certain distance from the soma<sup>11</sup>.

In practically all cells the effect of copper sulphate was reversible, and the amplitude of the IPSP, the ACh potential and the resting potential recovered after prolonged washing in snail Ringer solution (Fig. 1, A5; and Fig. 2, C4 and D4).

No effect of copper sulphate on the membrane resistance was observed when square pulses were passed across the neuronal membrane. Because copper sulphate has no effect on the membrane resistance, it can be assumed that it reduces the increased permeabilities to chloride and potassium ions elicited by ACh both at the somatic and synaptic regions of *H*-neurones. The participa-

tion of potassium in the mechanism generating the cholinergic IPSP and ACh potential is minimal, so that a fall in potassium permeability after administration of copper sulphate cannot by itself explain the present results. Furthermore, the absence of action of copper sulphate on neurones with non-cholinergic IPSPs which depend on potassium excludes an effect on potassium permeability. It may be then concluded that, as in the case of frog skin, copper sulphate in dilute concentrations markedly reduces the permeability of the neuronal membrane to chloride. The effect seems to be similar for the fluxes of chloride in both directions, because the hyperpolarizing and the depolarizing effects of ACh are reduced in the same proportion. This indicates that the effect of copper sulphate is not achieved through a modification of the chloride pump which probably exists in these cells<sup>2</sup>.

According to the Goldman-Hodgkin-Katz equation, copper sulphate should produce an increase in the resting potential by reducing the permeability to chloride. The fact that copper sulphate produces a diminution of the resting potential is rather puzzling, but further work is needed to elucidate this point.

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### Alterations in the Urinary Excretion of Pseudouridine and Deoxycytidine in Rats Induced by Glucocorticoids

ONE of the characteristics of corticoid action<sup>1-5</sup> is a marked catabolic effect on the lymphatic organs, such as thymus and spleen. This is demonstrated by the loss in weight of these organs<sup>6,7</sup> as well as by the decrease in the nucleic acid content<sup>7</sup>, and by the diminution of incorporation of the precursors of nucleic acids after administration of glucocorticoids<sup>8</sup>.

Degradation of nucleic acids is accompanied by an increased excretion of their catabolic products (pseudouridine and deoxycytidine) in urine<sup>9-11</sup>; this has been proved by the effect of ionizing radiation on the organism. The object of the present work was to ascertain whether the catabolic effect of corticoids on the nucleic acids in the spleen results in a similar degradation pathway.

Infantile male rats of the Wistar-Konárovics strain, weighing 55-65 g, in groups of five, were kept in metabolic cages and fed on a standard diet. Urine was collected every 24 h. Pseudouridine and deoxycytidine were determined in the urine using a method previously

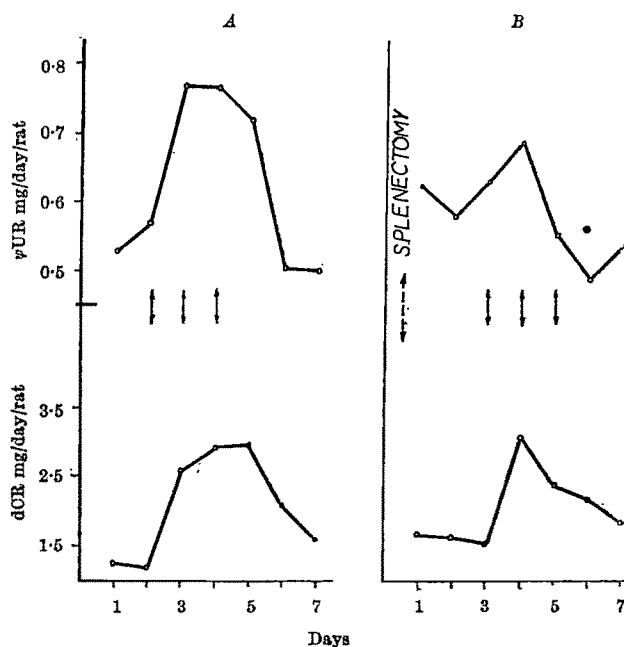


Fig. 1. The concentration of deoxycytidine (dCR) and pseudouridine (pUR) in the urine of eusplenic (A) and splenectomized (B) rats after administration of cortisone.

described<sup>10</sup>. Splenectomy was performed under ether anaesthesia 4 days before the administration of cortisone. In both splenectomized and normal animals cortisone was given in a dose of 2 mg/100 g of body weight for 3 consecutive days.

In normal rats cortisone produced a marked increase in the excretion of both catabolites 24 h after the first administration. This increase was maintained after the repeated administration of the steroid but ceased rapidly after the last dose (Fig. 1A). In splenectomized animals the course of the urinary excretion of these catabolites differed considerably after the administration of cortisone, in comparison with the findings in the normal animals. In this experiment the increase in their content occurred after the first dose only; repeated administration did not produce any effect (Fig. 1B).

On the basis of these experiments it may be assumed that the mode of catabolic degradation of nucleic acids is similar after both ionizing radiation<sup>9-11</sup> and glucocorticoid action in that in both cases degradation of nucleic acids takes place predominantly in the spleen.

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## Asymmetric Distribution of Materials sloughed by Hamster Intestinal Sacs

EVERTED sacs of the small intestine have been used widely for the investigation of transport and metabolism by the small intestine<sup>1</sup>. When such sacs are incubated with a buffer, they slough materials which can interfere with the assay of compounds being investigated and suitable corrections become necessary<sup>2</sup>. Despite this, there have not been any reports as to the nature and distribution of the liberated substances. The present communication gives preliminary data concerning the composition of some of these materials and emphasizes that their distribution is quite asymmetric.

Everted intestinal sacs were prepared from adult golden hamsters (*Mesocricetus auratus*) of both sexes. The animals were allowed food and water freely until they were killed. The small intestine was removed, washed with Krebs-bicarbonate buffer pH 7.4, and then everted. Three sacs were made from each small intestine. Individual sacs were filled with 1 ml. of buffer (serosal fluid) and placed in a 25 ml. flask containing 5 ml. of identical buffer (mucosal fluid). After gassing with 95 per cent oxygen (O<sub>2</sub>) + 5 per cent carbon dioxide (CO<sub>2</sub>), the flasks were stoppered and incubated at 37° C for 1 h. The fluids were drained and centrifuged at full speed in a centrifuge for 20 min in order to remove sloughed tissue. The mucosal and serosal solutions were then handled identically, but separately.

When run at a 1:31 dilution (with pH 7.4 Krebs-bicarbonate buffer), the average optical densities of the fluids (six sacs) were as shown in Table 1.

Table 1  
Serosal solution    Mucosal solution

260 mμ	0.18	0.07
280 mμ	0.12	0.07

It might be expected that the mucosal side would have a higher concentration of sloughed substances, since the mucosal cells tend to disintegrate histologically during incubation. Yet the optical density was higher in the serosal solution. In terms of total amount, since there was five times as much serosal as mucosal fluid, the mucosal solution contained about two-thirds of the quality of 280 mμ absorbing materials present in both fluids.

One-tenth millilitre of each liquid was dried at 105° C and treated with 3.0 ml. of 0.1 per cent ninhydrin in glacial acetic acid<sup>2</sup>. After 30 min, the optical density was read at 440 mμ. The average of the serosal solutions was 0.62, while the average of the mucosal liquids was 0.19. There was thus a concentration of ninhydrin-reacting materials within the sacs more than three times greater than that in the mucosal fluid.

Following ascending chromatography on Whatman No. 1 paper, strips were air dried and sprayed with 0.1 per cent ninhydrin in butanol. The strips were visually inspected 1 h later, following 2 min of heating at 105° C. Corresponding spots were observed in both mucosal and serosal solutions; there was a much greater intensity of colour with the serosal fluids.

Lipids in mucosal and serosal solutions were also extracted (one part to nine parts of 2:1 chloroform-

methanol) and spotted on glass plates that had been coated with 250μ of activated 'Absorbosil-1'. Thin layer chromatography was performed in a solvent system of petroleum ether (boiling point 30°-60°): diethyl ether (peroxide free): glacial acetic acid, 90:10:1. After 45 min the plates were air-dried and stained with iodine vapour. Spots were outlined with a pencil and a tracing made. Diglycerides, cholesterol, unsaturated fatty acids, saturated fatty acids, triglycerides and cholesterol esters were identified by their *R<sub>F</sub>* values (and comparison with standards). As judged by the intensity of the iodine stain, serosal fluids contained less triglyceride than the mucosal solution. The serosal fluid, however, appeared to contain more fatty acid. This suggests that triglycerides were being split by the intestinal sacs, with fatty acids then entering the serosal fluid.

Aliquots of the lipid extracts were evaporated at 4° C and an aliquot was transmethylated for 6 h at reflux temperature using 5 per cent sulphuric acid in anhydrous methanol. The fatty acid composition was analysed by using the resultant methyl esters and gas-liquid chromatography (see Table 3). Peaks were compared with known standards. The composition of the large "unknown" peak from the serosal fluid is uncertain. Its retention time on the chromatographic column was 4.8 times that of stearic acid and may represent oxidized fatty acids.

Table 3

Fatty acid	Mucosa (per cent)	Serosa (per cent)
14:0	3.2	2.2
16:0	20.6	17.2
16:1	3.9	4.0
18:0	10.9	7.2
18:1	24.2	14.8
18:2	23.1	12.1
18:3	3.2	3.6
20:0	—	1.7
20:4	1.8	1.4
Unknown	9.0	35.7

Asymmetric distribution of materials sloughed by intestinal sacs *in vitro* may be a manifestation of continuing transport from the mucosal surface toward the serosal surface. In addition, any persistent electrical potential difference across the sacs (mucosa negative with respect to the serosa) would also contribute to an asymmetry of the materials.

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## Influence of the Oviduct and Ovum on Cyclic Ovarian Activity in the Rat

THE muscular and secretory activity of the female genital tract has been implicated in the processes of gamete transport with emphasis on the role of the uterus and oviduct. It is well known that the physiological activity of these two portions of the female genital tract varies with respect to the hormonal phases of the ovarian cycle<sup>1</sup>. The ampullary portion of the oviduct plays still another important part as the site of fertilization in most mammals. In spite of the wealth of information concerning mechanisms of tubal transport, there has been a lack of knowledge concerning any additional interactions between the oviduct and ovary. It has been reported by Bishop<sup>2</sup> that secretions of the oviduct undergo changes in response to cyclic ovarian activity.

One major characteristic of mammalian ovarian cycles is the shedding of the ovum at the time of ovulation, and these ova, whether fertilized or not, are transported into

Table 2

Solvent system	<i>R<sub>F</sub></i> values of principal ninhydrin-reacting spots
Butanol : acetic acid : water (4 : 1 : 1)	0.08 maroon 0.20 red purple 0.28 purple 0.34 yellow 0.48 purple 0.58 purple
Phenol saturated with water	0.84 yellow with violet border
Butanol : ammonium hydroxide (4 : 1)	0.08 yellow 0.12 violet 0.16 violet 0.37 violet 0.48 violet

Table 1. EFFECTS OF OVIDUCT LIGATION AND EXTIRPATION ON CYCLIC OVARIAN ACTIVITY

Experimental group	No. animals	Pre-operative cycles (days)			Post-operative cycles (days)		
		No.	Range	Average	No.	Range	Average
(I) Sham operated	8	13	4-6	4.6	42	4-7	4.5
(II) Ligation utero-tubal junction	8	14	4-6	5.1	37	4-9	5.4
(III) Ligation ovarian end oviduct	7	22	3-6	4.4	36	4-9	4.6
(IV) Removal both oviducts	7	22	4-7	4.4	41	4-6	4.3

the uterus. Normally, the uterine environment is essential for implantation; however, in some instances the blastocyst may implant and develop within the oviduct as evidenced by tubal ectopic pregnancies in humans<sup>2</sup>. Scientific investigation has not shown if the transport of the unfertilized ovum and/or oviduct secretions into the uterus influence cyclic ovarian activity. It was the purpose of this investigation to determine if there existed any interrelationships between the oviduct and/or ovum and ovarian activity.

Mature female rats were divided into four experimental groups: sham-operated controls (I), those with permanent ligatures at the utero-tubal junction (II), those with permanent ligatures at the ovarian end of the oviducts (III) and rats in which both oviducts were removed (IV). The operations were performed under ether anaesthesia with the aid of a dissecting microscope. Daily vaginal smears were examined as the criterion for ovarian activity before and following surgical treatment.

The results of this preliminary investigation are shown in Table 1. The seventy-one pre-operative vaginal cycles were characterized by a range of 3-7 days in length and an average of 4.6 days. The one hundred and fifty-six post-surgical cycles showed a range of 4-9 days and an average length of 4.7 days. A statistical analysis of the data revealed no significant difference among the different experimental groups or between the pre- and post-operative cycles of each group.

It would appear that the presence of the ovum in the oviduct and uterus does not influence cyclic ovarian activity in rats as evidenced by females with permanent ligatures at the ovarian end of the oviduct and those with ligatures at the utero-tubal junction (groups II and III). It was also interesting to note that surgical opening of the ovarian bursa (essential for ligation of oviduct at ovarian end) did not appear to alter the vaginal cycles. In addition, five of the eight females in group II were characterized by fluid-filled bursal cysts on one or both ovaries at post-mortem examination, which indicates that there is a constant influx of oviduct secretions into the uterus. It would therefore appear that the oviducts are not essential for cyclic ovarian activity in the rat as evidenced by the removal of both oviducts in group IV. This would be in agreement with data obtained from women with a bilateral tubal ligation or bilateral salpingectomy who appear to have normal menstrual cycles, provided the surgical procedures have not impaired the ovarian blood supply.

These preliminary studies would suggest that the physiological activity of the oviducts is concerned primarily with gamete transport and fertilization mechanisms rather than the regulation of cyclic ovarian activity. It would also appear that the unfertilized ovum plays no part in the sequential aspects of the ovarian cycle. On the other hand, the present studies did not determine the long term effects of tubal ligation or bilateral extirpation of the oviducts on the cyclic ovarian activity of the rat.

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## RADIOBIOLOGY

### Chromosomal Alterations in Pearl Millet induced by $\gamma$ -Rays

To investigate the effect of  $\gamma$ -ray irradiation on *B* chromosomes, dry seeds of a stock of *Pennisetum typhoides* carrying *B* chromosomes were exposed to  $\gamma$ -ray irradiation in doses of 10 kr., 20 kr. and 30 kr.; the  $\gamma$ -ray source was cobalt-60. The treated seeds were sown in pots and those that survived were transplanted into the field later. Meiosis in the pollen mother cells was examined to determine the types of aberration produced. For cytological investigation young spikelets were fixed in acetic alcohol (in proportions of 1:4) and anther squashes were made in acetocarmine.

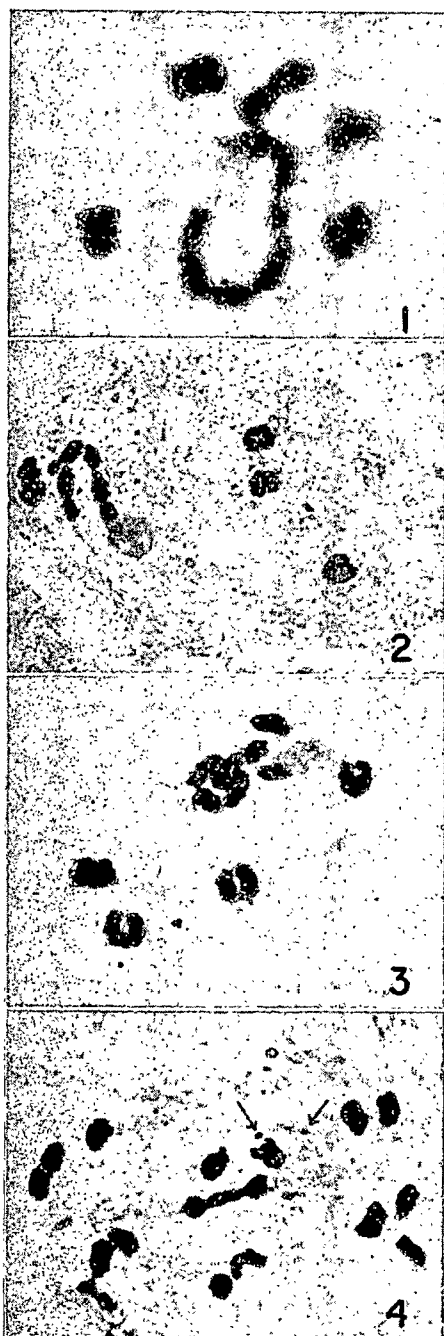
The most common aberration was a ring or chain of four chromosomes. When the chromosomes concerned with the organization of the nucleolus were involved in the translocations, a chain of four would form at first metaphase because chiasma formation is very rare in the satellited short arms of these chromosomes. The number of *B* chromosomes varied from three to five in these cells and they showed regular associations into trivalents when three *B* chromosomes were present; one quadrivalent or two bivalents or a trivalent and a univalent when four *B* chromosomes were present, and a trivalent and a bivalent when five *B* chromosomes were present.

An aberration resulting in the formation of a chain of six and four bivalents was rare and was observed in only one plant grown from seed exposed to 30 kr. Here the chromosomes with nucleolus organizers were involved and so only a chain of six occurs at first metaphase. The *B* chromosomes in these cells with chains of six showed the usual variability in number, from three to five in each cell. Regular pairing was observed among these *B* chromosomes to give rise to higher associations like trivalents and quadrivalents.

More complicated types of configurations of *A* chromosomes were encountered in one plant raised from seed exposed to 30 kr. This plant has fifteen *A* chromosomes instead of the usual fourteen, and a variable number of *B* chromosomes. The more frequent type of configuration was a chain of seven chromosomes and four bivalents (Fig. 1). Here also the chromosomes concerned with organization of the nucleolus were involved. In a few cases the extra chromosome was associated with one of the chromosomes of a chain of six to give an association of seven (Fig. 2). In two cells a chain of three, a ring of three and four bivalents and a univalent were observed (Fig. 3). The univalent was lying on the nucleolus but it was difficult to say whether it possessed a nucleolus organizer or not. In another cell was a ring of three and six bivalents. In all these cells with fifteen *A* chromosomes two dot-like fragments with a diameter less than that of the *A* chromosomes were observed regularly to be present. These fragments underwent regular first anaphase segregation and several times one of these divided and the two halves moved to the poles (arrowed in Fig. 4). The fragments regularly appeared during second division of meiosis. These facts, together with the fact that they passed through several cell generations to reach the pollen mother cell stage, show that they are centric fragments.

The *B* chromosomes paired normally in these cells with fifteen chromosomes; congression in first metaphase and segregation in first anaphase were also normal. Non-disjunction at first anaphase which was frequent in untreated material was not, however, observed here.

The effect of X-ray irradiation in *Pennisetum typhoides* has been investigated<sup>1</sup>. Dry seed was given a dose of 42,000 r. for 1 h, 2 h and 3 h. In the plants raised from these seeds there were rings of four, rings of six, two rings of four, rings of eight and rings of three along with a short fourth segment. Inversion was suggested by the presence of bridges at first anaphase. Aneuploidy and fragments



Figs. 1-4.

have not been reported. From a comparison of the results it is apparent that the types of chromosomal aberrations induced by X-rays and  $\gamma$ -rays to a large extent are alike.

Extra chromosomes have been reported by Caldecott and Smith<sup>2</sup> in plants grown from barley seed previously subjected to X-ray irradiation. In one spikelet they observed a single isochromosome and a total of fifteen chromosomes in a cell instead of the usual fourteen. The increase in number, according to them, is the consequence of the formation of an isochromosome. In the present material there is an indication that the extra chromosome might be an isochromosome, because a ring of three was observed in two cells. The presence of an isochromosome also explains the formation of a ring of seven.

The seeds were irradiated at the Biology Division of the Atomic Energy Establishment of Bombay. I thank Professor J. Venkateswarlu of Andhra University for

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## BIOLOGY

### Resistance to Cold in *Ceratomia catalpae*

SALT<sup>1</sup> has reported resistance to frost and to being frozen in several species of arthropods. He has also reported on the synthesis and accumulation of a low molecular weight solute (glycerol) in insects and has suggested that this may explain resistance to cold<sup>2</sup>. Lozina-Lozinsky<sup>3</sup> stated that in the caterpillar, *Pyrausta nubilalis*, which can tolerate extremely low temperatures, the proteins and nucleoproteins can resist an increase in concentrations of the electrolytes. Asahina and Tanno<sup>4</sup> found that the overwintering pre-pupal larvae of a sawfly, *Trichiocampus populi*, were able to survive freezing at  $-30^{\circ}\text{C}$  for a full day, and withstand liquid nitrogen temperature ( $-195.8^{\circ}\text{C}$ ) provided they had been previously frozen at temperatures lower than  $-20^{\circ}\text{C}$ .

Work in our laboratories has demonstrated freeze resistance in the larvae of the large American hawk moth, *Ceratomia catalpae*. Seven groups of ten larvae were cooled to  $+4^{\circ}\text{C}$  for 21 days. During this period there was no apparent change in diapause. When brought to room temperature, they began to move and feed in a normal fashion. All survived to the pupal stage (about 72 h). In a group of thirty that were precooled to  $+4^{\circ}\text{C}$  for 6 h and then taken to  $-20^{\circ}\text{C}$  for 12 h, sixteen survived and went on to pupal stage. A further group of thirty was precooled to  $+4^{\circ}\text{C}$  for 6 h, frozen at  $-20^{\circ}\text{C}$  for 12 h and then immersed in liquid nitrogen ( $-195.8^{\circ}\text{C}$ ) for 5 min. After allowing this group to remain at room temperature for 15 h, movement was recorded in only two individuals. In the final group of fifty larvae taken to  $+4^{\circ}\text{C}$  for sixty-four days, forty-six survived and went on to pupae. All individuals remained in diapause while at these temperatures.

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### Occurrence of *Teleonemia scrupulosa* on *Sesamum indicum* Linn. in Uganda

*Teleonemia scrupulosa* (Stål) (Hemiptera, Tingidae) is a native of the tropical regions of the Americas and has been extensively used as an agent for the biological control of the noxious weed *Lantana camara* Linn. (Verbenaceae). It was introduced first into Hawaii<sup>1</sup>; since then it has been introduced into Fiji<sup>2</sup>, Australia<sup>3</sup>, Indonesia<sup>4</sup> and other island groups in the Pacific. It is also established in India<sup>5</sup>, Mauritius, and South<sup>6</sup>, Central<sup>7</sup> and East Africa. In general the level of control achieved in these areas has

been disappointing, although severe local defoliation and partial control has been reported from many of these countries, and it is generally considered to be a useful addition to the insect complex used in the control of *Lantana*. Exhaustive tests in Fiji and Australia showed that this Tingid would feed on none of the plants tested except *Lantana*<sup>8</sup>. The bug was reported in Texas on *Callirhoe involucrata* (Malvaceae) and a plant of the mint family but was unable to breed or live long on them<sup>9</sup>. Its release in India was not recommended because laboratory tests showed that it could feed on teak (*Tectona grandis* Linn.) which is also a member of the Verbenaceae although breeding was slow and *Lantana* was preferred<sup>10</sup>. When it escaped at Dehra Dun, however, it became established on *Lantana* but could not be found on nearby teak<sup>5</sup>. The only other previous records of attack by *T. scrupulosa* on other plants are its occurrence on *Myoporum sandwicense* (Myoporaceae) and species of *Xanthium* (Compositae) in Hawaii, ebony in the United States, and *Lippia alba* (Verbenaceae) in the Antilles, according to the catalogue just published, which includes some hitherto overlooked records<sup>11</sup>.

*T. scrupulosa* (the lacebug) was first introduced into East Africa in 1952, from Hawaii, and was released on the Kenya Coast in 1953, where it became established<sup>12</sup>. Successful releases were made later at Nairobi and near Kisumu in Kenya, Zanzibar, Dar-es-Salaam and Morogoro in Tanzania, and in Uganda at Kawanda Research Station in 1960. In the mistaken belief that it had not become established at Kawanda, fresh material from Trinidad was also released there in 1962. In all these places its effect on *Lantana* conformed with that found previously and there have been no reports of its occurrence on crop plants. When adults collected from Kawanda were released at Serere Research Station in June 1963, severe defoliation around the release point to a distance of several hundred yards was caused in the dry season from November 1963 to March 1964. With the onset of the rains in April 1964 considerable regrowth and foliage production was observed and flowers developed. In the dry season of 1964-65 there was a massive population build-up of *T. scrupulosa* which was not followed by the expected regrowth of *Lantana* at the onset of the rains. New shoots and flowers were immediately attacked and defoliation around Serere Research Station was almost complete for a radius of 2-3 miles by the end of May 1965. By this time the lacebug had spread naturally over a wide area and has been recovered 18 miles from the place of release.

A disconcerting feature was the discovery of numbers of the insect on cultivated *Sesamum indicum* Linn. (Pedaliaceae) on the Research Station in early May and again on an experimental block planted in July 1965. The population on *Sesamum* increased until five to ten insects were present on each leaf. In addition, stems, flowers and nectaries were attacked. Copulation and oviposition were observed on *Sesamum*. Large numbers of eggs were laid, and viable nymphs emerged. Mortality of nymphs was high, but the insect was able to complete the cycle from egg to adult on *Sesamum*.

So far it has not been possible to carry out controlled experiments, but the following, largely qualitative, observations have been made. On *Sesamum* the bionomics of the insect were similar to those on *Lantana*<sup>8</sup>. The eggs were laid on the mid-rib or main leaf veins on the under surface of leaves, and caused severe necrosis. Up to twenty eggs were laid on one vein, and they frequently caused severe distortion around the point of insertion. Nymphs fed on the undersurface of leaves and were gregarious. Development was slower on *Sesamum* than on *Lantana*. A high proportion of nymphs bred on *Sesamum* failed to complete the final ecdysis successfully. The young adults died attached to the old cuticle. Approximately one hundred late instar nymphs collected from *Sesamum* in the field were caged in the laboratory on excised *Sesamum* leaves. From these, only twelve adults

were obtained; their emergence was staggered and none laid any eggs.

Defoliation occurred on plants severely attacked by the bug. A block of *Sesamum*, sprayed with dimethoate to control gall midge, *Asphondylia sesami* Felt., was clearly far less damaged than an adjacent block of the same crop deliberately left unsprayed. Far fewer capsules were produced on *Sesamum* which was attacked by *T. scrupulosa*.

These observations are of considerable importance in that *T. scrupulosa* is known to feed on few plants other than *Lantana camara* and allied species of the genus. It is also of interest that, though *Sesamum* is also grown in many of the countries where *T. scrupulosa* now occurs (for example, Mexico, Java, Kenya), no previous records of its occurrence on *Sesamum* can be traced. It has also been found feeding, but not ovipositing, on the wild *Sesamum angustifolium* Oliv. and on young silks of maize at Serere, and at Kawanda a few adults have been found on *Sesamum indicum*. It is as yet too early to predict the final outcome, but it seems likely that *T. scrupulosa* will be able to control *Lantana* in the Serere area.

The very poor survival rate of nymphs on *Sesamum*, and the fact that it is an annual crop in the ground for only 4 months, suggest that there is little danger of the development of a strain which is adapted to *Sesamum*. When the present overpopulation has subsided so that production of adults is in balance with the quantity of *Lantana* available and there is no great surplus of adult bugs unable to feed on their normal host, movement on to *Sesamum*, if it occurs, will be slight. Because the plant is available as a host for only a portion of each year, and because it is grown at the season least favourable to *T. scrupulosa*, it would then be unlikely that any adults which find it would be able to build up a damaging population.

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### Vitamin B<sub>12</sub>-controlled Biotin Avidity in Autotrophic *Euglena gracilis*

SEVERAL years ago, it was found that cells from recently isolated but impure (0.1 per cent contaminants) cultures of the autotrophic bacterium *Nitrosomonas europaea* contained more than 10 µg biotin/g dried cells<sup>1</sup>. Because such values are considerably higher than the 1.7-7.1 µg biotin per g of dried cells reported for five species of heterotrophic bacteria<sup>2</sup>, we suggested that autotrophs might require large amounts of biotin for utilization of their carbon source, carbon dioxide. Assay of biotin content of pure cultures of autotrophic bacteria revealed that they contained the same amount or only slightly less biotin than do heterotrophs: *N. europaea*

contained 1.6–2.4  $\mu\text{g}$  and *Nitrobacter agilis* contained 0.56–1.0  $\mu\text{g}$  biotin/g dried cells<sup>2</sup>.

We then revised our speculation to consider biotin uptake associated with biotin content of autotrophic cells. In other words, if biotin is essential for autotrophic life, then does an autotroph such as *N. agilis* with low biotin content take up more biotin than *N. europaea* which actually contains about twice as much biotin? The experimental test of this question showed that  $1 \times 10^8$  *N. agilis* cells took up and utilized 150  $\mu\text{g}$  of biotin while  $1 \times 10^8$  *N. europaea* cells did not take up detectable amounts of the vitamin<sup>3</sup>.

A more suitable tool for investigation of biotin avidity is an organism which can be cultivated both autotrophically and heterotrophically and which does not require biotin for growth. *Euglena gracilis* meets these requirements and was therefore used for the investigations on cellular biotin content and uptake presented in this report.

*Euglena gracilis*, Z strain, was grown in an autotrophic medium<sup>4</sup> containing either minimal (0.1  $\mu\text{g}/\text{ml}$ .) or sufficient (1.0  $\mu\text{g}/\text{ml}$ .) vitamin B<sub>12</sub> or in one of two heterotrophic media, either the autotrophic medium supplemented with glucose (10  $\text{mg}/\text{ml}$ .), glycolic acid (0.5  $\text{mg}/\text{ml}$ .) and excess vitamin B<sub>12</sub> (5.0  $\mu\text{g}/\text{ml}$ .), or Difco-Bacto 0532 *Euglena* B<sub>12</sub> assay medium<sup>5</sup> containing either minimal or sufficient vitamin B<sub>12</sub>. In all experiments 50 ml. cultures were grown in acid-washed 125 Erlenmeyer flasks with turbulence maintained by bubbling ordinary air through the cultures at the rate of 60 bubbles/min. Cultures were grown under constant illumination with warm white fluorescent lights at 24–26° C until the cultures were well into their exponential growth phase. With an inoculum of about 500–600 cells/culture, total incubation time took about one week.

To assay biotin content of cells, log phase cells were collected by centrifugation, washed, lyophilized, and stored in a cool, dark cabinet until the time of assay for biotin content. Free biotin content was measured in aliquots of suspended cell material, and free plus bound biotin content by hydrolysing 100–200  $\text{mg}$  of lyophilized cells in 6 N sulphuric acid, according to the method of Skeggs<sup>6</sup>. The hydrolysate was then neutralized to pH 6.5–7.0, added to the biotin assay medium, and assayed using the *Lactobacillus plantarum* method<sup>4</sup>. Bound biotin content was calculated as the difference between total and free biotin.

To measure biotin uptake, log phase cells were counted with a haemocytometer, centrifuged, and resuspended in a portion of their original medium so that there were  $3\text{--}8 \times 10^6$  cells/ml. Biotin, at a final concentration of 150  $\mu\text{g}/\text{ml}$ ., was offered aseptically to these cell suspensions and, except for a small sample of suspension immediately removed for assay purposes (zero time control), were incubated for 3 h under the same conditions which served for growth. A 3 h period is much less than the time required for one cell division of *E. gracilis*. Supernatants from both the zero time control and the 3 h incubations were separated from the cells by centrifugation, and aliquots of these supernatants assayed microbiologically<sup>4</sup> for biotin content.

The results from assay of our zero time controls showed that our assay was accurate to about  $\pm 5$  per cent, and that no biotin was produced by any cultures before addition of biotin. The amounts of biotin found in *euglena* cells grown with sufficient vitamin B<sub>12</sub> and grown under a variety of conditions are given in Table 1. Total biotin content was remarkably constant under all conditions tested.

Data of the biotin uptake experiments (Table 2) showed that the autotrophic, B<sub>12</sub>-sufficient *euglena* cells took up to 6  $\mu\text{g}$  of biotin/ $3.3 \times 10^6$  cells, while the autotrophic, B<sub>12</sub>-deficient and all the heterotrophic cells (regardless of conditions of their B<sub>12</sub> nutrition) did not take up detectable amounts of biotin. These results, which show vitamin

B<sub>12</sub> control of biotin uptake in autotrophically grown *Euglena*, can be interpreted as an example of metabolic homeostasis at the coenzyme level. Some biotin-enzyme catalysed and B<sub>12</sub> enzyme-catalysed reactions are closely associated metabolically, for example, in the formation of propionate from succinate<sup>7</sup>. With limitation of B<sub>12</sub>-reactions, as in B<sub>12</sub>-deficiency caused by supplying only minimal amounts of the vitamin, endogenous biotin suffices; but with B<sub>12</sub> in excess, the endogenous biotin supply now becomes the metabolic limiter and the cells show avidity for exogenous biotin (Table 2). Such limitation becomes critical for autotrophically grown *Euglena* where carbon dioxide fixation is the prime source of cellular carbon.

Table 1. BIOTIN CONTENT/G DRY WEIGHT OF CELLS OF *Euglena gracilis* GROWN IN THE ABSENCE OF EXOGENOUS BIOTIN

Growth medium	Grown in light			Grown in dark		
	0.5 $\mu\text{g}\%$ Vitamin B <sub>12</sub>	$\mu\text{g}$ Biotin	Per cent	$\mu\text{g}$ Biotin	Per cent	
	Free	Bound	Bound	Free	Bound	Bound
Autotrophic Medium I*	0.12–0.7	0.45–3.0	73–77			
Heterotrophic Medium IA†	0.15–0.6	0.9–2.0	73–80	0.11–0.3	0.8–3.0	86–90
Medium II‡	0.2–0.8	0.75–2.0	60–73	0.15–0.4	1.0–2.5	84–88

\* The autotrophic medium of Fuller *et al.*<sup>4</sup> supplemented with thiamine, 0.6  $\text{mg}$  per cent.

† The autotrophic medium I supplemented with glucose, 1.0 per cent, and glycolic acid, 0.05 per cent.

‡ *Euglena* B<sub>12</sub> assay medium (Guttman, 1963)<sup>5</sup>.

§ As determined by assay with *Lactobacillus plantarum*<sup>4</sup>.

Table 2. BIOTIN UPTAKE BY *Euglena gracilis*, GROWN IN THE LIGHT IN THE ABSENCE OF EXOGENOUS BIOTIN

Growth conditions	Vitamin B <sub>12</sub> in medium ( $\mu\text{g}$ per cent)	Cells/ml. suspended in biotin containing medium	$\mu\text{g}$ biotin* taken up/ml./3 h
Autotrophic medium I	10	$8 \times 10^6$	0†
	100	$3.3 \times 10^6$	6
Heterotrophic medium ‡	10	$3.5 \times 10^6$	0†
	100	$3.6 \times 10^6$	0†

\* As described by assay with *Lactobacillus plantarum*<sup>4</sup>.

† Less than 0.1  $\mu\text{g}$ .

‡ As described by Guttman<sup>5</sup>.

At least one carbon dioxide-fixing enzyme which requires biotin, pyruvate: carbon dioxide ligase (6.4.1.1) has been demonstrated in autotrophic bacteria (M. I. H. Aleem, personal communication).

For such a consideration to be of general importance, autotrophic cells which contain low levels of biotin should take up appreciable quantities of this vitamin while autotrophic cells with high endogenous biotin (that is, where biotin is not a limiting factor) should not. In the latter case a second coenzyme in a sequence which includes a biotin enzyme should be the limiting factor. The first part of this hypothesis was confirmed by our finding that one autotrophic bacterium, *N. agilis*, which contains half as much endogenous biotin as does another autotrophic bacterium, *N. europaea*, took up exogenous biotin while *N. europaea* did not. We thank the U.S. National Institutes of Health for a grant.

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### Timber, *Tetrameles nudiflora* R., resistant to Teredid Borers in Bombay Harbour

THE destruction by wood-borers of timbers used for marine construction has been a serious problem in both temperate and tropical waters. This communication directs attention to the resistance of the timber *Tetrameles nudiflora* to attack by marine boring organisms. This is interesting in view of the fact that this timber has been graded as coming under class III in respect of durability on land as assessed in grave-yard trials<sup>1</sup>.

Untreated panels of *T. nudiflora* (12 in. × 3 in. × 0.5 in.) were immersed in the sea at three widely separated sites in Bombay Harbour. Sixteen panels were immersed at each site together with an equal number of *Abies pindrow* (Himalayan fir) panels which served as controls. Damaged controls were replaced normally after every 3 months.

The exposures were carried out from July 1962 to September 1965. There were four separate exposures (Table 1).

Table 1

Exposure I:	from 9-7-62 to 21-8-63:	14 months
Exposure II:	from 9-7-62 to 20-3-64:	20 months
Exposure III:	from 20-3-64 to 17-10-64:	7 months
Exposure IV:	from 20-3-64 to 1-9-65:	18 months

The samples exposed to attack were examined at intervals of 3 months by sectioning to determine the extent of internal deterioration. The control panels were examined in a similar manner.

The teredid borers reported<sup>2</sup> from Bombay waters are *Bankia* (*Bankiella*) *carinata* G., *B. (Liliobankia)-companellata* M.R., *Teredo (Kuphus) manni* W., *T. (Bactronophorus) thoracites* G., and *T. (Teredo) elongata* Q. The

presence of *T. furcifera*, a synonym of *T. parksi*, and also *Lyrodus pedicellatus* has recently been observed by us. The incidence of the last two *Teredo* borers and *B. companellata* at the sites of exposure for the past 4 years has been very heavy.

Periodical examination of the test panels revealed that none of these was damaged by the teredid worms, *Teredo* and *Bankia*, at any time during their exposure. At one of the sites (Trombay) some panels did show attack of *Martesia* sp., but this was not surprising as this pholad is well known for its catholic preference. The control timber panels were heavily attacked and damaged by all types of borers within about 3 months of exposure.

Microscopic examination of the exposed test panels revealed the presence of innumerable entry holes or larval pits of 0.25 mm diameter, with the dead remains (chitinous shells) of the veliger larvae. It was therefore evident that although there had been initial anchorage or attack, there had been no progress in burrowing and the larvae had died. On almost all occasions, the larvae suffered death before their chitinous shells were transformed into calcareous ones. The superficial attack of the larvae did not impair the strength of the timber panels tested.

With a view to confirming the field exposure results described previously, the laboratory exposures of this timber were also made. The larvae of *T. furcifera* were used as the test organisms, as recommended by Becker<sup>2</sup>. This borer<sup>3</sup> has been found to be a very convenient test organism for the experimental work. Small pieces of *T. nudiflora* were offered for the attack of the teredid larvae. The timber pieces of *Abies pindrow* (Himalayan fir) were used as controls. Figs. 1a and b illustrate the damage suffered by *T. nudiflora* and *A. pindrow*. The results obtained in the laboratory tanks were similar to those obtained on the raft trials in the open sea.

I thank Shri S. K. Ranganathan and Shri C. P. De for their advice and encouragement.

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### New Type of Nuclear Life Cycle in *Hemileia vastatrix*

*Hemileia vastatrix*<sup>1</sup> is the cause of rust disease of coffee, and there have been many investigations of its life cycle. This rust is characterized by the formation of non-resting teliospores which germinate *in situ* and produce sporidia, which, however, are unable to reinfect coffee, which suggests that they are non-functional<sup>2-4</sup>. Pycnial and aecial stages of this rust are not known. We have conducted further investigations of the life cycle of and mode of perennation of this fungus, with special emphasis on nuclear behaviour.

Fresh leaf material in various stages of infection was fixed in Carnoy's fluid for 1 h, then kept in 'Craf' A and B for 24 h, washed, dehydrated and embedded in paraffin wax according to the usual procedure. Sections 10μ thick were cut. Nuclei were stained with iron haematoxylin, crystal violet, and Feulgen. Temporary mounts were made in acetocarmine.

The results obtained show that the parasitic hyphae are dikaryotic (A and B in Fig. 1) in the initial stages, and later become diploid and monokaryotic through fusion of the dikaryons (Q). This nuclear status is maintained in the stromatal hyphae, the sporogenous hyphae as well as the spore mother cells (D and F). The single diploid nucleus in the spore initials divides mitotically

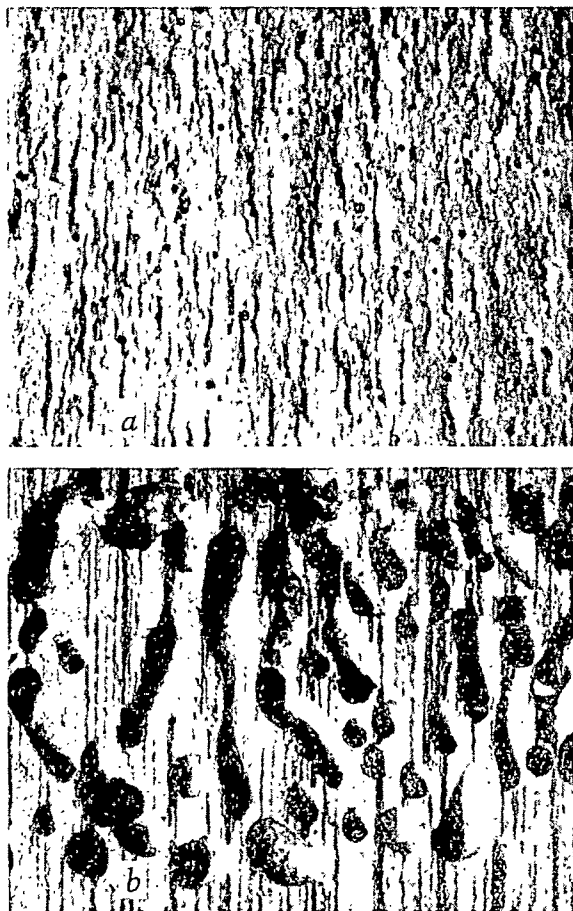


Fig. 1. Teredo attack under laboratory conditions. a, Surface view of the test piece of *Tetrameles nudiflora*; b, cross-section of the control *Abies pindrow*. (×3.)



(F), and one of the two daughter nuclei moves towards the base which ultimately becomes the stalk cell (G and H).

The diploid nucleus in the spore mother cell enlarges and undergoes a division, which shows all the typical features of meiosis, involving formation of bivalent pairs with chiasmata (J and K) and resulting in typical meiotic diads (L). The stage of meiotic diads is prolonged by the suspension of meiosis. Consequently, this meiotic diad has been mistaken previously for a dikaryophase associated with the formation of normal urediospores, but which actually represents a transient non-septate haplophase in the course of abnormally suspended or arrested meiosis.

Usually, nuclear fusion among rust fungi is followed immediately by initial or zygotic meiosis and is associated with teliospores. The abnormal phenomena described above thus represent unusual prolongation of the fused diplophase of the life cycle and an abnormally suspended meiosis following its first reduction division. The abnormal meiotic diad is prolonged and just precedes the infective phase after the completion of the second meiotic division. The nuclear constitution of this meiotic diad is not comparable with that of normal urediospores. This prolonged meiotic diad may therefore be designated as uredinoid teliospore for convenience.

On germination this uredinoid teliospore puts out a "pseudo-promycelium" which terminates in a structure like an appressorium (M), where the meiotic diad undergoes a second division of meiosis to produce a meiotic tetrad (N). The appressorium containing the meiotic tetrad puts out a bifid infection "peg", each arm of which receives a pair of haploid nuclei from the meiotic tetrad (O). If two nuclei out of four are sorted at random in each of the two infection "pegs", there could be some scope for genetic variation depending on the degree of genetic heterozygosity in the original somatic hyphal nuclei. Compared with the normal process of hybridization in other rusts, scope for genetic variations in such life cycles would be relatively restricted. This probably explains the abnormally low number of biotypes reported in this rust

fungus. In the last stage of the life cycle bifid infection "pegs" reinfect the host and repeat the life cycle (A).

I propose to name this new type of life cycle of rust fungi "Kamat type" after Prof. M. N. Kamat, head of the Mycology Division, M.A.C.S. Poona, India. I thank Dr. G. B. Deodikar, director of M.A.C.S., Poona, India, for his help.

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### Influence of Vesicular-arbuscular Mycorrhizas on the Uptake of Phosphorus-32 by *Liriodendron tulipifera* and *Liquidambar styraciflua*

ECTOTROPHIC mycorrhizas are capable of enhancing the uptake of nutrients by the host plant, and they have been shown to absorb and accumulate more ions than non-mycorrhizal roots<sup>1-3</sup>. It has also been shown that the fungus sheath can absorb ions and release a fraction of them to the host plant<sup>1</sup>. There is much less evidence, however, that nutrient uptake and ion accumulation are enhanced by the endotrophic vesicular-arbuscular mycorrhizas where there is no fungus sheath but only an extensive external and internal hyphal development<sup>4</sup>. Plants with vesicular-arbuscular mycorrhizas contain more nutrients than the non-mycorrhizal control plants<sup>5-7</sup>. Soil in which mycorrhizal plants have grown contains less phosphorus than soil in which non-mycorrhizal control plants have grown<sup>8,9</sup>. This evidence indicates that vesicular-arbuscular mycorrhizas may function in a manner similar to the ectotrophic type by increasing the absorption of ions by the host plants.

The purpose of our investigation was to determine whether vesicular-arbuscular mycorrhizas of sweetgum (*Liquidambar styraciflua* L.) and tuliptree (*Liriodendron tulipifera* L.) enhance phosphorus-32 uptake. The fungus used in our experiments, *Endogone fasciculata* Thaxter, has not been obtained in pure culture on artificial media and it was maintained on the roots of living maize grown in sterilized soil<sup>8</sup>. Sweetgum and tuliptree seedlings were inoculated by growing them in steam sterilized soil to which chopped maize roots containing *E. fasciculata* had been added. The control plants were grown in sterilized soil to which an equal quantity of autoclaved chopped mycorrhizal roots had been added. In order to introduce the contaminating bacteria and fungi present on the mycorrhizas into the control pots, the infected roots were washed in distilled water and the wash water was filtered through a sieve with openings 44 $\mu$  wide. Some of this filtrate (30 ml.) was added to the soil in each of the control pots.

In the first experiment, individual 90 day old mycorrhizal and non-mycorrhizal tuliptree seedlings were transferred to separate containers with an aerated solution of 1.083  $\times 10^{-4}$  molar phosphate. A carrier-free solution of phosphorus-32 containing 8.7  $\mu$ c. was then added to each container. After 4 days the plants were removed from the solution of phosphorus-32. The roots were then rinsed in a solution of 0.001 molar phosphate, rinsed twice in distilled water, blotted, and dried for 24 h at 45° C. Root samples were collected at random from each plant and wet washed in a mixture of nitric and sulphuric acid and neutralized with sodium hydroxide. The radioactivity in each sample was determined by placing a 0.1 ml. aliquot on a planchet and counting the samples under a gasflow detector connected to a scaler. Each sample was counted

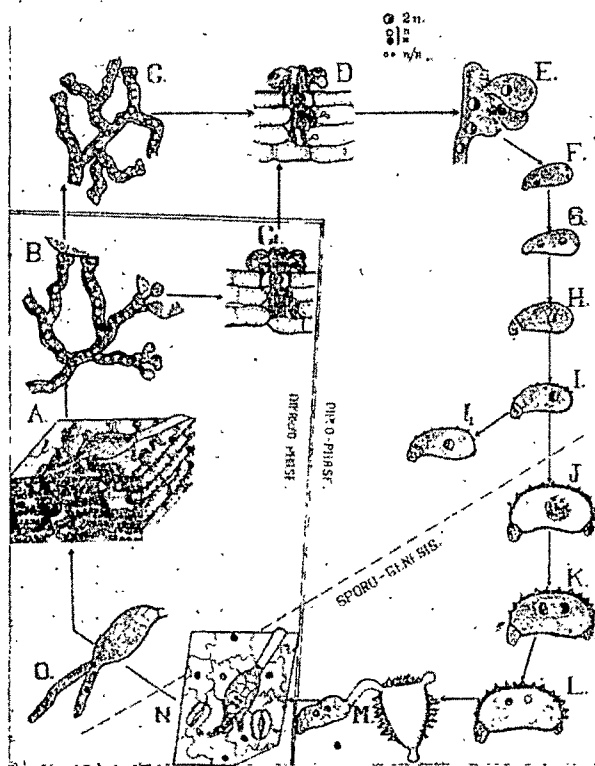


Fig. 1.

under the same conditions of sample geometry and counter efficiency. The radioactivity in the roots of the mycorrhizal tuliptree seedlings was greater than that in the non-mycorrhizal seedlings (Table 1). The experiment was repeated and similar results were obtained.

Table 1. RADIOACTIVITY OF THE ROOTS OF 90 DAY OLD MYCORRHIZAL AND NON-MYCORRHIZAL TULIPTREE SEEDLINGS AFTER 4 DAYS IN A SOLUTION OF PHOSPHORUS-32

	Mean activity (c.p.m./mg dry weight)*
Mycorrhizal tuliptree seedlings	6,416†
Non-mycorrhizal tuliptree seedlings	3,365

\* Means based on five replications.

† Difference between means significant at the 5 per cent level.

In another experiment, 95 day old mycorrhizal and non-mycorrhizal sweetgum seedlings were transferred to individual pots containing steam-sterilized soil, and allowed to grow for 40 days. A solution of phosphorus-32 containing 14  $\mu$ c. was injected into the soil of each pot in six places around each plant and the plants were grown for six days. To ensure uniform counting geometry for assay of radioactivity, duplicate circular leaf pieces, 5 mm in diameter, were taken from the first leaf below the developing terminal leaf of each plant. The radioactivity of these intact leaf pieces was then determined with a gas flow detector connected to a scaler. The foliage of the mycorrhizal plants showed much higher radioactivity than did the foliage of the controls (Table 2).

Table 2. RADIOACTIVITY OF THE FOLIAGE OF MYCORRHIZAL AND NON-MYCORRHIZAL SWEETGUM SEEDLINGS GROWN IN A STERILIZED SOIL CONTAINING PHOSPHORUS-32

	Mean activity in foliage (c.p.m./mg fresh weight)*
Mycorrhizal sweetgum seedlings	353†
Non-mycorrhizal sweetgum seedlings	24

Seedlings were 135 days old.

\* Means from 6 replications with 2 subsamples and 4 replications with 2 subsamples.

† Difference between means significant at 1 per cent level.

The results from these experiments indicate that vesicular-arbuscular mycorrhizas enhance the uptake of phosphorus-32 by the host plant from nutrient solution and from soil, and indicate that the function of vesicular-arbuscular mycorrhizas is similar to that of ectotrophic mycorrhizas.

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## MICROBIOLOGY

### Routine Method for Concentration and Partial Purification of a Murine Leukaemia Virus (Rauscher)

MURINE leukaemia viruses are generally obtained either from plasma or tissues of leukaemic mice or from tissue culture supernatants by differential centrifugation in a citrate buffer, as described by Moloney<sup>1</sup>. This method, however, results in loss of about 90 per cent of the initial leukaemic activity<sup>2</sup>.

We describe here a simple modification of the Moloney procedure, suggested by the work of Mrs. N. Plus on the sigma virus of *Drosophila*<sup>3</sup>, which enables concentration and partial purification of the Rauscher virus without any loss in leukaemic activity.

The source of Rauscher virus is plasma from *BALB/c* mice, 1 month old, inoculated intraperitoneally with 0.1 ml. of a suspension containing  $10^5$ – $10^6$  *E.D.*<sub>50</sub>/ml. of virus. The mice are killed 28 days later and their blood is collected and pooled; it is then prepared by one of two methods.

In Moloney's method blood is collected and supplemented with an equal volume of 0.306 molar potassium citrate buffer, pH 6.8. This is made clearer by low speed centrifugation in a refrigerated centrifuge; first at 1,200g for 20 min and then at 2,400g for 20 min. All later steps are carried out in the cold at 4° C. The upper and lower parts of the supernatant in the centrifuge tube are discarded; the middle part which represents two thirds of the supernatant is collected and the virus suspension is centrifuged for 2 min at 10,000g. The supernatant is finally centrifuged at 56,000g for 90 min. Following this procedure the virus pellet is resuspended with a 'Teflon' pestle homogenizer in a volume of 0.05 molar sodium citrate buffer, pH 6.8, equal to the original volume of the virus suspension just before ultracentrifugation.

In our proposed modifications leukaemic blood is collected in the same manner and mixed with a concentrated heparin solution in distilled water. The final heparin concentration is 10–15  $\mu$ g/ml. of blood. The same procedure as in Moloney's method is used for the low speed centrifugations. The supernatant of a third centrifugation at 10,000g for 2 min is mixed volume for volume with a solution containing 0.5 molar sucrose and 0.00075 molar *tris*-buffer, pH 7.2, and then ultracentrifuged for 90 min at 56,000g. The virus pellet is resuspended using a 'Teflon' pestle homogenizer, in a solution containing 0.25 molar sucrose and 0.00075 molar *tris*-hydrochloric acid, pH 7.2.

Bioassays for leukaemogenic activity are carried out by inoculation of tenfold dilutions of each sample into female *BALB/c* mice aged 5–6 weeks. Ten mice are inoculated intraperitoneally with 0.1 ml. of each dilution. Mice are killed 28 days later and the animals are considered positive if the wet spleen weight exceeds 150 mg. The titre unit expressed as *E.D.*<sub>50</sub> is the dose with which 50 per cent of the animals are positive and is calculated according to the cumulative method of Reed-Muench (standard deviation is  $\pm 0.30$  log). The results are quite reproducible and have been examined elsewhere (report by Levy, in preparation).

Table 1. LEUKAEMOGENIC ACTIVITY OF RAUSCHER VIRUS EXTRACTED FROM *BALB/c* MOUSE PLASMA

Experiment	Method	Leukaemogenic activity before ultracentrifugation ( <i>E.D.</i> <sub>50</sub> /ml.)	Leukaemogenic activity after ultracentrifugation ( <i>E.D.</i> <sub>50</sub> /ml.)*	Percentage of infectivity recovered (Per cent) (Average)
(1)	Citrate	$10^{4.56}$	$10^{3.55}$	6
(2)		$10^{4.10}$	$10^3$	5
(3)		$10^{4.08}$	$10^{3.50}$	18.5
(4)		$10^{4.08}$	$10^{3.87}$	15
(5)		$10^{4.10}$	$10^{4.10}$	9
(6)	Sucrose	$10^{4.08}$	$10^{3.87}$	0.5
(7)		$10^{4.50}$	$10^{4.45}$	71
(8)		$10^{4.56}$	$10^{4.78}$	130
(9)		$10^4$	$10^4$	100

\* In each instance, virus pellets were resuspended in a volume of buffer equal to the original volume of the virus suspension just before ultracentrifugation.

Comparative results of both purification procedures are given in Table 1. It can be seen that about 90 per cent of leukaemogenic activity is lost when the citrate method is used, while the sucrose method gives a complete recovery of the initial leukaemogenic activity. These data clearly demonstrate the usefulness of sucrose medium for the concentration and partial purification of Rauscher virus and possibly other murine leukaemogenic viruses.

Further purification can be achieved in sucrose gradients as we have shown by the more detailed work reported elsewhere (report in preparation).

These observations suggest that ultracentrifugation is not in itself responsible for inactivation. We have observed

already that very fast or long run centrifugation does not cause any further inhibition of the leukaemogenic activity of Rauscher virus. Furthermore, citrate medium is not directly responsible for the loss of activity, because virus kept in this medium for prolonged periods is preserved entirely<sup>2</sup>. These facts suggest that osmotic pressure or ionic effects alone are not directly responsible for virus inactivation which seems to be caused by the concentration in a salt solution. On the other hand, centrifugation in non-ionic gradients, such as polyglucose and polysucrose<sup>4</sup> or sucrose<sup>5</sup>, preserves the biological activity of Rauscher virus.

In addition it may be pointed out that after one log loss of activity after the preparation of pellets in citrate buffer, partial recovery (0.5–0.7 log) occurs when the virus is centrifuged in a sucrose gradient. This suggests that the virus concentration in ionic media strongly affects its leukaemogenic activity. This inhibition may be caused by an aggregation of particles.

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## CYTOLOGY

### Inhibition of Migration of Rat Peritoneal Exudate Cells by Ascites Tumour Fluid Fractions

FACTORS in the ascitic fluid of rats bearing a Walker 256 carcinosarcoma are capable of inhibiting the amoeboid motility of peritoneal exudate cells. This finding may be pertinent to evidence that a tumour interferes with the host's immunological mechanisms<sup>1</sup>.

The tumour was carried in male Wistar rats. Normal rats of the same strain were given an intraperitoneal injection of 2 ml. of a 10 per cent solution of sodium caseinate in 0.14 molar saline. Twenty-four hours after injection, the peritoneal cavity was washed with Hartman's solution. The cell population obtained consisted of approximately 80 per cent mononuclear cells and 20 per cent polymorphonuclear leucocytes. These cells were then made up to be 10 per cent by packed volume in minimum essential Eagle's medium fortified with 15 per cent normal rat serum and containing 100 u of penicillin G/ml. and 100 µg of streptomycin sulphate/ml. Small capillary tubes (non-heparinized, 1.3 to 1.5 mm in diameter, 75 mm long) were filled with the cell suspension, sealed at one end, and centrifuged. The capillary tubes were cut at the cell-fluid interface and the portions containing the cells were placed in Petri dishes and secured with a small drop of paraffin. Six millilitres of the medium used for suspension, or a combination of the medium and ascitic fluid in a total volume of 6 ml., were added to the Petri dishes, which were then placed in an incubator at 37° C and left for 24 h. The extent of migration of the peritoneal exudate cells was then measured by centrifuging from the culture medium the cells which had migrated out of the capillary tubes, resuspending in 0.2 ml. of Eagle's medium, and counting in a haemocytometer. The percentage inhibition of migration was expressed by

$$\frac{C - A}{C} \times 100$$

where *A* and *C* are the total number of cells migrating from the capillary tubes in cultures with and without the fraction to be tested, respectively (Fig. 1). *C* was  $1.4 \times 10^4$ – $2.1 \times 10^4$ , which represented 12 per cent to 18 per cent of the total number of cells originally in the capillary tubes. With increasing concentrations of ascitic fluid in the cultures, the inhibition of migration of the cells increases (Fig. 2). One hundred units of inhibitory activity were arbitrarily designated to be that amount of inhibition caused by 1 ml. of the tumour fluid.

Extracts of liver and kidney from normal animals were prepared by homogenization of the tissues of 3 volumes of Eagle's medium fortified with 15 per cent rat serum. The effect of the supernatant fractions of these extracts, obtained at 105,000g, on cell migration was then determined (Table 1). Increased concentrations of normal rat serum and serum from tumour bearing rats were also assayed (Table 1). These results indicate that the inhibitory effect is not an overall property of normal tissues.

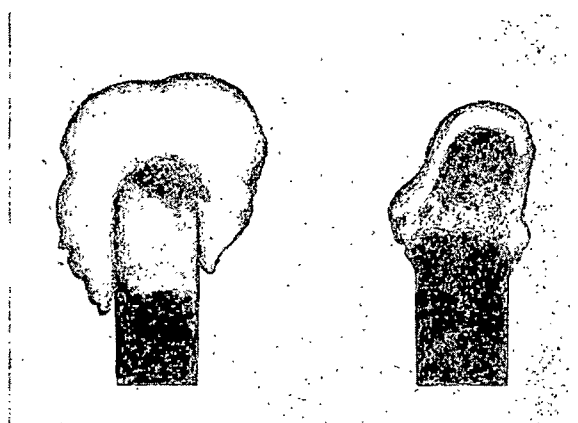


Fig. 1. Migration of peritoneal exudate cells in 24 h in typical culture containing minimum essential Eagle's medium with 15 per cent normal rat serum (left) and additionally 50 per cent Walker 256 ascites tumour fluid (right).

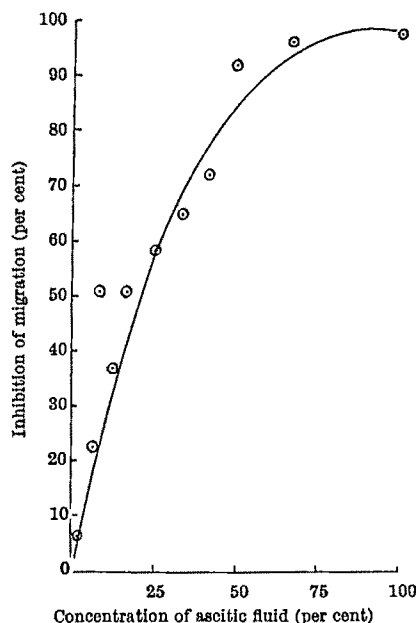


Fig. 2. Relationship of concentration of Walker 256 ascites tumour fluid in the cell culture to inhibition of migration of peritoneal exudate cells after 24 h of incubation. Values for 1.7, 6.7, and 25.0 per cent ascitic fluid represent the average of four determinations each. Values for 33.3, 41.7, 50.0 per cent represent the average of eight, eight, seventeen, six, six, and twenty-two determinations, respectively. Remaining values represent the average of two determinations. The curve was constructed from values obtained using ascitic fluid from thirteen groups of tumour bearing rats, each group containing six to twelve animals.

Table 1. EFFECT OF TISSUE EXTRACTS AND SERUM ON MIGRATION OF PERITONEAL EXUDATE CELLS

Fraction	Concentration in culture medium (per cent)	Inhibition* (per cent $\pm$ S.D.)
Liver	8.3	-51 $\pm$ 30
Liver	16.7	-79 $\pm$ 18
Kidney	8.3	-5 $\pm$ 5
Kidney	16.7	-26 $\pm$ 50
Normal serum†	50.0	-3 $\pm$ 16
Walker 256 serum	50.0	-3 $\pm$ 8

\* Values for percentage inhibition represent mean values  $\pm$  S.D., each based on four determinations. Negative values indicate that migration was stimulated rather than inhibited.

† Serum from non-tumour-bearing rats.

Table 2. EFFECT OF EIGHT TREATMENTS OF WALKER 256 ASCITES TUMOUR FLUID ON ITS ABILITY TO INHIBIT *in vitro* MIGRATION OF PERITONEAL EXUDATE CELLS

Treatment	Concentration in culture medium (per cent)	Inhibition (per cent)
Normal collection (22° C, 30 min)	50.0	92 $\pm$ 5
4° C, 13 days	50.0	58 $\pm$ 5
22° C, 8 h	50.0	55 $\pm$ 24
56° C, 30 min	50.0	-30 $\pm$ 24
Dialysis, 24 h, 4° C	50.0	91 $\pm$ 8
Lyophilization	50.0	54 $\pm$ 11
Normal collection (22° C, 30 min)	16.7	53 $\pm$ 13
-4° C, 1 month	16.7	59 $\pm$ 9
4° C, 24 h	16.7	49 $\pm$ 9
'Pronase', 2 PUK/mg of protein*, 24 h, 4° C	16.7	7 $\pm$ 18

Values for percentage inhibition represent mean values  $\pm$  S.D. Each value is the average of four determinations except normal collection (16.7 and 50.0 per cent concentration), dialysis, and 'Pronase'. These values represent 9, 21, 7, and 8 determinations, respectively. Dialysis was performed against three changes of glass double distilled water, pH 6.7.

\* 'Pronase' units are expressed according to Nomoto *et al.*<sup>2</sup>.

The results of preliminary studies aimed at the elucidation of the nature of the inhibitory activity are summarized in Table 2. These data suggest that the activity is associated with a labile macromolecule which is either a protein or is associated with a protein. It was necessary to carry out the 'Pronase'<sup>2</sup> incubation at 3° C, and so the action of this protease derived from *Streptomyces griseus* was possibly incomplete. That 'Pronase' alone had no effect on migration was shown by adding an equal amount of 'Pronase' to cultures containing only the culture medium.

We determined whether the inhibitory effect occurred in an ascitic fluid produced by an agent other than a tumour. Non-tumour ascitic fluid was produced with complete Freund's adjuvant according to the method of Munoz<sup>3</sup>. The non-tumour ascitic fluid which was allowed to clot exhibited no inhibition, whereas that which contained 1.2 mg of disodium ethylenediamine tetraacetate (sodium EDTA)/ml. as anticoagulant showed a moderate level of activity (Table 3). This observation indicated that either fibrinogen or a protein involved in the clotting mechanism may be responsible for the inhibitory effect. When plasma from healthy rats was assayed, it was also found to be inhibitory (Table 3).

The tumour ascitic fluid seldom clotted and the level of inhibitory activity was twice as high as that of the non-tumour ascitic fluid, and so it appeared that an inhibitory protein may be present in the tumour fluid which is not present in other fluids.

The Walker 256 ascites tumour fluid (sufficient to contain an average of 45 mg of protein) was subjected to preparative electrophoresis in 5 per cent acrylamide gel<sup>4</sup>, using *tris*-EDTA-boric acid buffer, pH 8.32 (ref. 5), at

Table 3. EFFECT OF ASCITIC FLUID AND PLASMA FROM NON-TUMOUR-BEARING ANIMALS ON PERITONEAL EXUDATE CELL MIGRATION

Sample	Treatment of sample	Concentration in culture medium (per cent)	Inhibitory activity* (U)
TAF†	None	16.7	100
N-TAF‡	Clotted	16.7	0
N-TAF	Sodium EDTA	16.7	50
Normal rat plasma	Sodium EDTA	16.7	103
	Sodium EDTA	—	20

\* Each value represents the average of eight determinations.

† Walker 256 ascites tumour fluid.

‡ Non-tumour ascitic fluid induced with complete Freund's adjuvant.

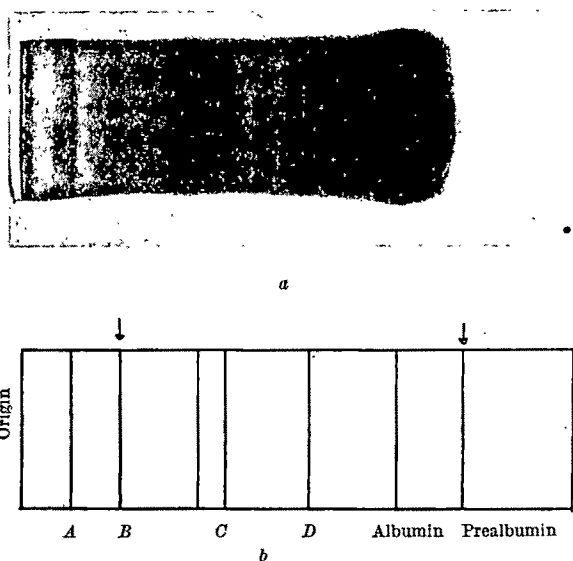


Fig. 3. Protein bands from Walker 256 ascites tumour fluid on acrylamide gel. The anode compartment was at the right as positioned here. Amido-Schwartz stain in acetic acid. (a) Photograph of preparative gel; (b) schematic diagram of the preparative gel,  $\times 0.9$  the scale (a), showing the approximate centres of regions later isolated.

300 V, which resulted in a current of approximately 150 mAmp when terminated after 3.5 h (Fig. 3). The protein fractions were subsequently recovered by elution convection electrophoresis<sup>6</sup> (Table 4). The total protein recovery averaged 50 per cent. The designations of these directly derived fractions were identical with the arbitrary regional labels given to the stained bands on the preparative gel (Fig. 3). Two of the elution convection electrophoresis fractions (designated B and prealbumin) were found to be inhibitory (Table 4, Fig. 3 at arrows); the other fractions stimulated migration. In the six experiments of Table 4, fraction B was found to be inhibitory in four experiments only, while the prealbumin fraction was inhibitory in all six.

Table 4. ASSAY OF PROTEIN FRACTIONS OF WALKER 256 ASCITES TUMOUR FLUID OBTAINED FROM ACRYLAMIDE GELS BY ELUTION CONVECTION ELECTROPHORESIS\*

Fraction	Inhibition (per cent)	Inhibitor activity (U)	Total protein in culture (mg)	Inhibitor specific activity (U/mg protein)
TAF†	46	100	21	4.8
Origin‡	-18	0	0.26	0
A†	-15	0	0.25	0
B†	8	15	0.22	36.4
C†	-26	0	0.52	0
D†	-38	0	0.95	0
Albumin‡	-40	0	1.89	0
Prealbumin‡	20	40	0.16	250.0

Negative values represent a stimulation of migration.

\* Values for Walker 256, fraction B, and prealbumin fraction represent the average of six experiments with four replicates in each experiment. Other values represent two experiments with four replicates.

† Unfractionated Walker 256 ascites tumour fluid.

‡ Fraction obtained from region so designated in Fig. 3.

Using analytical acrylamide gel electrophoresis the B fractions of tumour ascitic fluid and the fractions from the region comparable with B for sodium EDTA-treated non-tumour ascitic fluid, and for plasma, were each resolved into three components, whereas only two components were observed in the fractions from the comparable region to B derived from serum and from clotted non-tumour ascitic fluid. On the basis of these electrophoretic results, fraction B is thought to contain the protein responsible for the inhibitory activity previously mentioned found in plasma and in sodium EDTA-treated non-tumour ascitic fluid, that is, fibrinogen.

The prealbumin fraction from the tumour fluid was subjected to further electrophoresis in glycine and sodium hydroxide buffer, pH 9.6 (Fig. 4), and in sodium succinate

## AGRICULTURE

## Influence of Diet on the Behaviour Pattern of Sheep

BALCH<sup>1</sup> noted that under normal management adult sheep and cattle sleep little if at all. He suggested that this behaviour pattern may be related to the need for maintaining the thorax in an upright position for proper functioning of the reticulo-rumen, and to the requirement of time and consciousness for rumination. He defined sleep as a marked relaxation of consciousness and especially of vision and hearing ability. The following observations on the behaviour of ewes would seem to support the suggestion that the typical sleepless behaviour pattern is connected with the requirements for rumination.

Six ewes were kept in 3 m × 3 m pens, where they had free access to water and to pellets of finely ground dried grass. They also received daily 2 kg of a conventional concentrated mixture for ewes which was given in two meals at the two daily milking times. While the ewes were on this diet no rumination was observed. This is in accordance with earlier reports of an absence of rumination when animals are fed on diets in which all the roughage is finely ground<sup>2</sup>. The pens were brightly illuminated throughout the period. After an initial period of 14 days, during which the animals remained healthy and milked normally, experimental treatments were applied. These consisted of an hourly injection of 9 units of oxytocin through a semi-permanent jugular cannula. Every hour the ewes were taken from their pens and walked 20 m through a passage into the milking parlour, injected and then returned to their pens. After the first few hours of treatment the ewes began to show obvious signs of fatigue and even of exhaustion. Their walk became slow and they had to be pushed through to the parlour. Three of the ewes had periods of panting. Most striking was their behaviour between injections. They lay down in a quite atypical position with the head straight out between the forelegs and the thorax fully inclined. The position was similar to that of a sleeping pig or dog. Their eyes were closed and the ewes did not respond to any noise or the approach of the experimenter. It was necessary to prod them repeatedly in order to awaken them from what, in the terms of the definition above, was a deep sleep. The milk yield dropped 15 per cent over the 4 days of the treatment.

On the advice of Dr. C. C. Balch, roughage was added to the diet; the ewes were offered hay *ad lib.* for 1.5 h every evening. This resulted in a dramatic recovery; rumination was observed within 2 h of the first hay meal and within 4 h all the obvious signs of distress and exhaustion had disappeared. No panting was observed. Between injections the ewes lay down in a typical position, with the thorax held upright and eyes open; their hearing was alert, and on many occasions rumination was observed. When the experimenter approached the pens for the hourly injection the ewes stood up and moved towards the gate of the pen in anticipation. The milk yield increased at the morning milking following the first meal of hay and had regained the earlier level by the next evening. Although the injections were continued in this group of ewes for a further 5 days, and subsequently on other groups of ewes for similar periods, no recurrence of the unusual behaviour was observed.

These observations suggest that ewes which were not ruminating, because of the finely ground diet, needed to sleep for considerable periods and were greatly upset when disturbed hourly. This need for sleep disappeared when the ewes were given hay and they began to ruminate.

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<sup>1</sup> Balch, C. C., *Nature*, 175, 940 (1955).

<sup>2</sup> Ruckebusch, Y., and Marquet, J. P., *Rev. Méd. Vét., Toulouse*, 114, 893 (1959).

Fig. 4. Vertical acrylamide gel electrophoresis of prealbumin fraction of Walker 256 ascites tumour fluid in glycine buffer, pH 9.6. Origin was at top and anode compartment was proximal to base. Prealbumin applied was initially isolated from the tumour fluid by elution convection electrophoresis from acrylamide gels after electrophoresis in *tris*-EDTA-boric acid buffer, pH 8.3.

buffer, pH 5.2. In both buffers, this fraction migrated as a single band. This fraction was found to exhibit inhibitory activity at a concentration as low as 6 µg of protein/ml.

The hexose and hexosamine bound to protein were determined for the electrophoretically isolated prealbumin fraction from the tumour fluid according to the techniques of Winzler<sup>7</sup>. On the basis of Lowry protein determinations the prealbumin was calculated to contain 2.6 per cent hexose and 0.9 per cent hexosamine.

Work possibly relevant to the foregoing data includes reports of fibrin as a cancer-distinctive substance<sup>8</sup>; the finding that the intravenous injection of a polysaccharide fraction extracted from Ehrlich ascites tumour cells into rabbits results in impaired migration of peripheral blood leucocytes *in vitro*<sup>9</sup>; and a report that a mucoprotein fraction derived from human plasma and urine is inhibitory to cell migration<sup>10</sup>.

Statistical advice and critical comment were given by Dr. Roger Flora and Dr. Alfred J. Crowle respectively. This work was supported by a U.S. Public Health Service Cancer Training Grant.

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<sup>1</sup> McCarthy, R. E., *Cancer Res.*, 24, 915 (1964).

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<sup>3</sup> Munoz, J., *Proc. Soc. Exp. Biol. Med.*, 97, 757 (1957).

<sup>4</sup> Raymond, S., *Clin. Chem.*, 8, 455 (1962).

<sup>5</sup> Peacock, A. C., Bunting, S. L., and Queen, K. C., *Science*, 147, 1451 (1965).

<sup>6</sup> Raymond, S., *Science*, 146, 406 (1964).

<sup>7</sup> Winzler, R. J., in *Methods of Biochemical Analysis* (edit. by Gluck, D.), 2, 279 (Interscience Publishers, New York and London, 1955).

<sup>8</sup> Day, E. D., *The Immunochimistry of Cancer*, 33 (Charles C. Thomas, Springfield, 1965).

<sup>9</sup> Watanabe, T., and Tanaka, F., *Japanese J. Exp. Med.*, 30, 449 (1960).

<sup>10</sup> Ledvina, M., *Physiol. Bohemoslov.*, 12, 548 (1963).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, January 9

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "Recent Developments in Fuel Cells" opened by Dr. D. P. Gregory.

INSTITUTION OF ELECTRICAL ENGINEERS (joint discussion meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Meeting on "The Application of Data Processing in a Large Manufacturing Plant".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Great Highway" (colour films).

## Tuesday, January 10

PHYTOCHEMICAL GROUP (at the School of Pharmacy, Brunswick Square, London, W.C.1)—Symposium on "Plant Tissue Culture".

ROYAL SOCIETY OF MEDICINE, EXPERIMENTAL MEDICINE AND THERAPEUTICS SECTION (at 1 Wimpole Street, London, W.1), at 2 p.m.—Symposium on "Protein Metabolism".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. F. A. Benson and other speakers: "Glow and Corona Discharge Tubes".

INSTITUTION OF THE RUBBER INDUSTRY (at the Eccleston Hotel, Victoria, London, S.W.1), at 5.30 p.m.—Mr. J. Batchelor: "Practical Aspects of Rheology in Polymer Processes"; 7 p.m.—"New Polymers".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Sir Harrie Mussey, F.R.S.: "Problems of Science Policy" (Civil Service Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion meeting on "Are the Safety Arrangements on British Aircraft Electrical Systems Entirely Logical?" opened by Mr. D. O. Burns.

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Prof. G. B. Martinenghi (University of Milan): "New Process for the Simultaneous Refining and Deodorization of Fats and Oils".

PLASTICS INSTITUTE, LONDON SECTION (at Imperial Chemical House, Millbank, London, S.W.1), at 6.30 p.m.—Mr. W. R. Groves: "Injection Moulding of Thermosets".

## Wednesday, January 11

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 11 a.m.—Discussion: "The Newer Metals" (Meeting for Young Metallurgists).

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (joint meeting with the Society of Applied Bacteriology, at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 2.15 p.m.—Meeting on "Aspects of Air Sterilization".

GEOLOGICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. G. Whittle: "Photogeology in Overseas Geological Aid: Aspects of the Work of the Photogeological Division of the Institute of Geological Sciences" (Demonstration); Dr. J. V. Hepworth: "Photogeological Recognition of Ancient Organic Belts in Africa" (Paper).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "High-speed Fuses for the Protection of Diodes and Thyristors" opened by Mr. E. Jacks.

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. A. H. Weaving, Mr. T. A. Wesolowski-Low and Mr. J. A. Izacott: "Quantitative Safety".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Dr. B. L. Clarkson: "Acquisition and Analysis of Random Data".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 8 p.m.—Mr. G. Smith: "Eye Drops".

## Thursday, January 12

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. C. C. D. Miller: "Labour Aspects of Joining the Common Market".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Expediting the Road Programme" introduced by Col. S. Maynard Lovell.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "M.H.D. Generation" opened by Dr. P. R. Howard.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Lord Bowden: "Problems of Research in Universities and Government Establishments" (Civil Service Lecture).

## Friday, January 13

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the I.E.R.E. Medical and Biological Group, at Savoy Place, London, W.C.2), at 10.30 a.m.—Colloquium on "Standardization, Servicing, Maintenance and Organization of Medical and Biological Instrumentation".

ASSOCIATION OF APPLIED BIOLOGISTS (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 10.60 a.m.—General Meeting followed by a Symposium on "The Biology of *Heterodera*".

## Monday, January 16

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5 p.m.—Dr. J. E. Norris: "The Use of Insect Pathogens for the Control of Pest Species".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "Laboratory Work Policy" opened by Dr. K. R. Sturley and Mr. A. D. Collop.

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—The Right Hon. Viscount Watkinson, P.C., C.H.: "Industry" (first of four Cantor Lectures on "Some Aspects of the U.S.A. Today").

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Prof. R. Y. Jennings and Dr. P. E. Kent: "Sea Bed Discoveries and International Law".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURERS (2) IN THE DEPARTMENT OF ELECTRONICS in the fields of integrated circuit design, or digital systems, or computer element design—The Deputy Secretary, The University, Southampton (January 12).

LECTURERS OR ASSISTANT LECTURERS IN THE DEPARTMENT OF MATHEMATICS in one or more of the following subjects: (a) numerical analysis; (b) theoretical physics, in particular the theory of electrons or light scattering; (c) statistics; (d) theoretical mechanics—The Registrar, The University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (January 13).

ASSISTANT LECTURERS OR LECTURERS (2) (graduates with research experience either in inorganic chemistry or in the physical properties of polymers) IN THE DEPARTMENT OF CHEMISTRY—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (January 16).

LECTURER IN STATISTICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (January 16).

LECTURER (with research experience in physical chemistry) IN CHEMISTRY—The Registrar, University College of North Wales, Bangor, North Wales (January 16).

LECTURER OR ASSISTANT LECTURER (with interests in some aspect of either systematic or regional geography) IN THE DEPARTMENT OF GEOGRAPHY—The Deputy Secretary, The University, Southampton (January 17).

BIOCHEMIST (with an honours degree in science (majoring in biochemistry) or equivalent, and suitable research experience) IN THE SOIL BIOCHEMISTRY DEPARTMENT for research on legume nitrogen fixation—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin, 4, Republic of Ireland (January 26).

LECTURER IN THE LARGE ANIMAL TEACHING UNIT based at the Veterinary Field Station, Royal (Dick) School of Veterinary Studies, Easter Bush, Roslin, Midlothian—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (January 27).

SENIOR DEMONSTRATOR (with a degree in science, preferably with honours, and some teaching experience, with specialization in zoology) IN ZOOLOGY at the University College of Townsville, University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, January 27).

LECTURER (preferably with an interest in problems of the atmosphere) IN PHYSICAL OCEANOGRAPHY—The Registrar (Room 39, O.R.B.), The University, Reading (January 28).

LECTURER (with a special interest in some branch of atmospheric physics or in dynamical meteorology) IN METEOROLOGY—The Registrar (Room 39, O.R.B.), The University, Reading (January 28).

LECTURER IN APPLIED MATHEMATICS in the School of General Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 30).

BIOLOGISTS (2) to work on the behaviour and physiology of bees—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. No. 1052/95 (January 31).

LECTURER (with considerable field experience of geology) IN GEOLOGY AND MINERALOGY—The Secretary, The University, Aberdeen (January 31).

RESEARCH ASSISTANT (graduate (or equivalent) in physics, engineering or textile technology) IN THE DEPARTMENT OF TEXTILE TECHNOLOGY to work on the development of woven, knitted and other fabrics—The Registrar, The University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (January 31).

SENIOR RESEARCH FELLOW OR RESEARCH FELLOW IN CHEMISTRY—The Registrar, University of Kent at Canterbury, Canterbury, Kent, quoting Ref. A28 (January 31).

SENIOR TUTOR/DEMONSTRATOR IN GEOGRAPHY at the University of Sydney, Australia, to conduct, or to assist in, courses of human geography, with special reference to tropical areas of Africa and/or South-East Asia; and to assume some major responsibility in the operation of first-year practical classes—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 3).

ASSISTANT LECTURER OR LECTURER IN THE DEPARTMENT OF PHYSICS (research interests of the department include solid state and gaseous plasmas, low temperature physics, semiconductors, materials science and lasers)—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (February 4).

SENIOR LECTURER (with a Ph.D. or comparable academic qualifications in petrology, an interest in field-work, and awareness of economic as well as the theoretical aspects of igneous petrology) IN GEOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, February 10).

POSTDOCTORAL RESEARCH FELLOWS (2) IN THE DEPARTMENT OF PHYSICAL CHEMISTRY, to carry out a joint programme of experimental research and critical evaluation of reaction kinetic data—The Registrar and Secretary, The University, Leeds, 2 (February 14).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with the degree of Ph.D. or equivalent research experience, and normally below the age of 30) in various branches of Chemistry (including Inorganic Chemistry, Chemical Engineering, Engineering, Physics and allied subjects)—The Registrar, The University, Manchester, 13 (February 17).

LECTURERS AND ASSISTANTS (qualified in pure or applied mathematics) IN MATHEMATICS—Secretary of the University Court, The University, Glasgow (February 17).

RESEARCH ASSISTANT (with a Ph.D. or equivalent experience in biochemistry or organic chemistry) IN THE DEPARTMENT OF ORGANIC CHEMISTRY, to collaborate with Prof. J. Baddiley, F.R.S., in research in the field of bacterial walls, membranes and capsules—The Registrar, The University, Newcastle upon Tyne, 2 (February 28).

ASSISTANT OR ASSOCIATE PROFESSOR IN BIOPHYSICS—Dr. L. Katz, Head, Department of Physics, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.



ASSISTANT or ASSOCIATE PROFESSORS (with a Ph.D. degree) IN THE DEPARTMENT OF PHYSICS—Prof. J. L. Ollmenhaga, Head, Department of Physics, University of Victoria, British Columbia, Canada.

BIOCHEMIST (Basic Grade) to assist in the development of a laboratory for the investigation and management of patients with kidney disease—Clerk of the Governors, St. Bartholomew's Hospital, London, E.C.1., quoting Ref. No. ASC/759.

CHIEF TECHNICIAN IN THE BIOCHEMISTRY DEPARTMENT to supervise technicians and various activities of the department, and to help in planning for the move to Guildford in 1969—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

DEMONSTRATORS and RESEARCH ASSISTANTS IN THE DEPARTMENT OF CHEMISTRY—Dr. W. C. Howell, Department of Chemistry, University of Western Ontario, London, Ontario, Canada.

GRADUATE RESEARCH ASSISTANTS IN MATERIALS SCIENCE—Chairman, Department of Materials Science, Thornton Hall, University of Virginia, Charlottesville, Va., 22903, U.S.A.

JUNIOR RESEARCH FELLOW IN PHYSICS—The Secretary, Balliol College, Oxford.

JUNIOR TECHNICIAN or TECHNICIAN (preferably with "A" level chemistry) for biochemical work in a new Experimental Pathology Department—The Secretary, University College Hospital Medical School, University Street, London, W.C.1.

ORGANIC CHEMISTS (with a good honours B.Sc., or equivalent, and preferably experience in aliphatic or heterocyclic chemistry) to join a progressive research team—The Director, Arthur D. Little Research Institute, Inveresk, Musselburgh, Midlothian, Scotland.

POSTDOCTORAL RESEARCH FELLOW IN ORGANIC CHEMISTRY in the School of Chemistry, for work concerned with fungal metabolism, the structure of mold metabolites, and the biosynthesis of the gibberellins—The Registrar, The University, Leeds, 2.

PROFESSOR OF EXPERIMENTAL NUCLEAR PHYSICS—Dr. L. Katz, Director, Accelerator Laboratory, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

READER IN HUMAN NUTRITION—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

RESEARCH FELLOW (chemist and physicist with research experience) IN THE DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY for work on high-pressure chemistry, mainly on the solid state—Dr. R. S. Bradley, School of Chemistry, The University, Leeds, 2.

RESEARCH OFFICER (with a Ph.D. degree or equivalent research experience in polysaccharide chemistry) to undertake full-time research on the structure and metabolism of polysaccharides of tropical pasture legumes—The Warden, University College of Townsville, P.O. Box 999, Townsville, Queensland, Australia.

RESEARCH OFFICER (with a degree or equivalent qualification in chemistry or physics or electronics, and preferably experience in the maintenance, operation, and construction of scientific instruments) IN CHEMISTRY—The Registrar, University of Queensland, St. Lucia, Brisbane, Queensland, Australia.

RESEARCH TECHNICIAN (preferably with previous experience of histological and/or photographic techniques) for work on electron microscopy—Dr. R. M. Hardisty, Department of Haematology, Hospital for Sick Children, Great Ormond Street, London, W.C.1.

SCIENTIFIC INFORMATION OFFICER (with a degree in agriculture or related subjects and a flair for languages, including English) to join a team engaged in publishing the latest research findings on temperature and tropical grasslands and annual field crops in two journals of international repute—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berkshire.

SENIOR TECHNICIAN IN THE DEPARTMENT OF CHEMISTRY to be responsible for the teaching and postgraduate laboratories and for a team of four technicians—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

SENIOR TECHNICIAN (with considerable laboratory experience and preferably some experience of electronics and/or workshop practice) IN THE DEPARTMENT OF INORGANIC, PHYSICAL AND INDUSTRIAL CHEMISTRY, to assist in research projects using nuclear magnetic resonance and electron spin resonance techniques—The Registrar, The University, Liverpool, 3, quoting Ref. No. 315.

TECHNICIANS/JUNIOR TECHNICIANS (young men and women of 18 and over) in the general teaching laboratories and physical chemistry laboratories in the Department of Chemistry—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

*Corrosion Control Abstracts*, No. 1. (English translation of *Referativnyy Zhurnal, Korroziya i Zashchita ot Korrozi*.) Pp. ii+1-98. Bimonthly. Annual subscription: £20; \$60. (London: Scientific Information Consultants, Ltd., 1966.) [2410]

Report of the Agricultural Research Council for the year 1965-66. Pp. vi+82. (London: H.M. Stationery Office, 1966.) 7s. 6d. net. [2410]

Ministry of Technology. Forest Products Research Special Report No. 23: The Quality of Ash from Different Parts of Britain. Pp. iv+8. (London: H.M. Stationery Office, 1966.) 3s. net. [2410]

National Society for Clean Air. Clean Air Year Book 1966-1967. Pp. 124. (London: National Society for Clean Air, 1966.) 3s. [2510]

Institute of Personnel Management, 1965-1966. Pp. 37. (London: Institute of Personnel Management, 1966.) [2610]

Science and Irish Economic Development, Vol. 1: Main Report. (Report of the Research and Technology Survey Team appointed by the Minister for Industry and Commerce in November, 1963, in association with OECD.) (Pr. 8975.) Pp. xxix+197. (Dublin: Stationery Office, 1966.) 7s. 6d. [2710]

Proceedings of the Royal Irish Academy. Vol. 64, Section A, No. 10:  $\mu$ -Distributed Sequences. By B. H. Murdoch. Pp. 143-161. 6s. Vol. 65, Section A, No. 1: Multiplicities in Weight Diagrams. By James McConnell. Pp. 1-12. 4s. Vol. 65, Section A, No. 2: The Exhaustion Method of Nucleus Size Analysis. By P. J. Nolan and J. A. Scott. Pp. 13-25. 3s. Vol. 64, Section B, No. 23: The Distribution and General Ecology of the Irish Siphonaptera. By A. J. M. Classens and F. J. O'Rourke. Pp. 413-464. 8s. Vol. 64, Section B, No. 24: Character Study of Soils Developed on Weichselian Glacial Deposits in Ireland. By P. Ryan and T. Walsh. Pp. 465-508+plate 28. 8s. Vol. 64, Section B, No. 25 and 26: The Species of the Genus *Echytus* so far Found in Ireland (Hymenoptera: Ichneumonidae). By A. W. Steffox. Notes on the Species of the Superfamily Heloroidea so far Found in Ireland (Hy-

menoptera). By A. W. Steffox. Pp. 509-516. 1s. Vol. 64, Section B, No. 27: The Stratigraphy and Structure of the Dalradian Rocks North of Bessac, Connemara, Co. Galway. By W. M. Edmunds and P. R. Thomas. Pp. 517-528+plates 29-31. 5s. Vol. 64, Section B, No. 28: A List of the Proctotrupinae Found in Ireland (Hymenoptera). By A. W. Steffox. Pp. 529-540. 1s. 6d. Vol. 65, Section B, No. 1: The Flora of European Turkey. By D. A. Webb. Pp. 1-100. 20s. Vol. 65, Section B, No. 2: A List of the Species of Belytinae (Hym. Proctotrupoidea) so far known from Ireland, With a Few Records of Species Taken in Great Britain. By A. W. Steffox. Pp. 101-115. 2s. (Dublin: Royal Irish Academy, 1966.) [2710]

### Other Countries

Organization for Economic Co-operation and Development, Paris. Government Purchasing in Europe, North America and Japan—Regulations and Procedures. Pp. 115. 10 francs, 15s.; \$2.50. The Engineering Industries in North America, Europe and Japan—A Study by the Special Committee for Machinery. Pp. 260. 15 francs; 22s. 6d.; \$3.80. (Paris: Organization for Economic Co-operation and Development; London: H.M. Stationery Office, 1966.) [2410]

U.S. Department of Commerce: Coast and Geodetic Survey. Environmental Science Services Administration. Deep Circulation, Central North Pacific Ocean: 1961, 1962, 1963. By William D. B. Beebe. Pp. viii+104. (Washington, D.C.: Government Printing Office, 1965.) \$1.75. [2410]

Institut for Atomenergi, Kjeller, Norway. Kjeller Report No. 105: Theory of Rossi—a Experiment in Reactor Noise Analysis. By D. Babala. Pp. 21. Kjeller Report No. 106: Interval Distributions of Reactor Neutron Counts. By D. Babala. Pp. 8. (Kjeller, Norway: Institutt for Atomenergi, Kjeller Research Establishment, 1966.) [2410]

Commonwealth of Australia. Department of National Development: Bureau of Mineral Resources, Geology and Geophysics. Petroleum Search Subsidy Acts. Publication No. 22: A.A.O. Pickanjinie No. 1, Queensland, of Associated Australian Oilfields N.L. Pp. 36+plate 1. Publication No. 54: Summary of Data and Results, Perth Basin, Western Australia. Eneabba No. 1, Hill River Stratigraphic Wells, Woolmulla No. 1 of West Australian Petroleum Pty. Limited. Pp. 50+plates 1-9. Publication No. 55: Summary of Data and Results, Perth Basin, Western Australia. Jurien No. 1, Abbarwardoo No. 1, Eganu No. 1 of West Australian Petroleum Pty. Limited. Pp. 22+plates 1-3. Publication No. 63: Dalhousie Gravity Survey, South Australia, 1963, by French Petroleum Company (Australia), Pty. Limited. Pp. 28+3 plates. Publication No. 69: Summary of Data and Results, Great Artesian Basin, Queensland and South Australia. Innamincka-Betoota Aeromagnetic Survey; Oodnadatta Aeromagnetic Survey of Delhi Australian Petroleum Ltd. Pp. 19+plates 1-4. Publication No. 70: Summary of Data and Results, Bowen Basin, Queensland. A. F. O. Inderi No. 1 Well, A.F.O. Rolleston No. 1 Well of Associated Freney Oil Fields N.L. Pp. 24+plates 1 and 2. 1:250,000 Geological Series—Explanatory Notes. Betoota, Qld., Sheet SG/54-6, International Index. Compiled by W. Jauncey. Pp. 10. Mount Marumba, N.T., Sheet SD/53-6, International Index. Compiled by H. G. Roberts and K. A. Plumb. Pp. 15. (Parkes, A.C.T.: Bureau of Mineral Resources, Geology and Geophysics, 1964 and 1965.) [2510]

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(38)

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(57)

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(64)


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(56)

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(42)

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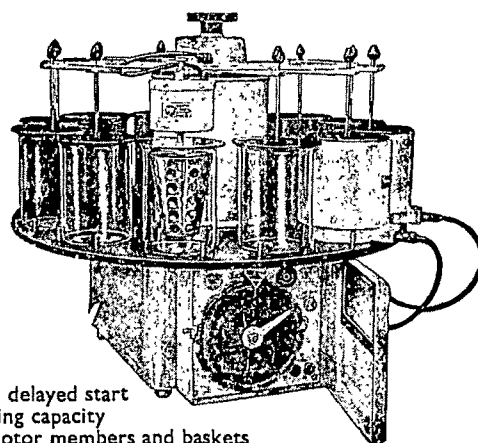
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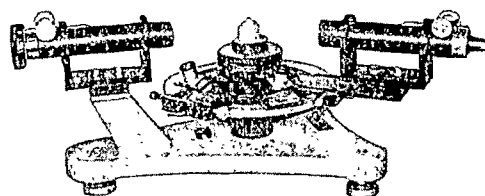
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## JOHN DEWEY'S GHOST

THE Plowden Committee on Primary Education has unfortunately produced a report which is more a textbook than a social document (*Children and the Primary Schools*, H.M.S.O., £1 5s.). On a number of important issues, the report is sensible and even adventurous, but its force is blunted by a web of exhortation and advice about the handling of the different parts of the primary school curriculum. It is also suffused with the attitude towards the education of the very young which has become characteristic of primary schools in Britain and elsewhere ever since the belated recognition half a century ago that even young schoolchildren must be treated as if they were real people. Of course they must—and it is high time that an influential authority should at last have said that corporal punishment must be forbidden in schools. It is also right that the examination used at eleven to channel children into different forms of secondary education should be discredited, for it is unnecessary as well as unjust. But does it follow that primary teachers should be as unconcerned as they are with what might be the intellectual consequences of their teaching? If it had wished to, the Plowden Committee might have asked whether the contemporary ethos of the primary school, humane though it may be, is a sufficiently tangible framework on which to build an educational process.

None of this detracts from the scheme the committee has devised for spreading the social benefits of primary education more equitably about the United Kingdom. There is now plenty of evidence that children from poor home environments are less likely to profit from the time they spend in school than children from better backgrounds. It follows that the best way of implementing the old slogan "equality of opportunity" is to see that children from poor environments are provided with better facilities at the schools they attend—better buildings, more teachers and more equipment. The Plowden Committee has had the courage to accept this principle, which is entirely to its credit. In doing so, it has designed a British alternative for schemes being tried elsewhere—the educational parts of the Poverty Program in the United States, for example. The committee proposes that local authorities should be encouraged to single out those of their schools at which children suffer from special social handicaps, and that they should then receive special help from public funds, partly so as to enable them to pay teachers better salaries. It remains to be seen whether the Government will make these funds available (as it should) and whether they will filter efficiently through the tortuous procedures that will no doubt be devised to see that public money is fairly spent. It might even now be wiser to devise a more rapid if less precisely equitable scheme for channelling

money to poor areas of the country. But the principle is sound, and should promptly be accepted.

The Plowden Report is also sound in its conclusion that there should be a more generous provision of nursery schools for children under five. Here, too, the case rests principally on social arguments, and is the now familiar springboard for schemes such as "Operation Headstart" in the United States. Educational stimulation at an early age is one way of helping children to liberate themselves from unfavourable backgrounds. There should be more of it. (With all this enlightenment, it is odd that the committee should be as complacent as it is about the psychiatric services for the primary schools.) On in-service training for teachers, the committee takes an entirely proper line, and even puts on record what many educationists have been saying for years—that every teacher should have an opportunity for intellectual refreshment at least once every five years. But is this enough? It is disappointing that the Plowden Committee has not made more of the schemes being tried out by a number of local authorities for giving primary teachers continuing help in certain fields, particularly in languages, mathematics and science. The committee will also have provided the British Government with a measure of support in its running battle with the teachers' unions by declaring that there is a need for more ancillary helpers in the schools, but its attempt to argue that the age of transfer from primary schools to secondary schools should be shifted from eleven to twelve is so much like an attempt to count the angels on the point of a needle that this issue will be thoroughly confused for years to come. That the committee should have made such heavy weather of this point is not, of course, a great surprise, given its confusion about the objectives of primary education.

Properly enough, the committee fought shy of some of the more obvious platitudes, but it describes the ideal school as a "community in which children learn to live first and foremost as children and not as future adults". To the extent that this formula encourages flexibility in primary schools, it is entirely admirable. Obviously the old pattern of primary education in which children of quite tender ages were regimented as if they were being put through a course of Pavlovian conditioning is thoroughly bad. But the ideal which the Plowden Committee has re-defined is also a licence for much that is shoddy in current teaching practice. All too often the assumption that there is a child's world distinct from that of adults allows primary school teachers to temper the rigours of the real world with the commendation of pointless effort. But, in reality, children would gain a great deal from a more open acknowledgment of the continuity between the

worlds which children and adults occupy. Even the Plowden Committee is pleased with the way in which the earlier acquisition of reading skills in British primary schools has made it possible for young children to participate at an early age in the adult practice of leisured reading. Many of the new mathematics programmes for young children have been consciously designed to give them a more mature appreciation of difficult matters without violating the well intentioned informality of the primary school. It is a great pity that the Plowden Committee has not followed its admirable definition of social goals by a more thorough search for intellectual goals as well. The progressive style of primary education for which John Dewey acted as an inspired impresario is better than what went before, but its practitioners are uncommonly smug in their conviction that knowing how to teach is more important than knowing what.

## DOOMSDAY 1967

THE British Broadcasting Corporation has an enviable reputation for the sober and honest treatment of scientific subjects on television, so that there was some surprise last week that the corporation should have welcomed the New Year with a programme called "Challenge" designed as if to draw attention to the catastrophe there will be if something is not done to call a halt to the spread of science and technology. With perverse thoroughness, the programme vividly delineated the problems occasioned by the introduction of new techniques of all kinds. Air pollution and water pollution were fully dealt with, as was fall-out. The emergence of strains of bacteria resistant to antibiotics was held up as a danger. The toll of human life on modern highway systems was well covered, as were several of the consequences of the rapidly growing population of the world. Tales were told of how it might one day be possible to use molecular biology for doing strange things with "the nature of life itself", and there was the worry about who should be given kidney machines and who should be left without. Altogether it was a performance to keep people awake at nights. It is no wonder that one of the participants reports a young married couple of his acquaintance to have resolved not to have children of their own. Unfortunately there is no reason to suspect that the programme was intended as a subtle means of solving what is called the population problem, so that its producers are not entitled to much credit on that account.

Indeed, they are entitled to no credit at all, for they seem to have fallen into the old doomsday fallacy which has been claiming social commentators as victims at least since the invention of gunpowder. It is easy and it is also tempting to let the mind dwell on the problems which change occasions. No doubt there were even Palaeolithic Cassandras who complained that some essential quality of primitive life would be lost if people settled down to keeping domestic animals and

growing crops (and there may be people now who say those ancient forebodings were correct). As things are now, with innovations of all kinds following each other in quick succession, the opportunities for gloomy prediction have multiplied enormously. But so, too, have the opportunities for avoiding disaster. Certainly it is wrong to make a fuss about the potential hazards of resistant micro-organisms without balancing this with an appreciation of the diminishing hazards of infectious diseases. It is equally misguided to forget that pollution in rivers may frequently be caused by the fertilizer plants upon which, in part, the survival of badly nourished people may depend. And wild talk about the potential hazards to society which may spring from recent work in biology is often simply wicked. The real problem is to find some way of balancing the benefits of change against the nuisances which change may often bring, or to find some way of eliminating the nuisances. The doomsday view does not help but, rather, hinders. It is no accident that petrified is often used as a synonym for frightened.

## FORWARD PLANNING

THE National Research Council in Canada has broken new and even unfamiliar ground in the planning of research by producing detailed forward forecasts of the resources of various kinds—people as well as money—that would be employed on university research for a decade or more ahead. First attempts in this direction were made in 1963 and, encouraged by the reception of its first forecasts, the National Research Council embarked on a more elaborate survey to use as the basis of a forward prediction, eventually published towards the end of 1966 (*Expenditures on Research in Science and Engineering at Canadian Universities*, NRC 9196, Ottawa, \$1.00). At this stage the greatest interest of the exercise will be the fact that the Forecasting Committee should have embarked on it at all. Nobody will seek to hold it too rigorously to the forecasts which have actually been made, particularly at the end of a decade in which there has been a four-fold increase in the enrolment of students at Canadian universities (where there are now more than 200,000 students), and when graduate enrolment is apparently increasing at something like 20 per cent a year. Yet the actual forecasts have a conservative flavour, perhaps because they have been compiled from the individual forecasts of universities which are accustomed, in the nature of things, to suspect that rapid change cannot continue indefinitely. Even so, total expenditure on university research in Canada may increase five-fold by 1975, from more than \$60 million a year to \$322 million a decade hence. Most of this growth is attributed to increases in the numbers of people engaged on research, both faculty members and students. The increasing cost of keeping one man fully occupied on research seems to be less worrying in Canada than it is elsewhere. It will be interesting to see how this balance shifts with time.

## NEWS AND VIEWS

### Computers Go Home?

THE decision by Bull-General Electric to withdraw from the market two computers designed and built by Machines Bull in France is likely to stiffen resistance to the involvement of American companies in French industry. General Electric obtained a half share in Machines Bull in 1964, and owns 51 per cent of the marketing organization, while the French concern owns 51 per cent of the manufacturing concern. The computers withdrawn are the Gamma 140 and 145, machines designed and built in France, but overlapping with the General Electric 400 series. General Electric says that the very large GE 600 machine, designed for use by a large number of people on a time sharing basis, is still for sale, although considerable disappointment with its performance has been expressed, and some orders cancelled. General Electric also has a foothold in the British market, through De La Rue-Bull Machines, of which 50 per cent is owned by Bull-General Electric, 25 per cent by the De La Rue Company and 25 per cent by General Electric. The computers cancelled were never part of the range sold by De La Rue-Bull in the United Kingdom, and the company has been at pains to point out that their range remains unchanged.

The cancellation is likely to confirm some French prejudices about the influence of American investment in Europe. Computer engineers certainly consider that the attitude of General Electric towards the British and French employees has been unsentimental even by the standards of computer companies who tend to hire and fire staff as the need for software waxes and wanes. The French *Plan Calcul*, which entered a new phase last month with the creation of the Compagnie Internationale d'Informatique, is hardly likely to founder as a result of the cancellations; indeed, it may well be strengthened by what will be interpreted as new evidence of American technological control. The new company, financed by the French Government, is to develop and construct a range of computers able to compete with the American machines; to do this, if it is possible, is likely to cost considerably more than the £50 m. which the French Government has so far decided to spend. Bitter about the General Electric decision, French computer engineers are likely to be making just this point.

### Contaminated Lead

It is becoming increasingly difficult for users of extremely sensitive counters to shield them from extraneous radiation. The problem is that some of the traditional materials used for shielding tend themselves to be contaminated. British steel, for instance, is likely to contain cobalt-60, and ruthenium-103 and -106. Cobalt-60 is introduced in the lining of blast furnaces as a means of checking the wear of the refractory bricks, and both ruthenium isotopes come from radioactive fall-out. One answer, which sounds facetious but is in fact quite serious, is to buy armour plate from old battleships built before the days of

fall-out and radio-tracer techniques. Another, adopted by a Swiss scientist, is to buy old railway lines and have them machined to fit together as a shield. Either course is likely to be very expensive.

The problem now seems to be spreading to lead, a common shielding material. Recently Mr. E. Reynolds of the Fisheries Radiobiological Laboratory reported that lead bricks bought to shield a scintillation counter were contaminated with silver-110 (*Nature*, **210**, 615; 1966), and Dr. J. Rundo of the Medical Physics Department at Harwell has found lead contaminated with antimony-125. Such contamination greatly increases the background levels and reduces the sensitivity of the apparatus, as well as confusing the issue by introducing a background level which continually decreases.

In both cases the source of contamination remains a mystery. One possibility is that there may be a link between the lead Mr. Reynolds has been using and a dismantled reactor—possibly the Merlin reactor, which was owned by Associated Electrical Industries and was demolished at the end of 1963. The Report of the Government Chemist for 1964 showed that the lead from a reactor contained silver-110. The demolition work at Aldermaston was supervised by the Nuclear Inspectorate of the Ministry of Power, which claims that it is very unlikely that any contaminated lead found its way on to the market. On the other hand, Mr. R. J. Philips, the Chief Designer of Graviners of Gosport (who supplied the bricks), believes it to be quite possible. The period between the demolition of Merlin and the discovery of the contamination is long compared with the half-life of silver-110 (270 days), which tends to argue against the reactor being the source of the contaminated lead. Another possible origin for the isotope suggested by Mr. Reynolds is its use by a mining company as a tracer to investigate the efficiency of extraction procedures, but nobody knows of a mining company using such a technique.

The origin of the antimony-125 in Dr. Rundo's lead seems equally obscure. Almost all lead contains antimony to improve its mechanical properties, but how a radioisotope was introduced cannot be explained. Add to this the fact that krypton from the atmosphere can no longer be used in proportional counters because it is contaminated with krypton-85, which has a half-life of ten years, and the magnitude of the problem becomes apparent. Neon must be used; it is less effective, but uncontaminated.

### Fall-out in New Zealand

ANXIETY in New Zealand about the French nuclear weapon testing programme in the Tuamotu Archipelago should be dispelled by a recent report to the Prime Minister. The report, by the directors of the Meteorological Service, the Institute of Nuclear Sciences and the National Radiation Laboratory, concludes that the French tests will add fractionally but not significantly to the long-lived radioactivity in New Zealand, and that the general level of radioactive contamination in the Southern Hemisphere will remain below that in the Northern Hemisphere. The total accumulation of strontium-90 from rain, for example, is more than four times greater in New York City than in New Zealand.

Continuous monitoring of fall-out in New Zealand since 1959 has indeed shown that the contamination of rain by strontium-90 since the late fifties increased until the end of 1964 much as in the Northern Hemisphere. Since then there has been a gradual decline of contamination with the disappearance of debris from nuclear weapons from the stratosphere, and the French tests have so far added very little in the way of long-lived material to the contamination being detected. In retrospect, it is remarkable how the deposition of radio-strontium in New Zealand has followed that in the Northern Hemisphere, though with a time lag and with a peak that never reached the same heights. No doubt the immediate consequences of the recent explosions in China will be less in the Southern Hemisphere than in the Northern.

## Cool Appraisal

SUPERCONDUCTORS have seemed for some time to offer attractive possibilities in power cables and transformers. It is argued that the greatly increased conductivity of cooled conductors or superconductors will more than make up for the cost of keeping them cool. Dr. K. J. R. Wilkinson, of the Central Research Laboratories of Associated Electrical Industries, has now carried out an investigation of the economic and technical feasibility of the idea (*Proc. Inst. Elec. Eng.*, **113**, 1509; 1966) which is likely to moderate some enthusiasm for superconductors.

Dr. Wilkinson has compared the use of niobium at 4° K (a superconductor), aluminium at 20° K (with a resistivity of  $3 \cdot 10^{-9}$  ohm/cm) and beryllium at 77° K (with a resistivity of  $2 \cdot 10^{-8}$  ohm/cm). His basic design uses two concentric annular cooling jackets with an evacuated space between them containing two radiation shields. For the extreme case of niobium at 4° K, Dr. Wilkinson shows that it will be more efficient to use several coolants for providing refrigeration, with the temperature dropping in smaller stages. The conductor would be in the form of a cylinder of niobium foil, with liquid helium circulating internally and liquid hydrogen and liquid nitrogen circulating externally. For the aluminium and beryllium conductors this design would be inadequate, and a wire stranded design is suggested.

Unfortunately, there seems little hope that the designs will ever be more than an academic exercise. The cost comparisons which Dr. Wilkinson makes show that only the niobium superconductor could be expected to offset its refrigeration costs by savings in conductor material and conduction losses. In this case, cost for a 3 phase circuit rated 760 MVA at 275 kV and 1,600 A would be about £12,000/km, against £13,000/km for copper, but this takes no account of construction and laying costs, which would certainly be enormously increased. For aluminium and beryllium the costs are much greater and work out at £24,000/km and £800,000/km respectively. In addition to the cost disadvantage, cooled cables are very vulnerable both to accidental damage and to fault overloads, and liquid hydrogen would represent a real hazard. For transformers the situation seems hardly more optimistic; although transformers wound with aluminium foil at 20° K might show a marginal advantage, this would be more than absorbed by the expenses of

installing, housing and supervising the plant, and looking after the liquid hydrogen.

## Directory of Directorates

UNESCO has produced the first volume of a series intended to be a much enlarged revision of the *Directory of National Science Research Councils* first published ten years ago. The first volume of the new series deals with Europe and North America (*World Directory of National Science Policy-Making Bodies, Vol. 1, Europe and North America*, Francis Hodgson, Ltd., £3 5s.). Later volumes will appear in the next few months. The intention is to include in the volumes those organizations which have a national responsibility for policy-making and the planning, organization and co-ordination of scientific and technological research. An attempt has been made to exclude bodies at "ministerial level" where they have responsibility not confined to planning and co-ordination of research, although clearly criteria for choice in this respect are bound to be exceedingly difficult to apply consistently.

## Mycoplasmas

from a Correspondent in Microbiology

THE Society for General Microbiology in conjunction with the Pathological Society held a timely and stimulating symposium on mycoplasmas in London last week. Papers on fine structure, antigenicity and identification provided a basis for a discussion of the pathogenicity of these microbes and their effects on animal cells. D. Taylor-Robinson proposed that identification schemes should consider the source of isolates—avian, mammalian, tissue culture, and the like—and metabolic attributes of isolates, such as glucose or arginine fermentation and their antigenic structure. Identifications based upon only a few criteria are clearly equivocal. Similarly, extreme caution should be exercised when interpreting electron micrographs purporting to demonstrate mycoplasma-like particles in malignant human tissues. (A warning to this effect was issued recently by the World Health Organization.) Another urgent problem in the study of mycoplasmas is the correct diagnosis of strains as the causal agents of disease. It is surprising and, indeed, disappointing that, in many reports of the isolation of mycoplasmas from disease conditions, these organisms are stated categorically to be the causative agents even though the crucial re-infection tests have not been made. In this context the investigation of swine enzootic pneumonia, described at the meeting by R. F. W. Goodwin and P. Whittlestone, is encouraging and exemplary. These workers obtained a number of mycoplasmas from pneumonic pigs only some of which would induce the disease when inoculated into healthy animals. The causal mycoplasma of the enzootic condition, *M. suis pneumoniae*, has very exacting growth requirements in comparison with other isolates. Thus the readily cultured and less fastidious types isolated from mixed mycoplasma populations may be wrongly identified as the pathogens, especially when the application of Koch's postulates is ignored.

Identification of causative factors is an acute problem in connexion with malignant and arthritic diseases.

Several reports have appeared on the association of mycoplasmas with neoplasms, but the significance of these associations is poorly understood. Some mycoplasmas have properties in common with tumour inducing viruses such as Rous sarcoma and polyoma. At the meeting, W. Russell discussed the transformation of *BHK21-C18* hamster fibroblast cells by mycoplasmas which closely resembled the transforming pattern of tumour viruses. In contrast to virus transformations, however, cells transformed by mycoplasmas showed no evidence of mycoplasma antigens or transplantation immunity, while tumour formation was not induced in hamsters inoculated with *M. fermentans*. Imbalanced nucleic acid metabolism in infected fibroblasts (see also Russell, W., *Nature*, **212**, 1537; 1966) might permit genetic changes which could produce neoplasms: such a role for mycoplasmas in oncogenesis is at present entirely speculative. In defining the current position on mycoplasmas and human leukaemias, R. J. Fallon concluded that there is no established cause and effect relationship even when mycoplasmal antibodies are associated with the leukaemic condition. The question of crypto-mycoplasmal infections not detected easily by ordinary culture methods may be significant here. The failure to isolate mycoplasmas from tissue cultures or rheumatoid synovial membranes probably reflects exacting nutritional requirements which would favour the establishment of obligate parasitism. The induction of, and the means of identifying, crypto-infections are important areas of investigation in attempting to elucidate the relationship, if any, of mycoplasmas to these types of diseases.

## Fluid Logic

SOME 360 delegates met at Cambridge last week at a conference on fluidics—the second of its kind to be held in Britain. The conference was organized by the British Hydromechanics Research Association in conjunction with the College of Aeronautics, the Institution of Mechanical Engineers, and the Society of Instrument Technology.

Fluidics is an abbreviation of the two words “fluid logic” and stands for one of the newest technological tools mainly concerned with small devices without mechanical parts in which, for example, flows can be switched by low pressure impulses, and in which small pressures can be amplified or compared so as to form the *and*, *or*, *nor* types of decisions. The technique offers a means of controlling processes and operations of devices which are insensitive to extremes of temperature, corrosion and the like, and which are also cheap and rugged. Many control problems are implicitly concerned with fluidics in any case, so that fluid logic is often a natural choice of a means of control, whether the fluid is sulphuric acid, radioactive liquid, burning gas, or simply air. Fluid logic devices have already been used successfully in the measurement of angular rate, high temperature, and also in controlling turbine speeds.

The fifty or so papers presented at the conference at Cambridge provided a comprehensive picture of what has happened since the first developments in fluidics seven years ago in the United States. Fundamental and industrial applications were dealt with in the

papers presented, and particular topics discussed included the application of fluidics to nuclear plant and machine tool operations of various kinds. It is encouraging that interest in fluidics has roughly doubled in the past 18 months, at least if interest is accurately reflected in the number of papers presented at the first and second conferences. The universities, the Ministry of Technology, the professional institutions and some sections of British industry are well aware of the challenge and the potential rewards of fluidics, but it is worrying that substantial sections of British industry are apparently unaware of the new technique.

## Ultra-centrifuge Anomalies

WHEN sedimentation and diffusion coefficients are calculated from ultra-centrifuge experiments, certain assumptions are usually made. Originally these were that there is a sharp free boundary between solvent and solute, that the ultra-centrifuge reaches a constant angular velocity immediately after starting and that the sedimentation and diffusion coefficients are independent of concentration. A solution due to Fujita and MacCosham used a realistic boundary condition instead of the sharp free boundary, and the assumption about instantaneous speed-up is incorporated by assuming the centrifuge to have started at an intermediate time equal to two-thirds of the time needed for acceleration. Speaking at a meeting of the physical biochemistry group of the British Biophysical Society, Dr. V. D. Barnett, of the University of Birmingham, suggested that even these modifications may not be enough to give consistent values for the coefficients.

Using an improved estimation procedure (based on a linearized least squares approach) which gives more accurate estimates of the coefficients, and calculating them for different times rather than for only one Schlieren frame, he had obtained estimates of the different coefficients of the process which showed an unexpected time-dependence. In an attempt to resolve this anomaly by relaxing the assumption of instantaneous starting, Professor H. E. Daniels, also of the University of Birmingham, has developed an expression in the form of a power series which describes the concentration gradient profile during the acceleration phase. Combining this with the solution for the profile after the switch-off in the constant angular velocity phase produces the solution for any general time. It is hoped that this modified solution will resolve the earlier anomalies, and data are being analysed to confirm this. In the discussion of Dr. Barnett's paper, some speakers expressed the fear that it may invalidate much previous work carried out with the ultra-centrifuge.

At the same meeting of the Biophysical Society, Dr. S. P. Spragg and Dr. R. F. Goodman, of the Department of Chemistry at Birmingham, discussed the problems associated with the acquisition of data from an ultra-centrifuge by an “on line” computer. Using an absorption technique, pulses are fed directly into the computer through a photo-multiplier, and are stored by the computer only when they are significantly different from the previous pulse. This means that the computer will only store useful information, and by pausing at certain points along the scan can improve the signal to noise ratio significantly.



## Transforming Communities

AGRONOMISTS and sociologists, sponsored by Shell International, have been helping the inhabitants of poor rural communities in Northern Italy, Nigeria, Venezuela and other parts of the world to increase their net income. Some of their work is described in the latest publication of the World Land Use Survey, *The Transformation of Rural Communities* (occasional papers No. 7).

Borgo a Mozzana is a *comune* (the smallest Italian administrative unit) in Tuscany. It has a population of 8,000 with about 1,300 small farms, mostly run by their owners, on poor acid soil. The main crops are cereals, olives, vines and potatoes; cattle are reared for veal and milk. An agronomist went to Borgo in 1954 and began to persuade farmers to improve their agricultural methods and materials. It was two years before he was fully accepted, but gradually new and better strains of crop plants, seeds and fertilizers were introduced. By 1963 the corn and wheat crops had increased, the quality of the vines had improved and the total value of oil produced had increased from 13 to 84 million lira.

A home economics adviser encouraged women of the community to improve child care and domestic hygiene. To improve the poultry, traditionally in the women's care, new breeds of fowls were introduced and a co-operative was formed for the organization of production and marketing of poultry and eggs. Such co-operation was the result of encouragement to the people to discuss their problems together. A consortium was formed for the production of seed potatoes in the hillside hamlet of Cune, and to make technical progress easier a road was built to link Cune with the centre of the *comune*.

In Southern Nigeria an agronomist and his assistant have begun to introduce pest control chemical fertilizers and better varieties of plants to the rural areas of Uboma. The oil palm, which is indigenous to Nigeria and is integrated into farming systems, has been improved as a cash crop, although it has proved difficult to convince farmers of the wisdom of marketing, which they tend to regard as "bad farming". Fish and poultry have been improved to increase the production of animal protein. Farmers have been organized into co-operatives based on religious or other groups; piped water has been supplied, and a health centre built.

In the Zulia district of Venezuela there is a project to improve the standard of agriculture which is based on family groups. Experts spent a year acquainting themselves with the area, and then began to demonstrate to the farmers how to improve their income from the land. Several new crops have been introduced, and weeds which have severely restricted crops in the past have been controlled, as have termites and ants.

The introduction of ploughs drawn by donkeys for fifty of the farmers has enabled the area of cultivation to be increased. Women and children have been encouraged to contribute with domestic trades, such as poultry rearing and bee keeping. The group working on this project consider that progress so far suggests that this will be a practical and relatively inexpensive way of promoting the transformation of traditional agriculture.

## University News :

## Aston in Birmingham

DR. W. O. ALEXANDER, at present technical director with Fosco International, has been appointed professor and head of the Department of Metallurgy.

## Appointments

DR. H. R. FLETCHER, Regius keeper of the Royal Botanic Gardens, Edinburgh, has been appointed Her Majesty's botanist in Scotland.

## Announcements

MR. E. GUDGEON, general sales manager, Harchem, Ltd., Toronto, has been elected chairman of the Chemical Economics Division of the Chemical Institute of Canada for 1967. He succeeds Lyall Fraser, of the United Shoe Machinery Co. of Canada, Ltd., Montreal.

At the annual general meeting of the Institution of Electronic and Radio Engineers on December 15 the following awards were presented for the outstanding papers published in the Institute's monthly journal, *The Radio and Electronic Engineer*, during 1965: *The Clerk Maxwell Premium* to Dr. G. O. Young for his paper, "Statistical Optimization of Antenna Processing Systems"; *The Mountbatten Award* and the *S. C. Bose Premium* to Professor S. K. Chatterjee and Mrs. R. Chatterjee for their paper, "Dielectric Loaded Waveguides—A Review of Theoretical Solutions"; *The Heinrich Hertz Premium* to J. S. Heeks, A. D. Woode and C. P. Sandbank for their paper, "The Mechanism and Device Applications of High Field Instabilities in Gallium Arsenide"; *The Lord Rutherford Award* to F. D. Boardman, E. L. E. Harrington and D. J. A. Carswell for their paper, "A Correlator for Investigating Random Fluctuations in Nuclear Power Reactors"; *The A. F. Bulgin Premium* to D. Harrison for his paper, "The Mechanism of Interference Pick-up in Cables and Electronic Equipment with special reference to Nuclear Power Stations"; *The Marconi Award* to J. Salomon, S. Pichafroy and P. Hurbain for their paper, "A Radar Receiving Array with I.F. Multiple-beam Forming Matrix"; *The Leslie McMichael Premium* to W. J. Battell for his paper, "An Analysis of Results obtained on an Aircraft Data Link out to 1,300 Nautical Miles (2,400 km)"; *The J. Langham Thompson Premium* to P. C. Young for his paper, "The Determination of the Parameters of a Dynamic Process"; *The Babbage Award* to P. I. Bonyhard and W. S. Carter for their paper, "Large Capacity Magnetic Film Stores—A Design Approach"; *The Dr. Norman Partridge Memorial Premium* to H. Lindskov Hansen for his paper, "A Groove Feed and Depth Control System for Gramophone Disk Cutting Equipment"; *The Rediffusion Television Premium* to F. G. Johannessen for his paper, "The Performance Requirements of a Television Monitor Receiver (Nyquist Demodulator) and Methods of Measurement".

THE 1966 Entomological Society of Canada Annual Gold Medal Award for outstanding achievement in Canadian entomology has been awarded to the late Dr. C. W. Farstad, who was director of the Plant Protection Division of the Canadian Department of Agriculture.

THE Paul Instrument Fund Committee, which is composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, and was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science", has made the following awards: Dr. R. D. DAVIES, senior lecturer in radioastronomy in the University of Manchester, £4,132 to enable him to extend from

120 to 192 the number of frequency channels on the spectrometer for radioastronomy using digital auto-correlation techniques; PROF. J. N. HUNT, professor of physiology, Guy's Hospital Medical School, £980 for the construction of a recording *in vivo* colorimeter; DR. W. J. JONES, University Chemical Laboratory, Cambridge, £500 for the purchase of equipment to enable him to increase the efficiency of the laser apparatus for the excitation of Raman spectra; DR. D. W. TURNER, reader in organic chemistry at the Imperial College of Science and Technology, London, £3,000 for the construction of a K-shell photoelectron spectrometer.

A NEW journal entitled *Chemical Physics Letters* is being published by North-Holland Publishing Company as from January this year. It will appear initially as a monthly journal and deal primarily with results of theoretical and experimental research in the field of chemical physics.

DR. B. C. J. LIEVEGOED, professor of social pedagogy at the Netherlands School of Economics and dean of the Faculty of Social Sciences in the new Twente Technological University Enschede, will give the first of the newly established Andrew Betts-Brown Memorial Lectures at Heriot-Watt University on January 16. The title of his lecture, which is open to the public, is "Consequences of Technological and Social Change".

THE eighth International Embryological Conference will be held in Interlaken during September 3-9. Further information can be obtained from Dr. A. Curtis, Zoology Department, University College, London, Gower Street, W.C.1.

AN international symposium on "Tropical Root Crops" is to be held in the University of the West Indies during April 2-8. Further information can be obtained from the Secretary, International Symposium on Tropical Root Crops, Department of Agriculture, Crop Production, University of the West Indies, St. Augustine, Trinidad.

A SYMPOSIUM on "Atomic Absorption Spectroscopy" is to be held in the Welsh College of Advanced Technology during April 6-7. Further information can be obtained from the Organizer of Short Courses, Welsh College of Advanced Technology, Cathays Park, Cardiff.

A ONE-DAY symposium on "Telemetry in Medical and Biological Research", organized by the Scottish Sections of the Institution of Electronic and Radio Engineers and the Institution of Electrical Engineers, will be held at the Royal Infirmary, Edinburgh, on March 9. Further information can be obtained from P. M. Elliott, Honorary Secretary, Scottish Section, I.E.R.E., 21 Craigmount Loan, Corstorphine, Edinburgh 12.

ERRATUM. In the communication by Dr. E. T. Gláz, E. Csányi and J. Gyimesi entitled "Supplementary Data on Crotoxin—an Antifungal Antibiotic" (*Nature*, 212, 617; 1966) the second sentence of the fourth paragraph should read "... the cytotoxic concentrations (in  $\mu\text{g}/\text{ml.}$ ) which resulted in 50 per cent inhibition of transplantability were as follows: crotoxin 400.0, crotoxin 250.0, trichothecin 20.0 and trichothecol (the alcohol part of trichothecin) 400.0".

CORRIGENDUM. In the communication "Renin and Euryhalinity in the Japanese Eel, *Anguilla japonica*" by Dr. H. Sokabe, Dr. S. Mizogami, Dr. T. Murase and Prof. F. Sakai (*Nature*, 212, 952; 1966), on line 4 "three to" should be inserted between "for" and "eleven", and on line 38 "of pH 7.4" should be inserted following "buffer".

CORRIGENDUM. In Table 1 of the article "Maternal Origin of the Group Specific (Gc) Proteins in Amniotic Fluid", by M. Usategui-Gomez and D. F. Morgan, in *Nature*, 212, 1600 (1966), in the column headed "Foetal sera" the sixth figure should be 2-1 and not 2-2.

## CORRESPONDENCE

### Origin of the Genetic Code

SIR,—I should like to make two points about the account of my talk to the British Biophysical Society which you published recently<sup>1</sup>. I do not in fact believe that the idea of a stereochemical relationship between all amino-acids and their anticodons, as suggested by Dunnill<sup>2</sup>, is likely. There is, in my opinion, suggestive evidence against it, but at the moment it is not enough to disprove the idea.

My second point concerns the main substance of my talk, which was an attempt to show that a plausible theory could be constructed without necessarily assuming any stereochemical interaction of amino-acids with either codons or anti-codons. I imagined the code to go through three phases:

1. The Primitive Code, in which a small number of amino-acids were coded by a small number of triplets.

2. The Intermediate Code, in which these primitive amino-acids took over most of the triplets of the code in order to reduce nonsense triplets to a minimum<sup>3</sup>. The codons produced by this process for any one amino-acid were likely to have been related.

Woese<sup>4</sup> has pointed out that this state of affairs could also have been produced by reading only a single base of a triplet, or by considerable inaccuracy in the reading of a few triplets.

3. The Final Code, as we have it today.

The crucial idea, already mentioned by Jukes<sup>5</sup>, concerns the transition from 2 to 3, which I certainly do not think was "unlikely to have taken place"<sup>1</sup>. Evolutionary theory suggests that a new amino-acid was incorporated into the developing code only if its introduction at that time gave a selective advantage to the primitive organism. This implies that its introduction did not disturb too much the proteins then being produced, and in addition made a significant improvement to at least one of them. This would have happened most easily if

- (a) the new amino-acid was "related" to the one previously coded by the triplet(s) in question<sup>6</sup>;
- (b) the organism coded rather few proteins;
- (c) these proteins were rather primitive in their construction.

Eventually as the number of proteins coded became larger, and their design more sophisticated, no possible new amino-acid could, on balance, be an advantage and the code would be frozen.

Such a theory could thus explain in a general way the non-random nature of the present code, since "related" amino-acids might well have acquired related codons. It is quite distinct from theories<sup>3,7</sup> which postulate that the code evolved as it did in order to minimize the damaging effects of present-day mutations on individual proteins.

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<sup>1</sup> *Nature*, 212, 1397 (1966).

<sup>2</sup> Dunnill, P., *Nature*, 210, 1267 (1966).

<sup>3</sup> Sonneborn, T. M., in *Evolving Genes and Proteins* (edit. by Bryson, Vernon, and Vogel, Henry J.) (Academic Press, New York and London, 1965).

<sup>4</sup> Woese, C. R., *Proc. U.S. Nat. Acad. Sci.*, 54, 1546 (1965).

<sup>5</sup> Jukes, T. H., *Molecules and Evolution*, 70 (Columbia University Press, 1966).

<sup>6</sup> Epstein, C. J., *Nature*, 210, 25 (1966).

<sup>7</sup> Goldberg, A. L., and Wittes, R. E., *Science*, 153, 420 (1966).

# Foot-and-mouth Disease—a World Problem

by  
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In the past few days there has been some evidence of infection by foot-and-mouth disease among animals in the south of England. In Britain the disease is controlled by slaughtering infected animals. The difficulties of control by vaccines were described by Dr. Brooksby in a lecture to the Royal Veterinary College, London, on November 30, 1966, and the following article is an extract of his lecture

THE problem of foot-and-mouth disease is worldwide. The animal population over a large part of the land surface of the globe is already affected by it and, because of the extraordinary facility with which the virus spreads, no area can be considered safe. It is true that the disease is not one of the major killer diseases, but it is usually considered to reduce animal productivity by something like 25 per cent so that it stands high on the list of diseases which the livestock industry would wish to see eliminated.

## Epizootic Areas

In large areas of Asia, Africa and even some regions of South America foot-and-mouth disease continues to spread virtually unchecked and uncontrolled. In such areas the disease is present at a continuously low level with, from time to time, periods of higher incidence. This situation reflects an interplay between the virus with its different variations and variable immunity in the cattle population. A widespread epizootic due to virus of one type is usually followed by a period when that type is of infrequent occurrence in the area concerned and when the incidence of the disease returns to its low level. The lesions observed in these areas, particularly in indigenous stock, may be slight and restricted to a small number of animals in the herd. From time to time, however, with a new wave of the disease (probably due to a type which has not occurred in the area recently), more severe lesions will be seen. In the absence of very recent infection, the antibody levels in general may be very low and may even be difficult to detect, although subsequently on re-infection a secondary response is obtained with rapid development of antibody.

The situation which has been described of an uneasy equilibrium between virus and susceptible species is only maintained so long as no outside factors are brought in. A new type of virus, or new hosts in the form of fresh animals moved into the area, upset the equilibrium and the disease becomes all too apparent. In Africa much attention is being directed to the problem of cloven-footed game animals as reservoir hosts. In the absence of controlled experiments it is difficult to assess the value of antibody studies in game animals as evidence of infection. In the few that have been studied, it has become noticeable that the highest antibody titres have been obtained in buffalo. In some other game animals such as kudu, the initial titre following infection has been quite low. Nevertheless, game animals have been found to be affected early in the course of an epizootic and in many cases the initial isolation of virus has been in a game animal. It is highly probable that the ecology of the disease in wild animals is not unlike that in the associated cattle. Viruses are found which are adapted preferentially to particular

species and it is possible that wild animals may become infected with strains which produce only minimal signs of the disease, while the same strain transferred to cattle may set up acute and severe infection. The discovery of the existence of carrier animals in domestic stock is an additional factor in bringing about the persistence of virus between outbreaks.

In many European countries, in quite extensive areas of South America and very much smaller parts of Africa and Asia, a determined attempt is being made to restrict the spread of foot-and-mouth disease and in the process its incidence is much more carefully observed. In such areas the characteristic picture is of fairly severe infection and, in the absence of vaccination, extensive spread through the affected herd. In these areas of better agricultural development, control measures restricting the movement of animals and animal products reduce spread and outbreaks can be restricted in this way. In vaccinated populations, severe epizootic spread is usually associated with the appearance of a new type or sub-type, and the same was true of Europe in the first 50 years of this century when recurrent waves of infection occurred at approximately 10-year intervals, with some subsidiary peaks between.

It is in such areas that the incidence of the disease poses a very real problem because the lesions are severe and the secondary complications which result are very damaging to the productivity of the livestock. The most energetic steps are being taken in such areas to reduce the significance of the disease.

"Fringe" areas can be defined as those in which the disease is eliminated for relatively long periods up to a year or even up to 5, 10 or 20 years. In general in such areas the control of the disease has been by eradication by slaughter, and the careful observation of animals after an outbreak has been controlled makes it highly unlikely that new outbreaks are derived from virus circulating in the population. New outbreaks occur rather from importation of the disease from enzootic areas and therefore geographical links with infected areas are not always necessary. The Scandinavian countries, the United Kingdom, the United States and Central America are in this category, although the actual number of outbreaks observed varies from country to country. In all these areas, unless vigorous control measures are applied, the virus spreads rapidly in the susceptible population and the outstanding feature is the ease with which chance contacts lead to secondary outbreaks.

## Free Areas

Some of the areas which are "free" from foot-and-mouth disease have achieved this position as the result of geographical isolation and maintained it by the application

of quarantine measures or restrictions on imports. Among such areas are Japan, Australia, New Zealand and North America. There are also areas, such as the southern part of Argentina, which although on the edge of areas affected by the disease do not themselves appear to become affected.

Although antigenic variation is not the only kind of variability encountered in foot-and-mouth disease virus, it is the one which is most spectacular and which is most frequently discussed. The situation in relation to antigenic variation becomes more complex year by year. At least forty distinct antigenic strains of the virus of foot-and-mouth disease are now known and there is no reason to believe that this list is exhaustive. The degree of antigenic difference between strains varies. The more extreme differences have allowed identification of the seven major type groups, which are known as the immunological types. Lesser differences within these groups are classified as sub-type differences. The seven major types and the dates of their identification are as follows:

- |                 |                               |
|-----------------|-------------------------------|
| (1) Type O      | Vallée and Carré (1922)       |
| (2) Type A      |                               |
| (3) Type C      |                               |
| (4) Type SAT 1  | Waldmann and Trautwein (1926) |
| (5) Type SAT 2  |                               |
| (6) Type SAT 3  |                               |
| (7) Type Asia 1 | Galloway <i>et al.</i> (1948) |
|                 | Brooksby and Rogers (1957)    |

The degree of difference between these types is such that an animal recovered from an infection with one remains susceptible to the others, although there is satisfactory immunity against re-infection with strains of the same type. Recently a number of strains have been found which although belonging to the same general immunological group do not confer the solid immunity indicated by such cross-immunity tests, and a proportion of animals will fail to become immune to challenge with some other strain within the same type group. There will, however, be no immunity to strains of other types.

In endemic areas the importance of types lies in the fact that successive waves of infection may pass through the area at relatively short intervals without the necessity for a time interval for the waning of immunity against the homologous virus. We have encountered three types within a few months in certain areas of Africa and we even have records of animals which have passed through infection with three different types of virus under natural conditions within six months. In the more highly developed areas where vaccination is practised, the type situation greatly complicates the planning and execution of vaccination campaigns.

Infection by sub-type strains within a type generally produces a satisfactory immunity to other sub-type strains of the same type. The range of difference between sub-types, however, is now varying from that which is not readily detectable unless fairly precise methods are applied, to a difference which approaches that in which the immunity of the recovered animals is broken down. In the field, sub-types are generally recognized first when vaccination fails to give the expected result.

### High Lability

Observations have recently been made on the possibility of change of sub-type in experimental conditions. Hyslop has shown that passage of the virus in immune animals or partially immune animals leads to the development of a new sub-type different possibly from any which have hitherto been recovered in the field. Parallel experiments have been carried out on the passage of virus in culture in the presence of serum and this also leads to the development of new sub-types. A third method for producing sub-type change is recombination of two virus strains grown in tissue culture. The demonstration of changes produced in this way in the laboratory shows how readily new sub-types might arise in the field and,

because the mutation rate of some strains of virus is of the order of  $10^4$ , many new strains must undoubtedly arise. Whether all of these reach the proportions of producing new outbreaks in animals is problematical, but it would seem more likely that only when conditions are highly favourable or the new strain is very widely different from strains which have been present before does preferential infection with the new strain as opposed to the one previously prevalent take place. Once a strain has broken through, it may persist through many serial transfers in animals. This is particularly true if it is widely different from the earlier strain.

Besides the variability of the virus in the antigenic sense, other changes in virus may be important in epidemiology. The most obvious of these is the change in virulence. It is commonplace that not all outbreaks of foot-and-mouth disease appear to attack the animals with equal severity and there seems little doubt that a change in virulence does occur. Many strains of virus from a major epizootic in Mexico were examined at Pirbright in the 1940s and these strains were shown to be of poor spreading power and of low virulence in the animals affected.

### High Endurance

An outstanding characteristic of the virus of foot-and-mouth disease is its capability of survival outside the body. Much of the early research work on the virus from the mid-1920s to the mid-1930s was devoted to the definition of the periods for which virus could survive under conditions simulating those to be found in the field and tests on virus dried by no particularly refined method on surfaces such as hay, straw, wood, etc., gave results in which the virus survived for periods of fifteen weeks or sometimes more. In the field there is a great deal of evidence of the survival of virus for long periods under natural conditions. On many occasions vehicles which have been used for the transport of animals in the early infective stage of the disease have, without proper disinfection, been used for conveying other groups of animals and frequently it has been found that the virus has survived for several days between two batches of animals being transferred by the same vehicle.

Some factors do militate against survival. Exposure of the virus to direct sunlight decreases survival time, so does a higher temperature. The spread of the disease in warm, sunny, dry climates does not appear to take place so easily by indirect methods as in colder, damp and dull climates. Unfortunately, however, we cannot rely on meteorological circumstances for prevention of spread, because even under adverse conditions the virus of foot-and-mouth disease appears to be very considerably tougher than most other micro-organisms.

Another aspect of virus survival which is important in epidemiology in regard to spread between countries is the survival of virus in meat and animal products which have been derived from infected animals. While the muscular tissue in slaughtered carcasses becomes acid during rigor mortis and so inactivates the virus which may be present, this is not true of certain offals such as liver, kidney and tripe or of the lymph nodes and bone marrow. Prolonged survival has been demonstrated in such tissues in animals slaughtered during infection, and it also seems likely that animals slaughtered in the incubative stages may be an unsuspected source of dissemination of the virus. There has been strong circumstantial evidence that meat from South America has been responsible for a fairly high percentage of the outbreaks in Great Britain, and improvement in control measures in South America, together with restriction on certain classes of animal products coming to Britain, is one of the factors which have contributed to the decrease in the amount of infection in Britain.

Although the virus of foot-and-mouth disease infects the cloven-footed domesticated and wild animals, differences in severity of the disease in various species are often

observed. Some strains of virus will infect pigs but not cattle and strains have also been found which infect cattle but not pigs. There are intermediate strains which affect both species equally readily. Under laboratory conditions it has not proved possible in a small number of passages to modify the virulence of one of those strains so that, for example, a strain normally virulent in pigs and not in cattle becomes virulent for both species. The number of attempts has been small, however, and there is little doubt that such a change must take place in the field and indeed there has been evidence of an increase in the number of cattle outbreaks in Holland. These findings suggest that species adaptation may have an epidemiological significance in that one species may act as a reservoir of infection for the other.

A similar relationship has been observed in the case of strains of virus which have shown a variable degree of virulence for sheep. The strain of SAT 1 which spread through the Middle East in 1961 undoubtedly produced a more severe disease in sheep than many other strains of virus, although at the same time it was capable of producing severe lesions in cattle. In general, however, the A<sub>22</sub> going through the same area has not been notably severe in sheep. Henderson and McLaughlan showed that the European hedgehog could be an important factor in the transmission of the disease from an initial focus of infection to a number of secondary cases in Norfolk. Recently, Capel-Edwards has drawn attention to the susceptibility of the coypu. Man appears to be marginally susceptible, but there are no records of transmission chains from cattle to man and back to cattle, nor, fortunately, strains which are preferentially adapted to man.

### Declining Immunity

An essential part of the epidemiology of a disease is the immune response of the affected animal. The immunity of an animal recovered from foot-and-mouth disease is generally conceded as lasting for 2-4 years or even longer against a homologous strain of virus, if this virus is presented to the animal by way of contact infection. There are certainly no records in the field of re-infection of animals with a strain of virus known to be identical with that from which the animal originally recovered. It must be realized, however, that, although the animal is immune for contact infection, its immune status is not constant throughout this period of recovery but is declining at a rate which seems to vary with the strain of virus involved in the original infection and with other unknown factors during the period of recovery. In general, duration of antibody persistence is correlated with the initial titre which is achieved. This may depend on the condition of the animals, their nutritional status, their age, and may even depend on the breed involved. The picture is one of a gradually declining immunity which leaves the animal progressively more susceptible to re-infection. Opposing this declining immunity is the virus which, by changing as described earlier, may be increasingly capable of infecting the animal in the face of the antibody which is circulating. Obviously, the extreme case is infection with a different type of virus altogether, which may take place within a few weeks even of the initial infection. A new sub-type may be resisted for several months but certainly will not be resisted for as long as the homologous virus. It is rare, however, for the situation to be as uncomplicated as this. In almost any endemic area animals will have been exposed to a variety of antigenic stimuli by different types or sub-types of virus, and the viruses circulating in the area will almost certainly be a mixture, if not of different types, at least of different sub-types which may preferentially infect particular animals. Under such conditions, how many minor infections which never become of serious importance do occur is a matter for conjecture. It is only when a sufficiently different type or sub-type appears in the region that a major epizootic does occur.

It is thought that, following infection with the virus, there is continuous multiplication for a period (depending on the strain of virus) of up to 15-18 months. This multiplication is at a low level and research has not yet demonstrated the transference of the virus from the carrier animal to a susceptible animal brought into contact. The epidemiological evidence in various parts of the world suggests that occasionally carrier animals may act as donors to recipient stock. A particularly important point is that modification of virus in respect of its antigenic constitution is possible while the virus remains in the animal. This would favour the selection of new variant or sub-type strains from time to time and, with waning immunity in the surrounding population, the initiation of a new epidemic.

The classical foot-and-mouth disease vaccine has always been an inactivated vaccine, although living vaccines have been applied on a scale that is rather more than experimental. Inactivated vaccines have given good results in areas where they have been intelligently applied and where prompt action has been taken on the incorporation of new sub-type strains as required. The figures for outbreaks in Europe over the past 15 years are sufficient tribute to the general efficacy of vaccination. Those for France and Holland are perhaps the most encouraging. In Argentina the determined campaign which has been waged for the past 4 years now covers almost all the infected areas of the country and, because an important part of the programme has been the improvement of vaccines by quality control, there are signs of a reduced incidence of the disease.

It must be emphasized that properly organized vaccination campaigns covering reasonably large areas are the only way in which control by vaccination will have a lasting effect. The sporadic use of vaccine in countries to protect particular herds during major epizootics is probably scarcely worthwhile because one dose of vaccine is unlikely to give a solid protection, especially if there is some difference between the vaccinating strain and the field strain. Vaccines have also been applied in the creation of barrier zones to prevent the spread of the disease into clean areas.

What then are the prospects for the control of foot-and-mouth disease by vaccination? Given the correct economic climate, the answer is encouraging. Where vaccination campaigns have been vigorously pursued, with due attention to the control of quality of the vaccine, the results have been good even allowing for the existence of a number of outstanding problems such as the difficulty of vaccination of pigs. In Europe and parts of South America we can look forward to the reduction of incidence to a level where affected animals are slaughtered and eradication is within the bounds of possibility.

In other regions, those bordering on the great endemic areas, the problem will be tackled piecemeal. With the vaccines at present available it is important to consolidate a campaign in a limited area and to avoid the temptation to make too great inroads into the unknown. Operations such as the maintenance of the barrier zones suggest that a start may be made on fringe areas, with extension in due course to the endemic area behind the barrier.

Research can help by leading to the production of more efficient and cheaper vaccines with improved keeping qualities under adverse conditions. It may also improve the prospects for living modified strain vaccines which would seem pre-eminently suitable for some operations in endemic areas. Developments in this field have not been so rapid or so successful as many of us had hoped. Where we are faced with the problem of long term protection for cattle in the more under-developed areas where game animals act as reservoirs of infection, it seems probable that modified strains may provide an answer.

On the world basis, the further that the frontier of disease control can be pushed into the endemic areas, the more secure is the position of those countries which have remained free or brought the disease under control.



# Water Balance in a Small Catchment

by

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The International Hydrological Decade (1965–75) has stimulated hydrological research of many kinds. One project is concerned with a small catchment area in Yorkshire

ALMOST a decade ago the International Geophysical Year was hailed with a blaze of publicity around the world and the letters IGY still evoke in Britain memories of stirring feats and considerable scientific accomplishment, particularly in Antarctica. In 1965 another world exercise in scientific co-operation, the International Hydrological Decade or IHD, began comparatively quietly, perhaps because of its less concentrated time span, so that even now, twenty months after its initiation, little is known about it except by the specialists directly concerned.

## Aims of the IHD

The background against which the concept of the IHD emerged is that of a world "shortage" of water. Quite obviously this shortage is not reflected in absolute terms, because the total amount of precipitation in the world far exceeds the total use of water; in Britain only 11 per cent of the precipitation is used as water supply. But the shortage is reflected in the non-availability of water in certain areas, perhaps because of inferior quality, the cost of exploitation or the physical maldistribution of water from one area to another. In each case there is an increasing need to locate and measure water resources. It is even more important that because the science of hydrology is still in a comparatively early stage of its development there remain large gaps in knowledge of the theory and fundamentals of the occurrence, distribution and movement of water over, under and through the surface of the Earth.

The activities of the Hydrological Decade are concentrated on these two principal aspects of the problem. According to a statement of the aims, "the programme will focus on science but will give strong consideration to utilitarian factors"<sup>1</sup>. Whereas in the case of the IGY, however, most of the principal objectives were achieved by means of intensive observations over a short period, the very nature of hydrological phenomena, and particularly the variability of hydrological regimes, means that a much longer study period is necessary. It is for this reason that a 10 year programme has been adopted.

In detail, the scientific objectives of the IHD are far reaching in their scope and implications. They include, first, an appraisal of the state of knowledge of world hydrology; second, the standardization of methods of data collection, compilation and reporting; third, the establishment of basic hydrometric networks and the improvement of existing networks in order to provide data about a wide range of hydrological systems varying in size from small catchments to the world as a whole; and fourth, research on particular hydrological systems, known as "representative basins", in selected geographical, geological and climatic environments. It is also intended that there should be research on specific hydro-

logical problems the nature and importance of which demand an international rather than a local effort. theoretical and practical training in hydrology and ancillary subjects, and the systematic exchange of information.

The principal objectives can thus be summarized as the collection of basic data, systematic research and the investigation of water balances of specific areas. Of these, I am concerned particularly with the third. In order to furnish the information that will be necessary if the objectives are to be attained, data are being collected from different types of recording stations and experimental areas. Most of the basic data are being provided by networks of observation stations concerned largely with precipitation, streamflow, evaporation and, in some cases, groundwater. At present, observation stations are standardized only for certain types of measurement; however, it is proposed that standardization will eventually be extended to cover all types of observation in order to reduce problems of data interpretation, and also that the density of existing hydrometric networks will be increased.

A further attempt to improve the international comparability and reliability of data involves the establishment of "decade stations" at which a high standard of accuracy, uniformity and continuity of data collection is maintained, so that the results may be confidently used by all hydrologists. In countries which are already well equipped, this has simply involved specifying certain observation stations as decade stations, but elsewhere the decade stations have had to be specially built in order to create an international network that is sufficiently dense and representative<sup>2</sup>.

The basic units for water balance investigations are the representative and experimental basins. Although sizes will obviously depend largely on natural conditions, the "representative basins" are usually the larger areas—although they rarely exceed 1,000 sq. km. They have been selected, so far as possible, in areas where the hydrological cycle has not been greatly disturbed by man. Nevertheless there will be no attempt deliberately to prevent human activities liable to have hydrological repercussions. Although, in some cases, little more may be involved than the installation of simple instrument networks for precipitation, stream flow and, where possible, groundwater level observations, most representative basins contain at least one decade station. The smaller "experimental basins" are those in which natural conditions, and particularly the vegetation cover, will be deliberately modified and an attempt made to determine the effects of this modification on the hydrological cycle.

Finally, there is a need for data from "benchmark basins" which are still in their natural state and the hydrological regimes of which are completely unchanged



by man. These data will indicate long term natural trends and will serve as a reference base against which data from experimental and representative basins can subsequently be compared.

### Water Balance Research in Great Britain

In Great Britain, water balance research along the lines suggested was given a considerable initial impetus by the Water Resources Act of 1963, which laid statutory obligations on the newly created River Authorities to survey the water resources of their areas and, in particular, to establish hydrometric schemes for measuring rainfall, evaporation and water flow, and to assess underground resources<sup>3</sup>. With the advent of the IHD, there was a rapid increase of research activity so that now, for the first time, widespread systematic and local regional water balance studies are being conducted simultaneously in many parts of the country. Many experimental catchment studies are being or have been established by river authorities, by university departments and by the larger research institutions such as the Road Research Laboratory, the Hydrological Research Unit of the Hydraulics Research Station, and the Water Research Association. An experiment designed by the Department of Geography at the University of Hull is typical of work being planned elsewhere, and will illustrate the scope and scale of the problems involved and will emphasize the need for the rapid collation and integration of results from individual experiments, not only in Great Britain but also on a world scale.

### The Holderness Catchment

The catchment selected for a 15 year programme of investigation covers an area of approximately six square miles in the plain of Holderness and is located some ten miles east of Beverley (Fig. 1). Holderness is a boulder clay plain the surface of which rises very gently towards the coast so that the drainage of most of the area flows into the Hull Valley or into the Humber. The land surface consists principally of hummocky moraines and ridges and shallow enclosed basins such as the one containing Hornsea Mere. Only at one point is a height of 100 ft. O.D. attained<sup>4</sup>. In the experimental catchment itself, the highest ground is about 80 ft. O.D. and the total amplitude of relief is approximately 55 ft., so that

most of the slopes are quite gentle. Soil types are varied, ranging from the light easily worked soils on the higher gravel areas to fairly sticky clay loams in the bottom of the depressions. Most of the area is under mixed farming with a rotation of grass and corn crops. The acreage of woodland is extremely low.

Average annual rainfall over Holderness ranges from about 24 inches in the south to approximately 26 inches in the north and is evenly distributed throughout the year with no one month receiving more than 10 per cent of the average annual total<sup>5</sup>. Intense storms are not characteristic of the area and those which have been recorded have occurred in the summer months between May and August<sup>5</sup>.

On the basis of all these considerations, and assuming an average annual evapo-transpiration total of 20 inches, it was calculated that the probable normal maximum discharge of the selected catchment area would not exceed 50 cusecs. There has previously been no continuous gauging of clay streams in this vicinity, although the upper reaches of the River Hull draining largely from the chalk of the Wolds have been gauged regularly or continuously at three stations. In the planning of this experiment there was thus little evidence to confirm the value of 50 cusecs, although some indication of its validity was provided by the fact that between 1953 and 1962 the daily maximum discharge of the West Beck, the larger of the two main headwater streams of the River Hull, ranged from 4.7 cusecs per sq. mile to 1.6 cusecs per sq. mile. Allowance was, of course, made for the fact that most of the 74 square miles of the West Beck catchment are located on chalk and that both daily totals and instantaneous values are, therefore, likely to be significantly lower than from an impervious clay catchment. In fact, during the very wet period since the beginning of the study in October 1965, the design flow from the experimental catchment has been briefly exceeded on a number of occasions.

A wide range of instruments has been installed with the view, initially, of quantifying the components of the general water balance equation

$$\text{water income} = \text{water losses} \pm \text{changes of water storage.}$$

Assuming that the catchment is geologically watertight, all water income is in the form of precipitation which must, therefore, be measured very carefully. As well as the existing network of standard 5 inch rain gauges, records from which are received by the Meteorological Office, and a number of Dines tilting siphon autographic rainfall recorders maintained by various authorities, two further Dines tilting siphon recorders together with 5 inch check gauges have been installed, one at each end of the experimental catchment. These autographic recorders have been fitted with the strip chart mechanism recently developed by the Meteorological Office and which makes it possible for the gauges to be left for up to four days before the chart must be renewed. This is important in a long term project, when daily attention to the more distant instruments may not be possible. Information is thus available from which the total amount, duration and intensity of rainfall on the catchment area may be readily computed.

The loss of water from the catchment area occurs in several ways, each of which must be accurately assessed. Streamflow from the entire catchment is measured by means of a trapezoidal flume in conjunction with a rotary type of autographic water level recorder. The flume is constructed in rot-proof timber and marine plywood to a design only slightly modified from that first used by the Water Research Association in 1962 (ref. 6). Subsidiary flow measurements are made as required, particularly during very high or low flows, using current meters at selected, stable stream or drain sections.

Apart from the removal of water by means of streamflow, evapo-transpiration constitutes the principal water loss and is also one of the most difficult parameters to

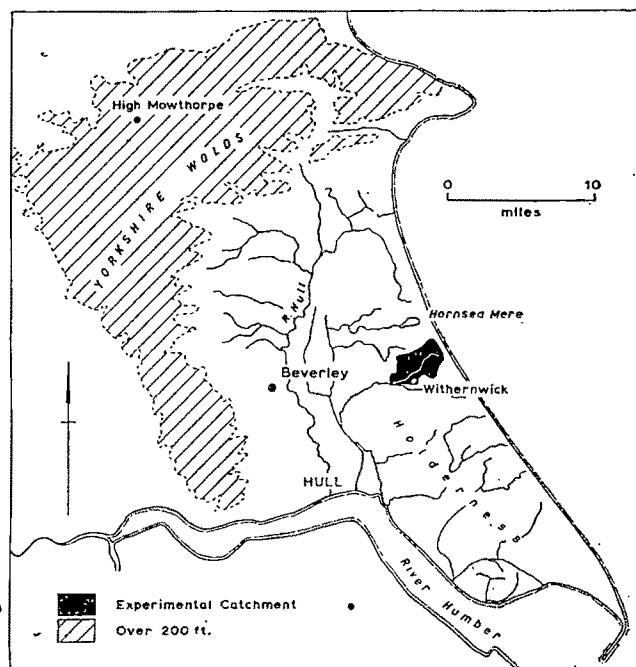


Fig. 1.

determine with precision. In the present study various techniques are used. Thus potential evapo-transpiration, which occurs when soil moisture supplies are at all times adequate for plant needs, is measured by means of an evapo-transpirometer with three grass covered soil tanks<sup>7</sup>. The amount of daily drainage from the base of each tank is subtracted from the amount of rainfall and irrigation on its surface, leaving a value which, when adjusted for changes of soil moisture storage, represents the amount of water used by evapo-transpiration. Open water evaporation is measured in a British Standard sunken pan (6 feet square and 2 feet deep) and also in a United States Weather Bureau Class A pan (4 feet in diameter and 10 inches deep) mounted on a timber framework. The relative merits of these instruments have previously been discussed at some length<sup>7,8</sup>. At this point it is sufficient that each is likely to give a different value of water loss as a result of variations in evaporating surface and exposure. In theory, the evapo-transpirometer should provide the most useful measurement of potential catchment water losses, provided that it is properly exposed and adequately maintained, although there are recent indications that equally valid results can be obtained more readily by using the British Standard pan. The Class A pan inevitably gives an exaggerated value of evaporation because of its exposure, although this can be readily corrected by applying a reduction factor or pan coefficient. This pan has been included in the present study in order to facilitate correlations with results from the many areas of the world in which it is widely used.

Measurements are also made of the meteorological data required to estimate potential evapo-transpiration. Mean daily temperature and humidity are derived from standard and recording maximum and minimum and wet and dry bulb thermometers and the run of the wind from a cup type of anemometer situated at a height of 2 metres above the ground surface. The Penman evapo-transpiration formula also requires the measurement or estimation of net radiation at the vegetation surface and for this purpose a net radiometer has been installed (C.S.I.R.O.). Because of the delicacy of this instrument, however, and particularly because of its susceptibility to damage by birds, net radiation measurements are supplemented by observations of the duration of bright sunshine. An estimate of net radiation can then be made, provided that a direct correlation between this parameter and the duration of bright sunshine has previously been established in the local area. Some measure of the soil heat flux, which is needed for a correct evaluation of evapo-transpiration losses, is obtained from continuously recording earth thermometers at depths of 1 and 4 feet.

Finally, a measurement of the actual evapo-transpiration loss occurring under the prevailing soil moisture conditions will be possible with the proposed installation of a simple weighing lysimeter similar to that being developed at the National Vegetable Research Station at Wellesbourne<sup>9</sup>.

Changes of water storage throughout the catchment are reflected in variations of soil moisture content and groundwater levels and in general the importance of these varies inversely with the time interval. Soil moisture content, like evapo-transpiration, is difficult to measure with precision even with instruments such as neutron probes<sup>10,11</sup>. Many of the common methods of measurement (tensiometers, porous blocks and so on) are either valid only over a narrow range of moisture content or are of doubtful accuracy. In the Holderness experiment, soil moisture variations are determined by weighing and drying samples taken in the field, although it is hoped that a neutron probe will eventually become available. Groundwater levels are measured regularly in existing wells and these data will shortly be supplemented by information from continuous water level recorders in a number of wells and boreholes in the catchment area.

The data collected from these instruments are not sufficient either for a proper evaluation of the general water balance of the catchment area or for more detailed studies of the interactions and relationships between selected parameters. Thus, for example, understanding the interaction between rainfall and run-off also requires detailed information about time and space variations of infiltration capacity, areal variations of crop and vegetation cover and steepness of slopes.

## Conclusions

This brief account of the use of specially designed catchment experiments and their instrumentation will have indicated that although the scale of such studies may be small—merely 6 square miles in this experiment—the physical problems of collecting the necessary data are considerable. But the problems of data processing and analysis are even more taxing. Computers must be used in order to reduce the work to manageable proportions. There are further problems in relating the results from one catchment study to those from similar experiments elsewhere. Fifteen such experiments in the British Isles alone are in active operation and many more are at the design stage. In the United States more than one hundred and fifty have been set up<sup>12</sup>. Rapid and concise publication of results and preliminary analyses is obviously a pressing need if an unmanageable backlog is to be avoided.

<sup>1</sup> Anon, *UNESCO Chronicle*, 10, 196 (1964).

<sup>2</sup> Batiase, M., *UNESCO Courier* (Special Issue), 4 (July–August 1964).

<sup>3</sup> Gregory, S., *Geography*, 48, 310 (1964).

<sup>4</sup> de Boer, G., *Shore and Beach*, 13–15 (December 1959).

<sup>5</sup> Meteorological Office, *Hydrological Memoranda* No. 9 (1963).

<sup>6</sup> Barsby, A., *Water Res. Assoc., Tech. Rep.*, No. 28 (1962).

<sup>7</sup> Ward, R. C., *Geography*, 48, 49 (1963).

<sup>8</sup> See, for example, Nordenson, T. J., and Baker, D. R., *J. Geophys. Res.*, 67, 671 (1962).

<sup>9</sup> Winter, E. J., *J. Hort. Sci.*, 38, 160 (1963).

<sup>10</sup> Holmes, J. W., and Turner, K. G., *J. Agric. Eng. Res.*, 3, 199 (1956).

<sup>11</sup> Bell, J. P., and McCulloch, J. S. G., *J. Hydrol.*, 4, 254 (1966).

<sup>12</sup> *Representative and Experimental Watersheds, U.S.A.*, 153 (American Geophysical Union, 1965).

The problems of water resources and allocation of water in the U.S.A. are discussed by J. L. Fisher in *Future Environments of North America* (edit. by Darling, F. F. and Milton, J. P.) (The Natural History Press, New York, 1966). He says: "Undoubtedly, demand for water will increase considerably in the years ahead as it has in past decades. Here again improvements in management of water supply, such as treatment and re-use, can make it possible for fairly large increases in demand for specific purposes to be met without a severe general problem of shortage. In the eastern part of the United States plentiful rainfall fairly evenly distributed indicates that the chief problem will not be one of sheer supply, but rather one of maintaining or improving water quality. In the West the chief problem will probably remain that of assuring sufficient supplies, but even in quite arid places, some shift in use away from irrigation toward municipal and industrial uses would make possible a continued population increase and industrial development. In the West more than 90 per cent of water withdrawn from streams and underground sources, and actually depleted or used up, goes for irrigation of crops many of which are in surplus supply.

"The spread of environmental pollution in many forms is one of the more noticeable and objectionable trends of our times. In addition to water pollution, the atmosphere in many metropolitan areas at certain times is heavily polluted, thereby imposing costs and irritations for large numbers of people. Much has been written recently about what may be called the pollution of agricultural land, lakes and streams, and the ground and foliage, generally as a result of the way in which certain pesticides are used."

## BOOK REVIEWS

### GENETIC APOCALYPSE?

#### Genetics and the Future of Man

Edited by John D. Roslansky. (A Discussion at the Nobel Conference, organized by Gustavus Adolphus College, St. Peter, Minnesota, 1965.) Pp. xii+204. (Amsterdam: North-Holland Publishing Company, 1966.) 18 guilders; 36s.

IN 1963 the Nobel Hall of Science was dedicated at Gustavus Adolphus College, St. Peter, Minnesota, as a memorial to Alfred Nobel. In January 1965 the first Nobel Conference was held there, and among the participants were, we are told, four Nobel laureates. According to the dust cover of this book, the conference was attended by eight thousand people and the lectures delivered are presented in *Genetics and the Future of Man*.

The conference was introduced by Polykarp Kusch, and consisted of six lectures. Of these, two, by Sheldon C. Reed and Bentley Glass, are fairly standard and uncontroversial accounts of the genetical adjustments which human populations might undergo in stable and unstable physical environments. Edward L. Tatum follows with an account of some of the things that might be achieved by techniques being developed for treating human cells like cultures of micro-organisms. William B. Shockley discusses population control and eugenics as it appears to a professor of engineering science and does so in a rather "grass roots" kind of way, in the course of which he falls into one of the classical genetical errors of confusing the apportionment among contributory agencies of the causation of a character itself with the apportionment of causation of the variation it is observed to show. The geneticist may find it difficult not to regard parts of this presentation as bordering on the unsophisticated, but at least Shockley emphasizes the need for "a continuing objective fact finding approach to these enormously controversial, enormously significant problems" even if the problems themselves are neither clearly set out nor clearly agreed by the participants in the symposium—perhaps because the necessary facts are not yet available.

Moral and religious implications are discussed at length by Paul Ramsey, who holds a chair of religion at Princeton. He takes his text largely from Muller and the genetic Apocalypse which he sees Muller as foretelling, and leaves at least one reader with the feeling that his discussion, closely argued as one would expect from a student of religion and ethics, would have had more impact if it had taken the genetic Apocalypse less for granted and had insisted on a firmer assessment of the genetical problem with which our ethics would have to cope. Lastly, Kingsley Davis analyses the sociological aspects of genetic control. He exposes the weakness of many of the arguments for genetic control as based on the assumed genetic consequences of social practice itself rather than on independently ascertained facts. He points to the resistance to any drastic programme of hereditary improvement inherent in the stability system of existing societies, and envisages change as more likely to be precipitated by a genetic crisis, possibly consequent on a nuclear holocaust, but sees some genetic change as essential if man is to overcome the limitation to his socio-cultural evolution—*itself* a novel and by no means obviously valid notion.

This book touches on subjects of vital interest to everyone and it makes interesting reading if only for the contrasts and disparities of assumption that it brings out. One wonders what the eight thousand who attended the

conference made of it—perhaps that before we can discuss fruitfully the problem of controlling the genetic structure of mankind we need to know more certainly what is now happening to that structure and to decide more clearly how far the continuing biological evolution of man (as distinct from the control of hereditary disability) is really as essential to continuing social progress as some of the speakers seem to have taken for granted.

KENNETH MATHER.

### WEATHER

#### Physical Climatology

By William D. Sellers. Pp. viii+272. (Chicago and London: University of Chicago Press, 1965.) \$7.50; 56s.

PROFESSOR SELLERS'S book has the same title as the well known book by H. Landsberg, but the two texts are very different. Professor Sellers concentrates more on physics and less on climatology, and his treatment is rather more advanced than that of Landsberg.

The variety of subject matter covered in the thirteen chapters of this book may be judged from a few chapter titles, such as "The Radiation Balance", "Heat Transfer in Soil", "Atmospheric Diffusion" and "Paleoclimatology and Theories of Climatic Change". To me the most attractive chapters are those which deal with radiation and the water and energy balances.

The basic facts and theories of solar and terrestrial radiation have no doubt been treated adequately in many books, including Brunt's classical *Physical and Dynamical Meteorology*, but Professor Sellers succeeds in stimulating the reader even in his tables and diagrams. For example, one table strikingly compares the energy equivalents of many natural and man-made phenomena; thus it appears that the quantity of solar energy received each day on the Earth is equivalent to the energy released by  $10^4$  hurricanes or by  $10^8$  Nagasaki atomic bombs. Apart from the value of his tables, it is pleasant to see so much up to date information; for example, the data on surface albedo were published by Kung *et al.* as recently as 1964. At the same time, Professor Sellers never fails to quote old authorities whenever their work is still relevant. Appropriately, the well known empirical formulae for effective outgoing radiation from the Earth's surface formulated by Ångström (1916) and Brunt (1932) are discussed with later equations developed by Elsasser and Budyko and others, including two formulae of Swinbank (1963) which have, surprisingly, only surface temperature as the independent variable. Equally appropriately, in a summary of estimates of the annual poleward heat flux at various latitudes of the northern hemisphere required to balance the radiative sources and sinks of energy in the atmosphere and at the surface of the Earth, it is interesting that G. C. Simpson's figures of 1929 are substantially the same as the latest estimates made by Budyko and London.

Professor Sellers clearly demonstrates the connexion between rainfall, evaporation (or evapotranspiration), run-off and the convergence of the flux of moisture through the water balance equations for the Earth's surface and the atmosphere, based on the principle of the conservation of matter. Climatological estimates of the various terms are given for particular geographical regions, for the oceans and the continents and for different seasons; they are of fundamental importance in connexion with the rather local problem of water conservation, in the classification of climates and in the general circulation itself. The author next links together radiation and moisture (or water) in balance equations based on the conservation of energy. The energy balance equations are thoroughly discussed, with the aid of informative graphs, for the Earth's surface, for the atmosphere and for the combined system of Earth and atmosphere.

Professor Sellers's book brings together various loosely connected topics which are not at present available in English. His writing is clear and economical, and the bibliography is up to date. The book is strongly recommended to students, but it should also be very useful to many meteorologists.

R. MURRAY

## HISTORY FOR SCIENTISTS

### Chymia

Annual Studies in the History of Chemistry, Vol. 10. Edited by Henry M. Leicester. Pp. 258. (Philadelphia: University of Pennsylvania Press; London: Oxford University Press, 1965.) 40s. net.

*Chymia*, the tenth in the series of annual publications, contains the following articles: "How Old is Hydrochloric Acid?", by L. Reti; "Christophle Glaser and the *Traité de la Chymie, 1663*", by R. G. Neville; "Berthollet, Proust, and Proportions", by S. C. Kapoor; "Unpublished Letters from H. Sainte-Claire Deville to B. S. Yakobi", by Y. I. Solov'ev; "Induced Oxidation-reduction Processes, the History of a Chemical Paradox", by E. Farber; "A Precursor of the American Chemical Society—Chandler and the Chemical Society of Union College", by E. K. Bacon; "Historical Sketch of the Electron Theory of Organic Chemistry", by G. V. Bykov.

The criteria for judging this book are provided by the publishers in a statement on the dust-cover: "The publication of scientific literature has reached such vast proportions in recent decades that the specialist is hard pressed to keep up with developments even in his own limited field. The history of science is thus largely neglected in favour of current findings and it has become increasingly difficult for the reflective scientist to maintain a sense of development, over centuries, of his area of study. *Chymia* is an attempt to supply, in the field of chemistry, information necessary to this perspective".

With the exception of those by Solov'ev and Bykov (the latter is excellent, and will greatly interest physicists as well as chemists) the articles show no evidence of having been written to provide interesting perspective for reflective scientists. The reason that the hard-pressed specialist neglects the history of science in favour of recent findings is that this book is the sort of material that he is offered by the historians. It is specialist, pedantic history written for a diminishing circle of specialist, pedantic historians. Do the publishers really believe that scientists in search of perspective will pay £2 of their own money for a book consisting mainly of long and obscure accounts of historical niceties adorned with the familiar archaic trappings so beloved by venerable scholars (untranslated passages of Latin, reproductions of the title pages of old books, hundreds on hundreds of footnotes, the main purpose of which seems to be to demonstrate that the authors have access to well-stocked libraries)?

If science historians are genuinely concerned about being neglected they must make an effort to present articles that will stimulate and interest scientists who are not historians. They must interpret the past; they must, as Bykov does so well, relate the past to the present. It is not enough to write in detail about the past and expect non-historians to be interested simply because the events described happened long ago, or to describe rare books and expect interest simply because the books are rare. If, on the other hand, they are oblivious to neglect by non-historians (which I suspect to be the truth) they will continue to ignore their important role as interpreters and will continue to write articles like these for each other to read. This is fine—scholarship for its own sake is a necessary human activity, and an enjoyable one—but if the scholar ignores society, he has no right to complain if society ignores the scholar.

R. G. PARTINGTON

## MEN OF STEEL

### The Sorby Centennial Symposium on the History of Metallurgy

Cleveland, Ohio, October 22–23, 1963. Edited by Cyril Stanley Smith. (Metallurgical Society Conferences, Vol. 27.) Pp. xxii+558. (New York: Gordon and Breach; London and Glasgow: Blackie and Son, Ltd., 1965.) 156s.

In this, thirty-six authors from six countries combine to cover in thirty-three chapters the lives of a number of pioneers of metallurgical science technology and techniques and the history of a variety of metallurgical phenomena, methods and theories mainly relating to iron and steel. The mass, or more correctly the volumes, of technical literature with which scientists and technologists are faced today is physically burdensome and mentally overwhelming since almost by definition most worthwhile books require sustained concentration if their reading is to be effective. The contents of the chapters of this centennial tribute by three American societies, principally to H. C. Sorby and other English metallurgists, can be read in relaxed fashion and the chapters, which are largely unconnected with each other, can be taken up at random.

The chapters divide into four groups of different interests—people, phenomena, techniques, particularly metallography and ferrous based materials. As would be expected of so many authors, the style, approach and readability are variable. If generalization is possible, the chapters about people—those individuals who initiated and brought some metallurgical feature to successful fruition—are composed like curriculum vitae, factually incontrovertible but a dull method of presentation; in support one can cite the chapters on J. F. Stead and J. O. Arnold and even the autobiographical chapters by Z. Jeffries and E. C. Bain.

The chapters I most enjoyed, because of the sense of participation in and the feeling of association with the events they describe, were related to phenomena (or lack of them). Today, scientific literature is written in a formalized impersonal fashion<sup>1</sup>. But "The  $\beta$ -iron Controversy" as reported by M. Cohen and J. M. Harris (and the mysterious "jargonium" of Sorby, pp. 37 and 38), "Dislocations in Plasticity" by E. Orowan, "History of Precipitation Hardening" by H. Y. Hunsicker and H. C. Stumpf and "The Development of the Theory of Alloys" by W. Hume Rothery, all had a personal imprint of their authors' sense of excitement. And where individuals write of their own contributions to metallurgical history, it is interesting to compare the introverted, factual, succinct description of the "Note on the Early Stages of Dislocation Theory" by G. I. Taylor with the extroverted exuberant expansive but still factual account of the Hungarian, E. Orowan. These differences lend a great deal of colour as well as surprise to the book.

The early history of metallography and the advanced level to which the technique was brought by Sorby and others should induce humility in the reader and practitioners of the method.

The descriptions of the development of materials—"Iron Manufacture in Britain in the Decade 1850–60", "History of Silicon-iron", "Soft Magnetic Materials", "Tungsten" and "Powder Metallurgy in Development of Dispersion-hardened Materials"—are interesting in that they show some kind of order in the history of the development of these materials even although to the individuals who lived through these developments the successive improvements were achieved in spite of a confusion of facts and misconceptions. It would be pleasing to be able to say that all future developments in metallurgy will be made without having to go through the same bewilderments that faced the several pioneers in this volume, but if this book teaches anything it is that trial and error—with emphasis on the error—is the likeliest way for future developments.

At 156s. all libraries can afford and should have this book, which should be borrowed for bedside reading. I doubt whether individuals even in an affluent society will spend this sum, but since books are still given as prizes in universities and technical colleges, this is useful volume to be kept in mind.

H. M. FINNISTON

<sup>1</sup> See "Is there too much literature?", supplement to *Nature*, 212, 1003 (1966).

## CHEMICAL TECHNOLOGY

### Encyclopedia of Chemical Technology

By Kirk-Othmer. Vol. 6: Complexing Agents to Dextrose and Starch Syrups. Pp. xiv+932. 338s. Vol. 7: Dialysis to Electron Spin Resonance. Pp. xvi+903. 338s. Second completely revised edition. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1965.)

VOLUMES 6 and 7 of the second edition of Kirk-Othmer need little introduction, except that one can congratulate the editors on their efforts, on their choice of another unique list of subjects, and on maintaining the usual high standard.

In this modern age every technologist must be conversant with and capable of using the techniques dealt with under the headings "Computers", "Data, Interpretation and Correlation", "Dimensional Analysis" and "Economic Evaluation". All these articles are concise and informative; they include mathematical derivations and often give examples of commercial and industrial applications. For example, the article on "Dimensional Analysis" explains how this technique has been used successfully to simplify experimental and correlation work applied to the power characteristics of simple turbines. On the other hand, the article on "Computers" could have paid more attention to illustrations of commercial application as in road building or power station construction.

To satisfy the needs of industrial technologists there are reviews on "Conveying", "Distillation", "Crystallization", "Diffusion", "Electrodialysis", "Electrodeposition" and "Electrolytic Machining Methods". In all of them emphasis is placed on industrial applications and on automated processes. The article on diffusion is especially geared to the atomic age, and deals adequately with the theoretical and practical problems associated with isotope separations. The technique of electrolytic machining sounds very attractive and promises much for the future.

Three types of corrosion are discussed in these volumes—corrosion by electrolytic solution, corrosion by gaseous environment, and corrosion by liquid metals or fused salts. Much of the work in the last category has been stimulated by problems arising from the use of nuclear reactors. Volumes 6 and 7 also deal with a wide range of commercial chemicals and raw materials of which only a few can be mentioned. There is a long informative review under "Dyestuffs", and an interesting account of the fluctuations of the world trade in them. The use of Zeigler catalysts in the synthesis of the stereo-specific polymers is dealt with under "Dienes", and of equal importance is the article on "Elastomers" which includes the A.S.T.M. definition of rubber now accepted as providing a boundary line between rubbers and plastics. The proposed use of the vulcanized EPT terpolymers (ethylene-propylene-dicyclopentadiene) in the tyre industry in 1968 is interesting. These volumes give detailed accounts of some important raw materials, including the processing, packaging, preservation and general handling of them. In the article on "Drying Agents" special attention is given to the theories and uses of molecular sieves. In food chemistry there are useful articles on "Confectionery" and "Eggs". The article on "Contraceptives" provides detailed accounts of the chemistry of the hormones. The article on "Dental Materials" is particularly informative and deals with the structure and chemistry of ceramic and polymeric materials

used as cements, and the porcelains, elastomers, amalgams and metal organic products used in dentistry.

K. FIELD

## QUANTUM STATISTICS

### The Theory of Quantum Liquids

Vol. 1: Normal Fermi Liquids. By David Pines and Philippe Nozières. Pp. xi+355. (New York and Amsterdam: W. A. Benjamin, Inc., 1966.) \$15.

### Quantum Field Theoretical Methods in Statistical Physics

Second edition. By A. A. Abrikosov, L. P. Gor'kov and I. Ye. Dzyaloshinskii. Translated from the Russian by D. E. Brown. English translation edited by D. ter Haar. (International Series of Monographs in Natural Philosophy, Vol. 4.) Pp. xii+365. (London and New York: Pergamon Press, Ltd., 1965.) 70s. net.

THE success of the BCS theory of superconductivity and a growing unrest with a basis of the theory of metals have led to an increased interest, and optimistic hope, in the use of the second quantization formalism for the study of the many body problem. This formalism is a necessary part of quantum field theory, but also provides the most convenient framework for all but the simplest many body problems; yet it has two great drawbacks. First, apart from the simplest problems it becomes very complicated both in the precise way in which physical problems are translated into mathematical problems, and in the sheer algebra involved. Second, it is not at all clear how far we have truly advanced in solving these problems, for both superconductivity and the screening of Coulomb forces are only rigorously understood in certain weak coupling limits. There is no doubt, however, that this language will be the one used in new advances and everyone who wishes to follow the present ideas must learn it.

The book of Pines and Nozières, the first of two, gives an account aimed at the graduate student, the experimentalist of low temperature or solid state physics, and the theorist who is not specialized in the field. It leans heavily on the quasi-particle concept put forward by Landau, and emphasizes physical ideas omitting the full ferocity of the theoretical literature. It is a well written book in which the ideas of many body theory are shown to give a basis for the concepts of solid state physics which, though hallowed by time, are only now in the course of being justified. Many solid state effects are discussed in detail, and at the end of the book a realistic account of the validity of current ideas is given.

The book of Abrikosov, Gor'kov and Dzyaloshinskii is very different. It is a full theoretical treatise. This edition was apparently planned as a first translation, but it was forestalled by an American publisher. With additions and revisions amounting to about one sixth of the original it is now presented as the second edition. This is not a book for experimentalists, but it is well established as a theoretical text in its first edition and is a mine of detailed calculations giving in particular a full account of Soviet work in this field. S. F. EDWARDS

## NUCLEAR SAFETY

### The Safe Transport of Radioactive Materials

Edited by R. Gibson. Pp. xi+290. (London and New York: Pergamon Press, Ltd., 1966.) 80s. net.

THE 1964 revised edition of the I.A.E.A. *Regulations for the Safe Transport of Radioactive Materials* is a considerable advance over the first, 1961, edition especially in the classification of radionuclides, more detailed criteria for fissile materials and detailed objective standards for



packaging design. A record of the technical and other parameters on which the revised regulations were drawn up may assist in their application and will provide useful reference material in any future revision. The book fulfils both roles admirably, more particularly as the majority of persons who contributed to it are themselves experts who assisted the International Atomic Energy Agency in the revision.

Part I of the book deals with the regulations themselves and their technicalities. A good overall perspective of the increasing degree of control required as a function of the potential hazards is given in Chapter 7, which ably describes the security considerations to be given to the movement of a large radioactive source. Chapter 1 is an excellent and detailed step by step guide to the requirements of the regulations. It provides an introduction to the comprehensive technical accounts which are given in respect of the control of external radiation, requirements for fissile materials, standards for packaging design, relaxations for materials of low specific activity and the classification of radionuclides, all of which are written in a very clear and readable manner. The administrative body in the regulations is the Competent Authority, particularly important for international transport. The duties of that authority are well described and in detail in Chapter 2; it would have been useful, however, to summarize the philosophy of the regulations in respect of unilateral and multilateral approvals as a function of the availability, or otherwise, of detailed standards and criteria.

The I.A.E.A. regulations are mandatory only for the I.A.E.A.'s own operations; in all other respects they are recommendations to be used as a basis for national regulations, and to be applied in international transport. Part II provides a very useful section in which an account is given of the extent to which the recommendations have been used. It is encouraging to read how widely they have been adopted.

The provisions of the I.A.E.A. transport regulations reflect consideration of both normal and accident conditions in the transport environment. Part III of the book is a concise review of the accidents and incidents involving radioactive materials. Such discussion leads naturally to questions of insurance and indemnity against third party liability in the transport of radioactive materials. Part IV provides a clear and readable description of how such arrangements have been provided for on a European regional, and a national, basis respectively.

G. J. APPLETON

## PROGRAMMING LANGUAGES

### Formal Language Description Languages for Computer Programming

Edited by T. B. Steel, jun. (Proceedings of the IFIP Working Conference on Formal Language Description Languages.) Pp. 330. (Amsterdam: North-Holland Publishing Company, 1966.) 40 guilders; 80s.

THE conference, the proceedings of which appear in this book, marks a significant stage in the development of automatic programming languages. In the early stages of development interest was solely in the writing of compilers; as Dr. Johnson said in another context, it was not done well, but we were surprised to find it done at all. A major advance was made when it was realized that the syntax of a programming language, that is, the rules defining the sentences which can be written in the language, could be expressed formally, and that compilers could be written to work from this formal syntax, thus systematizing their construction. To define a programming language, however, it is necessary to specify not only the permissible constructions—the syntax—but also the meaning of each construction—the semantics. Hitherto, the semantics of a language have been described in an

informal and often pragmatic way, the ultimate definition being the behaviour of the compiler on a particular machine. Attempts are now being made to establish methods for formally defining the meaning of programming languages, and the conference brought together almost every worker in this field at the time.

The methods proposed for formal definition of semantics fall into two broad classes. The first class comprises those methods which develop a meta-language in which to describe the meaning of the programming language. This involves the definition of a number of fundamental concepts of structures in terms of which the more complicated concepts and structures of a programme can be described. The alternative method is to define an idealized machine, often called an evaluating mechanism, and to define formally the action of the machine in response to a piece of programme. In this way the meaning of a programming language is defined by describing what the idealized machine will do when presented with a programme as an input text. The twenty papers in this volume between them cover many variations of these two broad themes. None of the papers attempts very much in the way of comparing and contrasting the various approaches, but at this stage in the development of the subject it is valuable just to have all the different ideas collected in one volume.

For this reason the book is essential reading for anyone starting work in this field, and it should find a place in the library of all computer scientists interested in programming languages.

D. W. BARRON

## GETTING THE SALT OUT

### Principles of Desalination

Edited by K. S. Spiegler. Pp. xiv+566. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 168s.

SCIENTIFIC and industrial activity in the field of desalination is increasing rapidly, and this book will make a very useful introduction to the topic for those engineers and scientists who find themselves drawn into this type of work.

Although the book is well balanced as a whole, it seems clear that those authors responsible for the separate chapters must each have been given different terms of reference, for most chapters treat their topics in quite different ways. For anyone approaching the subject for the first time, the existing order of chapters would certainly not be the best. Chapter 9, on the "Preparation of Ultrapure Water", would have been a more suitable first chapter.

The concept of energy contained in the second chapter, on the theory of thermoeconomics by Evans, Crellin and Tribus, is not a concept to which an engineer can easily re-orientate after many years familiarity with energy, entropy and the normal manipulation of mass and heat balances. Examples of the use of this new parameter were given in Tribus's paper to the International Symposium on Water Desalination, where its utility was more in evidence than it is in the book.

Silvers's chapter on distillation is a very good fundamental analysis of the mechanics of distillation in large plants. Almost all desalination plants based on land or on ships are of the distillation type and one might have expected Mr. Spiegler to include a chapter on engineering design of distillation plant; perhaps, even, at the expense of the length of the excellently written chapter on vapour reheat.

Lof gives a very good account of the research work on, and the potential of, solar stills, but I cannot agree with his statement that "solar distillation occupies a favoured position among desalination processes in capacity ranges up to 50,000 or perhaps 100,000 gallons/day".



Not many people would be prepared to buy a solar distillation plant with a capacity of 100,000 gallons for \$1 million when the same sized distillation plant would cost nearer a fifth of this amount.

Should anyone wish to obtain a well based knowledge of the theory and practice of electrodialysis, he would be well advised to read the admirable chapter by Shaffer and Mintz; it covers a very wide range of topics in the electrodialysis field in a clear and concise manner. Apart from some operating data from the large electrodialysis plants in America, and which have only fairly recently been published, this chapter covers most of the available electrodialysis information of interest to the desalination engineer. The same words could be echoed in the case of the chapter on reverse osmosis, though both chapters suffer from one small omission—pictures of actual operating equipment. The chapter on freezing tends to go to the other extreme with its almost exclusive devotion to operating plant description, but it does not discuss the difficulties of designing the more intractable components such as the compressor and the wash column; these are some of the main obstacles to the progress of this method of desalination.

Chapter 10 on scale formation and prevention is a useful brief introduction to the subject, but the serious reader would be advised to extend his reading before applying the information here. The final chapter in the book, entitled "The Cost of Conventional Water Supply", applies only to the United States; it does, however, give desalination engineers in other countries a very good idea of the type of local information with which he should be familiar.

On the whole, I and others in the field of desalination have found the book an extremely interesting and well balanced treatment of the subject, though a chapter on steam cycles for combined electric power and desalination plant and one on ion exchange would have made it more complete. A second volume on the same lines but filling in the gaps would make them both a valuable contribution for all those working in the field.

C. R. BENNETT

## BIOCHEMISTRY, 1966

### Annual Review of Biochemistry

Vol. 35. Edited by Paul D. Boyer. In association with Alton Meister, R. L. Sinsheimer and Esmond E. Snell. Part 1: Pp. viii + 1-456 + 91. Part 2: Pp. iii + 457-908 + 91. (Palo Alto, California: Annual Reviews, Inc., 1966.) \$11.50 the two parts.

SINCE it was first published in 1932, the *Annual Review of Biochemistry* has until now appeared each year in a single volume. But the 1966 edition has undergone binary fission and now appears in two parts, each of approximately 450 pages, containing about a dozen scientific articles. In addition, there is the usual introductory autobiographical essay by an eminent biochemist, the contributor on this occasion being A. C. Chibnall, who writes under the title "The Road to Cambridge". Author and subject indexes, together covering both parts of the volume, are printed in each of the two parts, as also is a list of the contents of the whole volume. In addition, there is a cumulative index of chapter titles and contributing authors which covers Volumes 30-35.

As might be expected, the biochemistry of amino-acids, peptides and proteins occupies much space, but a particularly interesting topic is "Water-insoluble Derivatives of Enzymes, Antigens, and Antibodies" by Israel H. Silman and Ephraim Katchalski, the last article in the volume. The "Biochemistry of Bacterial Cell Walls" by Hans H. Martin also covers a new and rapidly expanding field of study which, from an experimental point of view, can be said to date from 1951, when Salton and Horne

described the first practicable method for isolating cell walls from whole bacteria on a preparative scale. Bacteria use building materials of complex composition, the macromolecular organization of which differs from that of the classical linear homopolymers of plant cell walls, and various names have been suggested for this class of substance. Hans Martin has adopted the name "murein", recently proposed in analogy with the term "protein". Whether this terminology will ultimately be used generally remains to be seen.

The review articles in general maintain their expected high standard, and one can say with certainty that the process of fission has had no discernible effect on the contents of the volume but has made available two parts of a size which is most convenient to handle.

F. G. YOUNG

## ARTHROPOD NEUROCHEMISTRY

### The Neurochemistry of Arthropods

By J. E. Treherne. (Cambridge Monographs in Experimental Biology No. 14.) Pp. viii + 156. (London: Cambridge University Press, 1966.) 30s. net; \$6.

To a considerable extent the limitations of the book are the limitations of knowledge about the neurochemistry of arthropods; its highlights depend on the topics that have so far interested the research workers. There is a concentration of research into the neurochemistry of insects and crustaceans mainly because of the availability of these animals.

The central nervous system (CNS) of the insect has a sheath around it which may limit the transfer of material between it and the haemolymph. Water soluble materials pass from the haemolymph into the CNS easily, however, and Treherne suggests that this may explain the problems of the vertebrate blood-brain barrier. In vertebrates there is a restricted extracellular system while in insects there is a large extracellular volume in the CNS.

Plant eating insects such as *Carausius* have blood with an interesting ionic composition; it is poor in sodium (15 mmoles/kg compared with 156 mmoles/kg for *Periplaneta*) and rich in magnesium (53 mmoles/kg compared with 5.3 mmoles/kg for *Periplaneta*). The nerve cord also has a high concentration of magnesium (22 mmoles/kg compared with 2.9 mmoles/kg for *Periplaneta*). Could  $Mg^{++}$  instead of  $Na^{+}$  be entering the nerve axons during nerve activity?

There is still uncertainty about the nature of the chemical transmitters in the arthropod nerve-nerve and nerve-muscle systems. It is likely that acetylcholine is a central transmitter. One difficulty has been that fairly high concentrations of acetylcholine ( $10^{-3}$  g/ml.) have to be added to the CNS to produce a clear effect but, as Treherne and Smith have shown, this is most probably due to the high concentrations of choline esterase that lie beneath the sheath of the CNS. With regard to peripheral transmitters, the evidence for glutamate as the excitatory transmitter in insects and crustaceans is discussed and the more recent papers are mentioned in an addendum to this volume. There is fairly good evidence that gamma-aminobutyric acid may be the inhibitory transmitter at the nerve-muscle junction.

It would have been useful if there had been a chapter on neurosecretion in arthropods, but this might have made the volume too long. There is a limitation placed on the authors of these monographs with regard to space and it is certainly an advantage to the reader if the material is described concisely and succinctly. The present volume provides the best available general account of the neurochemistry of the arthropods—a subject that will be the basis of our understanding of the control system of arthropod behaviour, insecticides, clock mechanisms, and the like. The book should do much to stimulate work into this somewhat neglected subject.

G. A. KERKUT

## ENZYME INHIBITORS

### Enzyme and Metabolic Inhibitors

By J. Leyden Webb. Vol. 2: Malonate, Analogs, Dehydroacetate, Sulphydryl, O-Iodosobenzoate, Mercurials. Pp. xx+1237. 272s. Vol. 3: Iodoacetate, Maleate, *N*-Ethylmaleimide, Alloxan, Quinones, Arsenicals. Pp. xx+1028. 256s. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.)

THE subject of this book is an analysis of action of selected enzyme inhibitors in terms of the physicochemical mechanisms of their effect on isolated enzymes and the metabolic consequences of enzyme inhibition on more integrated cellular functions. The effects on animal, microbial and plant cells are discussed without discrimination between species.

Volume 2 contains four chapters—on malonate, analogues of enzyme reaction components, dehydroacetate and sulphydryl reagents respectively. Volume 3 has seven chapters, on iodoacetate and iodoacetamide, maleate, *N*-ethylmaleimide, alloxan, quinones, arsenicals, and comparison of sulphydryl reagents.

Although the author deserves considerable credit for collecting large amounts of material, it is difficult to avoid directing attention to significant shortcomings. The most general is an apparent weakness of logical structure, leaving the impression of a more or less random collection of information which has not been sorted out authoritatively. It is almost unrealistic to expect authoritative knowledge from a single author in as many diverse fields as are dealt with in this work. It would have been less risky to have left many unsolved problems in the form of accurate descriptions of phenomena without attempts at interpretation. The penalty for the author's encyclopaedic ambitions is an unbalanced presentation of both excellent and unreliable references, some inaccuracies of quotation, and occasionally interjections of unfounded subjective opinions. Perhaps the most creditable aspect of the contents of the two volumes is a tendency to emphasize physicochemical considerations. It is unfortunate, however, that important advances in the field of enzymatic mechanisms, particularly relationships of protein structure to catalytic activity, have been virtually ignored. It is equally disappointing that significant advances in molecular biology which provide interesting examples of mechanisms of action of inhibitors are not to be found in these volumes.

A few examples illustrate these shortcomings and may serve as a warning to the reader. On page 44, Volume 2, we find the remarkable conclusion that the active site of succinic dehydrogenase has a "slit or tubular" structure, a statement which, in the absence of more serious physical information, is no comfort to enzyme or protein chemists. Effects on isolated enzymes and on enzyme systems (for example, electron transfer systems) are sometimes confused (that of the calcium ion, for example, on page 47). It is unfortunate that some well-known biochemical mechanisms appear to be *terra incognita* if one wishes to depend on this text for information (including uncoupling of oxidative phosphorylation, Crabtree and Pasteur effects, pages 115, 119, 127; interpretation of lipid metabolism, page 137; omission of participation of carbamyl phosphate in the urea cycle, page 157, etc.). The chaotic method of presenting diverse subjects ranging from inhibitors of fumarase to vitamin analogues is especially evident in Chapter 2 of Volume 2. There are some misquotations of published papers (for example, on page 334), and also noticeable omissions, faults which introduce uncertainty in the text. Although Chapters 5 and 6 of Volume 3 are more carefully organized, they tend to be repetitious.

These volumes will remain sources of varied information without the assurances of accuracy, reliability and up to date interpretation which one expects from an authoritative handbook.

E. KUN

### Polarography 1964

Edited by Graham J. Hills. (Proceedings of the Third International Congress, Southampton.) Vol. 1: Pp. xvi+1-686+xvii-xxviii. Vol. 2: Pp. xv+687-1164+xvii-xxviii. (London: Macmillan and Co., Ltd., 1966.) 315s. the set.

It is sometimes difficult for those actively engaged in electro-analytical chemistry—and impossible for those not so engaged—to appreciate the wide interest in, and the possibilities of, the technique simply described as polarography. Only by perusal of volumes like these does there come a realization of the tremendous impact that polarography has made and continues to make on electrochemistry.

These volumes are a record of the 1964 International Congress of Polarography organized by the Polarographic Society and held at the University of Southampton. The record consists of eighty-nine papers. Among them are reviews such as that by Kolthoff of the fundamentals of polarography in inert solvents; recent trends in organic polarography by Zuman; recent developments in direct current polarography and recent advances in high-resistance polarography. For the specialist there is a range of papers covering as wide a selection as the polarography of oxidized and reduced forms of diphosphopyridine nucleotide to the kinetics of fast reactions. Fully to appreciate the possibilities, it will be necessary to purchase both volumes, but those who do so will find them worth while.

R. J. MAGER

### The Fundamentals of Mathematical Analysis

Vol. 1. By G. M. Fikhtengol'ts. Translation edited by Ian N. Sneddon. (International Series of Monographs in Pure and Applied Mathematics, Vol. 72.) Pp. xxvi+494. (London and New York: Pergamon Press, Ltd., 1965.) 50s. net.

THIS book was originally published in Russian in 1960 and consists of the usual development of analysis at the university level. It starts with the Dedekind definition of irrationals, and uses the concept of variables in discussing the idea of function. Limits are introduced using infinitesimals (null sequences), and the normal development through continuity, differentiability to the Riemann integral and functions of several variables follows. There are applications of the theory to problems of maxima and minima, length of a curve, indeterminate forms and areas and volumes. It concludes with a historical survey of the early works in differential calculus. The main defect from the student's point of view is that there are no exercises, though there are several worked examples.

R. L. PERRY

### The Fundamentals of Mathematical Analysis

Vol. 2. By G. M. Fikhtengol'ts. Translated by Ann Swinfen. Translation edited by Ian N. Sneddon. (International Series of Monographs in Pure and Applied Mathematics, Vol. 73.) Pp. xxi+518. (London and New York: Pergamon Press, Ltd., 1965.) 55s. net.

IN this volume, G. M. Fikhtengol'ts continues the development of analysis through the usual university syllabus. A discussion on the convergence of series is followed by a considerable section on uniform convergence and the interchange properties associated with it. A continuous, nowhere differentiable function is constructed. The text continues with the theory of implicit functions, multiple integrals and Fourier series and integral. Interspersed with the development of the theory are sections on the historical background. The book concludes with a brief introduction to the further developments of analysis. It is a satisfactory, if somewhat lengthy text, covering a little more than is usually included in a specialist pure mathematical course in analysis, though there is no theory of sets or mention of enumerability. Together the volumes contain more than 1,000 pages.

R. L. PERRY

### Chemical Carcinogenesis and Molecular Biology

By Pascaline Daudel and Raymond Daudel. Pp. 158. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 53s.

IN spite of the recent and, in some respects, more detailed book by Clayson on chemical carcinogenesis, this book will be welcomed by biologists. The title is somewhat optimistic as there is still no good tie-up between the understanding of the chemical structure and biochemistry of carcinogens, and the basic molecular biological phenomena. The twelve chapters cover the field briefly but clearly, each containing a useful list of references. Some of the conclusions are less cautious than ideal; for example, the fact that more skin papillomata appear if the carcinogen is given near midnight, and the fact that mitotic frequency in the skin is lower at midnight than at noon; these two observations do not suggest that carcinogens act primarily on cells which are synthesizing DNA. Generally, a more critical approach to the literature would have been useful.

The translation retains some gallicisms: "sensitivity" instead of "sensitivity", "works" of this kind instead of "experiments"; and it is a pity that in the text the letter "l" and the number "1" are sometimes indistinguishable. This may confuse those not expert in the particular field discussed. There are some spelling mistakes, and minor points which might irritate the experts (for example, sulfanoxyalkanes and nitrosamines are classed together), but this readable little book will be useful for students and biologists interested in the complex field of chemical carcinogenesis.

L. G. LAJTHA

## OBITUARIES

### Lord Brain

LORD BRAIN, who died on December 29, aged 71, was neurologist, philosopher and writer.

Walter Russell Brain was educated at Mill Hill School and at Oxford, where his teachers included Sir Julian Huxley, J. B. S. Haldane and Sir Charles Sherrington. His major hospital appointments, until his retirement in 1960, were at the London Hospital, where he was a general and consulting physician, and at the Maida Vale Hospital for Nervous Diseases. From 1924 he was engaged in research, in addition to his practice as a neurological physician, and he published numerous original papers.

At the London Ophthalmic Hospital (Moorfields) he became interested in the ocular symptoms of thyroid disorders, especially exophthalmos, and he described the syndrome exophthalmic ophthalmoplegia—a condition which involves paralysis of the muscles of the eye. In his later clinical neurological work, Brain was particularly interested in carcinomatous neuropathy—the relation between cancer and degenerative changes in the nervous system. He was involved in surveys of patients with cancer, which have shown that there is a connexion between the incidence of cancer and of neurological disorders such as motor neurone disease (amyotrophic lateral sclerosis). He also investigated disorders of the joints of the neck and of the spinal cord as a cause of neurological symptoms. He was an authority on disorders of speech, about which he wrote one of his books, and on neurological disturbances after damage to the major hemisphere of the brain.

Lord Brain, who was a Quaker, was always interested in philosophy, and particularly in the relation between the brain and the mind in perception. In the Linacre lecture, delivered at Cambridge in May 1965, he described perception, in neurophysiological terms, as a method of conveying information to the brain. He also applied his specialized knowledge to literature, which with philosophy was his main pleasure apart from work, and to the many public offices which he held, particularly after his retire-

ment. He was the president of the Migraine Trust, set up last year to finance a programme of research into this disorder, as well as being chairman of the Standing Committee on Drug Addiction, and a member of, among others, the Royal Commission on Marriage and Divorce and the Law Relating to Mental Illness. He had been president of the Royal College of Physicians and the British Association, and was active in the House of Lords; in the committee stage of the Abortion Bill he moved two amendments which were accepted. One amendment replaced as a ground for abortion the possible birth of a child with no prospect of reasonable enjoyment of life with the possible birth of a child with a serious mental or physical handicap. He remarked that the prospect of reasonable enjoyment of life is not something which a doctor can calculate.

### Professor F. E. Ray

DR. FRANCIS E. RAY, aged 68, research professor in pharmaceutical chemistry at the University of Florida in Gainesville, died on November 25 in Hong Kong while he was on a world tour before going to the ninth International Cancer Congress in Tokyo.

Ray obtained his B.Sc. and D.Sc. with Professor Chattaway from the University of Oxford, when he was a Rhodes scholar, and Professor W. A. Noyes supervised his M.S. thesis at the University of Illinois. Ray was a member of the faculty of the University of Cincinnati for many years, and he and his students were pioneers in the preparation of isotopically labelled organic compounds for cancer research when he was the director of the Laboratory of Radiochemistry. In 1949 Ray was appointed director of the Cancer Research Laboratory at the University of Florida, and in 1960 he assumed his last position.

Ray, early in his career, discovered the sulphonium equilibrium and wrote an authoritative review with G. Rieveschl on the chemistry of the polynuclear hydrocarbon fluorene. This proved to be the starting point for Ray's interest in the carcinogenic derivatives of this hydrocarbon, a field in which he and his students made many important discoveries. Thus, his name is associated with new derivatives of the carcinogen 2-acetylaminofluorene; with the induction of adenocarcinoma of the glandular stomach in rats by means of 2,7-diacetylaminofluorene; with the production of minimal deviation hepatomas with *N*-2-fluorenylphthalamic acid, and with the preparation of pharmaceutical products labelled with chlorine-36 and sulphur-35 for metabolic investigations.

Ray was the author of four text-books on experimental, general and organic chemistry which were at one time adopted by many of the major universities in the United States. He was an active member of many learned societies, among them the American Association for Cancer Research, the American Chemical Society, the Society of Nuclear Medicine, the Society for Experimental Biology and Medicine, Sigma Xi, and he was a fellow of the Ohio, the Florida, and the Iowa Academies of Science. He served on a panel on carcinogenesis of the International Union Against Cancer, and as director of the Florida Division of the American Cancer Society and as a member of its Research Committee. He was a consultant to the Medical Division of the Oak Ridge Institute of Nuclear Studies. Ray was an authority on the chemical causes of cancer and he testified on this subject before a committee of the U.S. House of Representatives.

To his students and associates, Ray was more than a teacher; he had a curiosity to delve into the unknown, to read and follow the literature on a broad basis, and the wisdom to develop concepts and ideas based on and guided by experimental facts. Ray loved music not only as a listener; he played the flute in the Civic Orchestra of Cincinnati and the University Orchestra in Gainesville. He liked poetry and expressed his talent for form as a photographer. He was proud of the tropical plants in his garden in Florida.

JOHN H. WEISBURGER

# Absolute Magnitudes of Quasi-stellar Radio Sources

by

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Absolute magnitudes of quasi-stellar sources, plotted against the logarithms of their red-shifts, fall between two limits. The interpretation of the diagrams varies according to the type of model universe chosen

A NUMBER of recent investigations<sup>1-5</sup> have dealt with the relation between the red-shift and the optical apparent magnitudes or the observed radio flux densities of quasi-stellar radio sources. The object of the present article is to analyse the absolute magnitude of fifty-four quasi-stellar radio sources for which the red-shift,  $z$ , and visual apparent magnitude,  $m_v$ , are available<sup>6-8</sup>. In order to calculate the absolute magnitude of a luminous source from its observed  $m_v$  and  $z$ , it is of course necessary to know the equivalent of its "distance". If it is assumed that the red-shifts are cosmological in origin, the theory of uniform models of the universe may be used to provide the "distance". In fact<sup>9</sup> the formula for the absolute magnitude in a model universe is

$$M_v + C = m_v - (K_v + A_v) - 5 \log D \quad (1)$$

Here

$$C = 5 \log (cT_0/10) \quad (2)$$

$T_0$  is the reciprocal of the Hubble constant, the distance  $cT_0$  is in parsecs,  $K_v$  is the visual  $K$  correction and  $A_v$  the correction for absorption in our galaxy. The function  $D$  of  $z$  is different in each model of the universe and is calculable as soon as numerical values are assigned to the acceleration parameter  $q_0$  and the density parameter  $\sigma_0$ <sup>10,11</sup>. Hence a given  $m_v$ ,  $z$  and  $C$  does not uniquely define  $M_v$ ; the model also contributes to its value.

## The $q_0 = \sigma_0 = 1$ Model

This model possesses the property that  $D=z$  so that (1) becomes

$$M_v + C = m_v - (K_v + A_v) - 5 \log z \quad (3)$$

Fig. 1 shows the plot of absolute magnitude against  $\log z$  for this model. The left-hand ordinate is graduated in terms of  $M_v + C$  and is therefore applicable for any assumed value of the Hubble constant  $1/T_0$ . The right-hand ordinate shows  $M_v$  itself, calculated for  $1/T_0 = 100$  km/sec/Mpc. The straight line  $m_v = 19^m0$  has also been

included in the figure. The large scatter of the points is similar to that found by other investigators<sup>1-5</sup>, but there is also a general slope of the points nearest to the  $\log z$  axis, downwards from left to right, which has been indicated by the dashed line. The extreme left-hand point (3C 273) has been excluded: this quasi-stellar source is quite exceptionally bright for its red-shift. Clearly the upper boundary of the points, exemplified by the line  $m_v = 19^m0$ , can be explained by the limited ability of telescopes to detect faint objects. As was pointed out by McCrea<sup>6</sup>, the area above this line in Fig. 1 may contain many faint objects. But the lower boundary, indicated by the dashed line, is harder to explain. Sandage (personal communication) and McCrea<sup>6</sup> have suggested that it is caused by a volume effect. It is supposed that there are only a small number of intrinsically bright objects which should make them detectable (statistically) only at large distances, that is, in large volumes. We have therefore used the tables of Refsdal, Stabell and de Lange<sup>12</sup> to examine this volume effect.

Table 1

$z$	0.0-0.7	0.7-1.1	1.1-1.5	1.5-1.9	1.9-2.2
Volume $10^3$ Mpc	8.3	9.5	9.9	8.4	6.6
Number with $M_v + C < 16^m5$	0	0	1	1	1
Number - density	0	0	0.10	0.12	0.15
Number with $M_v + C < 17^m0$	0	1	2	1	4*
Number - density	0	0.11	0.20	0.12	0.61*
Number with $M_v + C < 17^m25$	1	3	3	2*	4*
Number - density	0.12	0.32	0.30	0.24*	0.61*

\* These values are somewhat affected by selection.

Table 1 shows the volumes between the intervals of  $z$  indicated in the first line of the table. The third line contains the number of sources with values of  $M_v + C$  less than  $16^m5$  in the relevant volumes, read off from Fig. 1. The corresponding number-densities are given next. Numbers and number-densities are also tabulated for values of  $M_v + C$  less than  $17^m0$  and  $17^m25$ . From

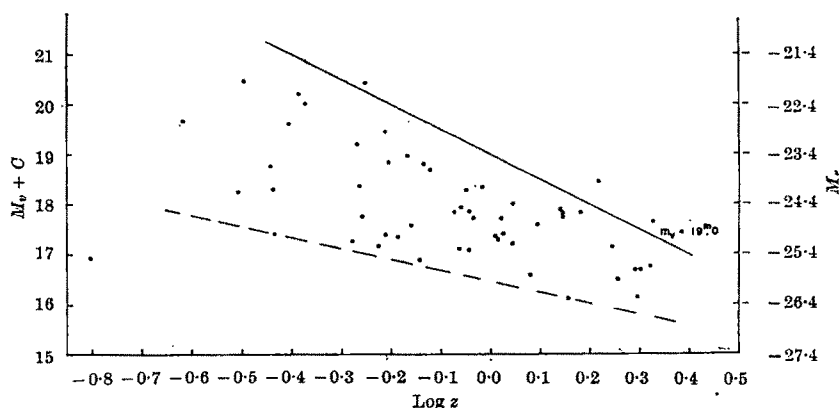


Fig. 1. Absolute magnitudes  $M_v + C$  (arbitrary scale) or  $M_v$  (scale for  $H = 100$  km/sec/Mpc) plotted against  $\log z$  for model  $q_0 = \sigma_0 = 1$ .

Table 1 the dearth of objects with small red-shifts, indicated in Fig. 1, seems to be significant. Hence the volume effect does not appear to be sufficient to account for the empty region below the dashed line, though it must certainly play a minor part. A corresponding result has been found for the steady-state theory by Sciama and Rees<sup>3</sup>, and also by McCrea<sup>5</sup> in discussions wherein the radio flux densities of the sources were used instead of optical apparent magnitudes.

The lower boundary that appears in Fig. 1 may be accounted for by one or both of two secular effects: (a) secular changes in the number of sources per unit physical volume; (b) secular changes in the intrinsic luminosity of each source. If the number of intrinsically luminous sources per unit volume was larger in the past than it is now, then the diagram should show more bright sources at large than at small  $z$ , because a large value of  $z$  means that a portion of the universe as it was in the remote past is under observation. If the intrinsic luminosity of a source decreases secularly, the diagram should show the same effect, namely, a greater number of intrinsically bright objects at large red-shifts (at an earlier epoch) than at small red-shifts. The general slope of the points in the diagram is therefore, on this view, a counterpart of the conclusions drawn from number-counts of extragalactic radio sources many of which are no doubt quasi-stellar sources. McVittie<sup>13</sup> showed in 1957 that the secular effect (b) could be used to interpret the counts made by Mills and his co-workers. More recently the alternative effect (a) has been applied to the counts made at the Vermilion River Observatory<sup>14</sup>. Longair<sup>4,15</sup> favours the secular effect (b) and uses the Einstein-deSitter model ( $q_0 = \sigma_0 = 0.5$ ) in his interpretation of the Cambridge counts.

### The Effect of the Model

There is another quite different conclusion that may be drawn from the basic formula (2). The same fifty-four quasi-stellar sources may be used with a different function  $D$ , that is with a different model universe. The tables of Refsdal *et al.*<sup>12</sup> can be used to recalculate  $M_v + C$  for a number of models. A measure of the magnitude differences (DELMAG) in the tables is  $5 \log (D/3)$ . Fig. 2 shows the results obtained for Milne's model defined by  $q_0 = \sigma_0 = 0$ . The slope of the points in the diagram has been steepened from left to right. The secular effects (a) and (b) have become more pronounced. The model  $q_0 = 0, \sigma_0 = 3$  yields Fig. 3 in which the slope has to all intents and purposes vanished. Secular effects are not required in this case. Finally Fig. 4 is obtained for the  $q_0 = -2, \sigma_0 = 1$  model. Here the slope of the lower boundary has been reversed. This is due to an effect investigated by Sandage<sup>16</sup> and by McVittie<sup>17</sup> by which, in some models, the apparent brightness of a source of given intrinsic luminosity, after decreasing in the usual way up to a limiting value of  $z$ , begins to rise again as  $z$  increases beyond the limiting value.

It is thus seen that the interpretation of diagrams such as those found in Figs. 1 to 4, or of similar diagrams in which apparent magnitudes or observed flux densities are used, must be carried out with great care. All methods employ  $\log z$  as one co-ordinate and it is only in the  $q_0 = \sigma_0 = 1$  model that  $z$  is proportional to luminosity distance. Interpretations that rely solely on this model, or on the Einstein-deSitter or on any other specific model, are likely to be model dependent and thus do not necessarily yield conclusions valid in all possible models.

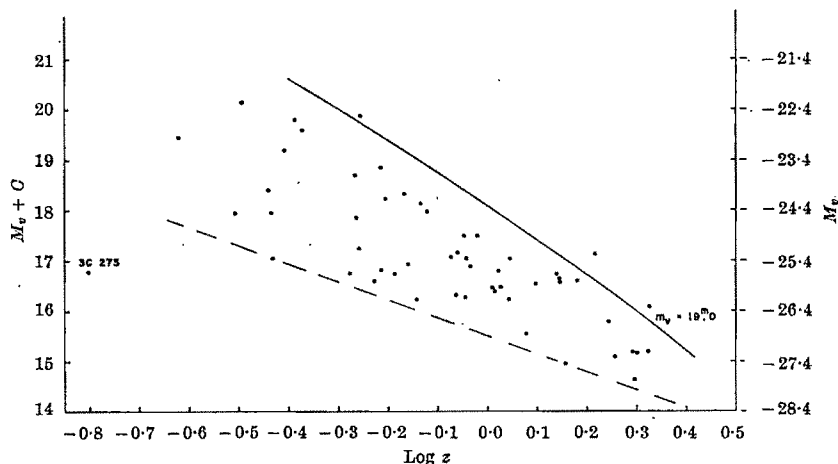


Fig. 2. Absolute magnitudes  $M_v + C$  (arbitrary scale) or  $M_v$  (scale for  $H = 100$  km/sec/Mpc) plotted against  $\log z$  for model  $q_0 = \sigma_0 = 0$ .

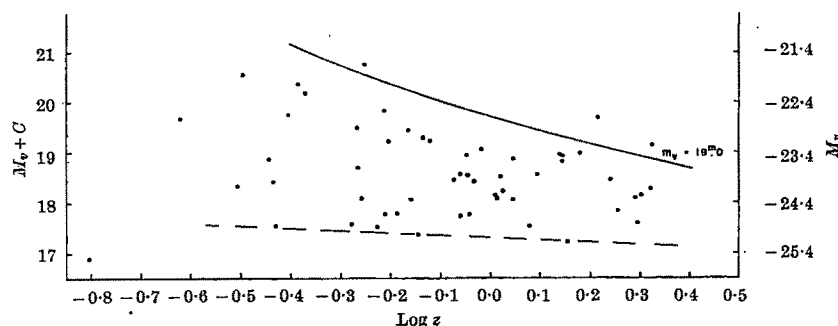


Fig. 3. Absolute magnitudes  $M_v + C$  (arbitrary scale) or  $M_v$  (scale for  $H = 100$  km/sec/Mpc) plotted against  $\log z$  for model  $q_0 = 0, \sigma_0 = 3$ .

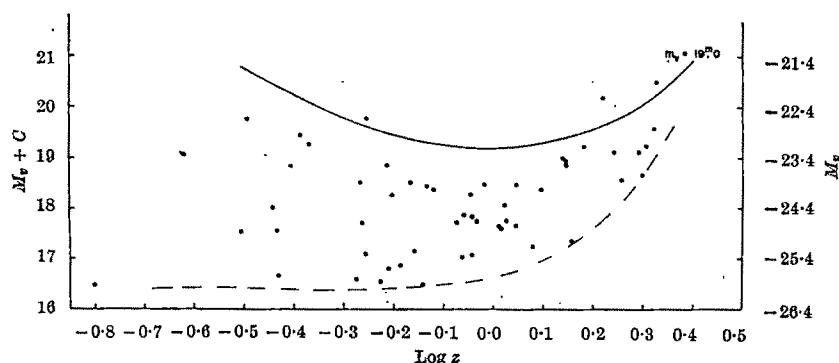


Fig. 4. Absolute magnitudes  $M_v + C$  (arbitrary scale) or  $M_v$  (scale for  $H = 100$  km/s/Mpc) plotted against  $\log z$  for model  $q_0 = -2$ ,  $\sigma_0 = 1$ .

Caution suggests that a variety of models should be investigated before final conclusions are drawn. In the present instance, secular effects have been invoked to account for the diagrams of  $M_v$  against  $\log z$ , when the model universe to which the observed universe conforms is of the type exemplified by the  $q_0 = \sigma_0 = 1$  or the  $q_0 = \sigma_0 = 0$  models. But such secular effects would be unnecessary if the model turned out to be similar to the  $q_0 = 0$ ,  $\sigma_0 = 3$  model of Fig. 3.

#### Local Theory of Quasi-stellar Sources

Fig. 2 also throws some light on the local theory of quasi-stellar sources. Suppose that the sources are assumed to have been ejected simultaneously from some point at, or near, our galaxy and that they have subsequently moved at high speeds in the space-time of special relativity. Then those with highest speeds (large  $z$ ) are more remote today than those with smaller speeds (small  $z$ ). Milne's model—which is that of special relativity—therefore applies and Fig. 2 may be used. But since  $C$  is now unknown, the values of  $M_v$  given on the right-hand ordinate are inapplicable. Nevertheless, a large value of  $M_v + C$  still implies an intrinsically fainter object than does a small value of  $M_v + C$ . The presence of the lower boundary (dashed line) for the points in Fig. 2 would imply the existence of a correlation between

speed of ejection and intrinsic luminosity of the ejected object. The physical cause of an explosion which had this property is not apparent. As always, 3C 273 appears as an anomalous object: it is moving too slowly for its intrinsic luminosity. If the quasi-stellar sources are supposed to have been ejected from the explosion centre at different times in the past, or from a number of comparatively nearby centres, however, no conclusions can be drawn from Fig. 2 because then the distances of the sources would be quite arbitrary.

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## Determination of Longitudinal and Transverse Optical Constants of Absorbing Uniaxial Crystals—Optical Anisotropy of Graphite

by  
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Equations relating the minimum values of the refractive and attenuation indices to the longitudinal and transverse optical constants are developed. The longitudinal optical constants of graphite are thus obtained

A UNIAXIAL crystal may be regarded as a stratified medium, that is one whose properties are constant throughout each plane perpendicular to the optic axis. To define the medium optically we need to know the dielectric constants along the optic axis and perpendicular to it. To be in harmony with the definition of transverse electric and transverse magnetic waves adopted by Born and Wolf<sup>1</sup>, we shall define the optical properties of the graphite as transverse when the electric vector is per-

pendicular to the optic axis and as longitudinal when the electric vector is parallel to the optic axis. Many uniaxial crystals exhibit natural surfaces perpendicular to the optic axis; hence, measurement of reflectance at normal incidence to such surfaces leads to the determination of the transverse optical properties.

The simplest experiments to obtain the longitudinal optical constants would involve reflexions at normal incidence from the ( $h k 0$ ) faces; one of the extreme values



of the reflectance, the minimum in the case of graphite, is a function of the optical parameters along the optic axis only. Unfortunately such faces suitable for optical measurements are not often available, as in the case of graphite. It therefore becomes necessary to resort to experiments involving oblique incidence of polarized light at the basal planes or to employ crystal faces inclined to the basal planes if normally incident polarized light is used. Although such experiments have been carried out with graphite, the optical constants of this material along the optic axis have not been determined. McCartney and Ergun<sup>2</sup> measured the reflectance at normal incidence in differing immersion media from the 10 $\bar{1}$ 2 face (the so-called *o*-face) of graphite and reported the minimum and maximum values of the refractive and absorption indices at 5461 Å. To calculate the optical constants along the optic axis they made use of the uniaxial indicatrix largely because rigorous equations had not been developed for the analysis of the directional optical properties. The indicatrix is applicable in the case of non-absorbing crystals. In this article, equations are developed which relate the minimum values of the refractive and attenuation indices to those along the optic axis (longitudinal) and perpendicular to the optic axis (transverse). Also, for the first time, the longitudinal optical constants of graphite are given.

The optical properties of anisotropic absorbing crystals are characterized by their dielectric ( $\epsilon_{nm}$ ) and conductivity ( $\sigma_{nm}$ ) tensors. In the case of uniaxial or other crystals of high symmetry, the principal axes of the two tensors coincide. If we take the co-ordinate axes in the direction of the principal axes of the two tensors, Fresnel's equation may be broken into two factors<sup>1</sup>

$$\begin{aligned} (\mathbf{v}_p')^2 &= \mathbf{v}_x^2 \\ (\mathbf{v}_p'')^2 &= \mathbf{v}_x^2 \cos^2 \delta + \mathbf{v}_z^2 \sin^2 \delta \end{aligned} \quad (1)$$

where  $\mathbf{v}$  is the complex velocity defined by  $\mathbf{v}_p = c/\mathbf{n}$ ,  $\mathbf{v}_x = c/\mathbf{n}_x$ , etc.,  $c$  is the velocity of light,  $\mathbf{n}$  is the complex refractive index defined by  $\mathbf{n} = n(1 + i\kappa)$ ;  $n$  and  $\kappa$  are the refractive and absorption indices respectively; and  $\delta$  is the angle which the wave normal makes with the  $z$ -axis. The first equation applies when the electric vector is perpendicular to the principal plane, that is the plane formed by the optic axis  $z$  and the wave normal  $\mathbf{s}$ ; the ray is referred to as the ordinary wave. Its velocity is independent of  $\delta$ . The second equation applies when the electric vector lies in the principal plane and the ray is referred to as the extraordinary wave; thus light polarized in any other direction may be expressed in terms of these two equations.

From the first of equations (1) it follows that

$$n = n_x, \kappa = \kappa_x \quad (2)$$

In transforming the second into more useful forms, the case of weakly absorbing substances is generally considered, and it is assumed that the second power of the attenuation index may be neglected in comparison with unity<sup>1</sup>. This assumption leads to serious error in the case of graphite, especially in the ultra-violet. Replacement of the complex velocities by refractive and absorption indices leads to

$$\frac{\sin^2 \delta}{n_x^2 (1 + i\kappa_x)^2} + \frac{\cos^2 \delta}{n_x^2 (1 + i\kappa_x)^2} = \frac{1}{n^2 (1 + i\kappa)^2}$$

Separating the real and imaginary parts of the last equation we obtain

$$\begin{aligned} \frac{(1 - \kappa_x^2) \sin^2 \delta}{n_x^2 (1 + \kappa_x^2)^2} + \frac{(1 - \kappa_x^2) \cos^2 \delta}{n_x^2 (1 + \kappa_x^2)^2} &= \frac{1 - \kappa^2}{n^2 (1 + \kappa^2)^2} \\ \frac{\kappa_x \sin^2 \delta}{n_x^2 (1 + \kappa_x^2)^2} + \frac{\kappa_x \cos^2 \delta}{n_x^2 (1 + \kappa_x^2)^2} &= \frac{\kappa}{n^2 (1 + \kappa^2)^2} \end{aligned} \quad (3)$$

The physical significance of equations (2) and (3) is as follows. For the ordinary wave, the medium behaves like an isotropic material having an apparent refractive index  $n_x$  and absorption index  $\kappa_x$  regardless of the direction of the wave normal. For the extraordinary wave, the medium again behaves like an isotropic material with an apparent refractive index  $n$  and absorption index  $\kappa$  defined by equations (3). It is seen that  $n$  and  $\kappa$  are functions of  $n_x$ ,  $\kappa_x$ ,  $n_z$ ,  $\kappa_z$  and  $\delta$ . The determination of these apparent indices is strictly an experimental problem.

In the case of graphite, freshly peeled or cleaned basal planes serve as suitable surfaces for optical measurements<sup>2</sup>. At normal incidence reflectance becomes a property of  $n_x$  and  $\kappa_x$  and it is not necessary to employ polarized light. Reflectance using two immersion media is sufficient to measure  $n_x$  and  $\kappa_x$ . If an inclined face (for example, a 10 $\bar{1}$ 2 face, for which  $\delta = 57^\circ 49'$ ) is used with the electric vector perpendicular to the  $z$ -axis, the reflectance at normal incidence is the same as that from the basal planes. When the electric vector is rotated  $90^\circ$ , then  $n$  and  $\kappa$  determined are defined by equation (3). We now can evaluate  $n_z$  and  $\kappa_z$  using equation (3). If we let

$$\begin{aligned} p &= \frac{1 - \kappa^2}{n^2 (1 + \kappa^2)^2 \sin^2 \delta} - \frac{1 - \kappa_x^2}{n_x^2 (1 + \kappa_x^2)^2 \tan^2 \delta} \\ q &= \frac{\kappa}{n^2 (1 + \kappa^2)^2 \sin^2 \delta} - \frac{\kappa_x}{n_x^2 (1 + \kappa_x^2)^2 \tan^2 \delta} \end{aligned} \quad (4)$$

we obtain

$$\begin{aligned} n_z^2 &= \frac{1}{2} \frac{\sqrt{(p^2 + 4q^2)} + p}{p^2 + 4q^2} \\ \kappa_z^2 &= \frac{\sqrt{(p^2 + 4q^2)} - p}{\sqrt{(p^2 + 4q^2)} + p} \end{aligned} \quad (5)$$

Experiments by McCartney and Ergun yielded  $n_x = 2.15$ ,  $\kappa_x = 0.66$ ,  $n = 2.04$  and  $\kappa = 0.01$ . The value of  $\kappa$  is too low, because it leads to negative values of  $\kappa_x$  using equations (4). If we assume that the measured value of  $\kappa_x$  is correct and  $\kappa \approx 0$ , then  $\kappa$  would have a value of 0.08. This increase would correspond to an error of about 0.5 per cent in the intercepts of the straight line plot the authors obtained. It appears to be reasonable, therefore, to assume that  $\kappa_x$  is too small to be measured by simple optical procedures.

Table 1. THE OPTICAL PROPERTIES OF HEXAGONAL GRAPHITE AT 5461 Å

Optical property*	On the $x$ - $y$ plane	Along the $z$ (optic) axis
Refractive index, $n$	2.15	1.81
Absorption index, $\kappa$	0.66	Not detectable
Dielectric constant, $\epsilon$	2.61	3.28
Conductivity, $\sigma$	$1.68 \times 10^{11}$ sec. <sup>-1</sup>	Not detectable
Reflectance in air, $R$	0.280	0.083
Emissivity, $1-R$	0.720	0.917

\*  $\epsilon$  and  $\sigma$  are defined by  $\epsilon = n^2(1 - \kappa^2)$ ,  $\sigma = n^2\kappa$ .

The same argument may be extended to the conductivity along the optic axis. Using the above equations, the value of  $n_z = 1.81$ . In Table 1 are listed the optical properties of graphite at 5461 Å. Its optical anisotropy is rather marked.

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# Chemiosmotic Hypothesis of Oxidative Phosphorylation

by  
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Dr. Moyle and Dr. Mitchell answer criticisms of their interpretation of tests of the hypothesis proposed by Dr. Mitchell in 1961 to explain ATP synthesis in the inner membrane of mitochondria and of chloroplasts by a fuel-cell type of mechanism

Tager, Veldsema-Currie and Slater<sup>1</sup> have criticized our interpretation of measurements of the proton translocation to oxygen quotients ( $\rightarrow H^+/O$ ) observed during pulsed oxidation of succinate and  $\beta$ -hydroxybutyrate by rat liver mitochondria<sup>2,3</sup>. Their criticisms stem from two main points, one theoretical and the other experimental.

Their theoretical criticism is based on our finding (which appears to confirm an earlier observation discussed by Chance *et al.*<sup>4</sup>) that a certain amount of slack or "backlash" exists between the flow of oxido-reducing equivalents through the respiratory chain and the initiation of respiratory control in rat liver mitochondria isolated by an orthodox procedure<sup>2</sup>. We attributed this backlash to the presence of a limited number of charged particles (probably including calcium ions) which can move across or within the mitochondrial cristae membrane, so that the establishment by the respiratory chain system of the controlling membrane potential across the membrane would be delayed to an extent stoichiometrically related to the quantity of movable charge. Slater *et al.* say that the existence of such a backlash effect would be "difficult to reconcile with the fact that oxidative phosphorylation operates at maximal efficiency at very high ratios of  $[ATP]/[ADP] \cdot [P_i]$  and with the relative inefficiency of ATP compared with a functional respiratory chain in reversing the respiratory chain". We used the mechanical term "backlash" with the deliberate intention of making it easier to appreciate that, as in a machine (such as a bicycle with a fairly slack chain), the efficiency of energy transmission is usually independent of the backlash once this has been taken up<sup>3,5</sup>, but that there is little opposing force within the slack region of the backlash. According to our reasoning<sup>2</sup>, proton translocation stoichiometries can be measured accurately only when the conditions are so arranged as to "minimize the unknown extent of back flow or exchange of  $H^+$  ions and  $OH^-$  ions across the membrane system"; the forces contributing to the back flow should be minimal within the backlash region, and hence the choice of this region for our investigations.

The more serious criticism by Slater *et al.* stems from experiments intended to check ours. We described<sup>2</sup> a routine procedure for measuring  $\rightarrow H^+/O$  quotients in rat liver mitochondria by injecting pulses of oxygen (as air-saturated 150 mmolar potassium chloride) into anaerobic mitochondrial suspensions in a medium containing 150 mmolar potassium chloride, 3.3 mmolar glycylglycine and 2 mmolar substrate at 25° C, and recording the pH with a sensitive glass electrode system. Usually we used quantities of oxygen in the pulse corresponding to some 1  $\mu g$  atom of oxygen/g mitochondrial protein, and we observed  $\rightarrow H^+/O$  quotients approaching 4 and 6 with succinate and  $\beta$ -hydroxybutyrate, respectively, as substrate—indicating that one oxido-reduction cycle of nicotinamide-adenine dinucleotide (NAD) was accompanied by the translocation of two protons.

Slater *et al.*<sup>1</sup> say that in experiments "carried out in exactly the same way as described by Mitchell and Moyle", oxidation of reduced nicotinamide-adenine dinucleotide (NADH) did not occur with  $\beta$ -hydroxybutyrate as substrate unless ADP was added to the mitochondrial suspensions. This statement is based on: (a) chemical estimations of NAD, using perchloric acid to stop the oxido-reduction reactions; and (b) estimations of the transition from NADH to NAD *in situ* in mitochondrial suspensions using an Aminco double beam spectrophotometer. They could not detect significant oxidation of NADH by their chemical procedure with oxygen pulses of some 5  $\mu g$  atom oxygen or more per gram mitochondrial protein.

Incidentally, the backlash limit corresponds to 2  $\mu g$  atom of oxygen in our experiments<sup>3</sup>. Using the Aminco double beam spectrophotometer, however, they observed that the decline of the absorbance difference  $A_{340-374} m\mu$  could have indicated the oxidation of NADH. Nevertheless, they rejected this interpretation because the decline of the absorbance difference,  $A_{340-374} m\mu$ , occurred even when oxygen was added in the presence of rotenone, which blocks the oxidation of NADH in the respiratory chain. They concluded that the absorbance change, with  $\beta$ -hydroxybutyrate as substrate in the absence of rotenone, must result from oxidation of the cytochromes<sup>1</sup>. We are unable to accept that the Aminco traces of their Fig. 3, plotted without absorbance or time scales, and showing no absorbance maxima, constitute sufficient evidence to warrant the conclusion that no NADH oxidation occurs.

In Fig. 1 we show some measurements of the time-course of absorbance differences ( $A_{340-374} m\mu$ ) observed with an Aminco double beam spectrophotometer during routine oxygen pulse experiments<sup>2</sup> using  $\beta$ -hydroxybutyrate as substrate. The experiments represented by Fig. 1a were done with normal rat liver mitochondria, but in those in Fig. 1b, respiratory chain-linked NADH oxidation was blocked by the presence of 25  $\mu g$  rotenone/g mitochondrial protein. The oxygen pulses in the Fig. 1 experiments corresponded to 0.73  $\mu g$  atom of oxygen/g mitochondrial protein.

Referring to the curves of Fig. 1, if the absorbance decrease  $A_{340-374} m\mu$  shows oxidation of respiratory pigments, the presence of the rotenone evidently inhibits the reduction of the oxygen pulse. It is therefore concluded that there is a reduction of oxygen under the conditions of these experiments which is sensitive to rotenone; and it follows that a substrate, presumably the  $\beta$ -hydroxybutyrate, is oxidized by way of an NAD-linked dehydrogenase sensitive to rotenone. By observing oxido-reduction of cytochrome  $a$ , using the absorbance difference,  $A_{605-630} m\mu$ , and by simultaneous oxygen electrode measurements, we have confirmed that the oxygen pulse is virtually reduced to completion in less than 2 sec in the normal mitochondrial suspensions of Fig. 1a,

but that it takes about 30 sec in the mitochondria inhibited by rotenone (Fig. 1b).

The trace of Fig. 1a for  $A_{340-374} \text{ m}\mu$  appears to consist of a fast initial spike and a slower more persistent component. We therefore suspected that the traces might represent changes in more than one respiratory carrier. In particular, we suspected that the carrier causing the fast and relatively stable absorbance difference of the mitochondria inhibited by rotenone in Fig. 1b might not be the same as the carrier causing the slower absorbance change in the normal mitochondria of Fig. 1a. We investigated this possibility by making repeated absorbance difference measurements with a fixed reference wavelength (374 m $\mu$ ) but changing the test wavelength ( $\lambda$ ) so that differences in the speed of absorbance change at different wavelengths might be shown up and difference spectra might be plotted (Fig. 2).

In Fig. 1, the initial spike—which is more prominent at  $A_{315-374} \text{ m}\mu$  than at  $A_{380-374} \text{ m}\mu$  in the normal mitochondrial suspension—indicates the oxidation of a pigment that returns towards the reduced state about 1 sec after the oxygen pulse. The changes of absorbance of this pigment are approximately synchronous with the oxido-reduction of cytochrome *a*. The biphasic character of the absorbance changes is particularly clear in the curve for  $A_{315-374} \text{ m}\mu$  in Fig. 1a. On the other hand, as indicated by the curves of Fig. 1b, the absorbance changes observed in the mitochondria treated with rotenone show no evidence of complexity at any wavelength pair in the range 305–374 m $\mu$ . Accordingly, difference absorbance spectra  $A_{\lambda-374} \text{ m}\mu$  of the pigments oxidized at 0.5, 1, 2, 4, and 10 sec after the oxygen pulse in normal mitochondria and those treated with rotenone are shown respectively in the plots of Fig. 2. The extinction due to NAD is virtually zero for the range 305–374 m $\mu$  and therefore the standard absorbance spectrum is equivalent to the absorbance difference spectrum for the transition from NAD to

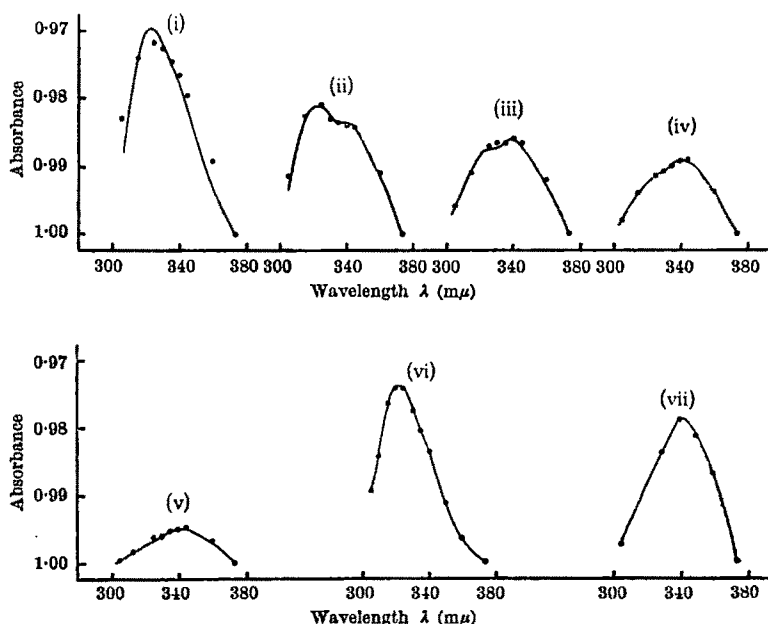


Fig. 2. Absorbance difference spectra ( $A_{\lambda-374} \text{ m}\mu$ ) obtained from traces corresponding to the samples shown in Fig. 1. Plots (i)–(v) show the spectra for normal mitochondria at time 0.5, 1.0, 2.0, 4.0 and 10.0 sec, respectively, after the oxygen pulse. The curves drawn through the points are composed of the curves of plots (vi) and (vii) as explained in the text. Plot (vi) is the spectrum obtained over the time interval 0.5–30 sec for mitochondria inhibited with rotenone. Plot (vii) is minus the standard absorbance spectrum ( $A_{374-\lambda} \text{ m}\mu$ ) of NADH obtained by adding 37.5  $\mu\text{mole}$  NADH to steady state anaerobic mitochondrial suspensions (32.0 mg mitochondrial protein) for various values of  $\lambda$  under the conditions of the experiments of Fig. 1a.

NADH. A comparison of the curves of plots (vi) and (vii) shows that the pigment oxidized in the mitochondria inhibited with rotenone has a difference absorbance spectrum (with a maximum near 320 m $\mu$ ) that is easily distinguished from that of the transition from NADH to NAD. In the normal mitochondria, however, it is evident that the difference absorbance spectrum at 0.5 sec closely resembles that of the mitochondria inhibited with rotenone, but that during the following period this gives place to a spectrum approximating more and more closely to that of the transition from NADH to NAD. In order to analyse

the plots (i)–(v) we have drawn through the experimental points curves which are composed of the curves shown in plots (vi) and (vii). The composite curves have been drawn by scaling the absorbance values for the transition from NADH to NAD (plot (vii)) and the “320 m $\mu$  pigment” (plot (vi)) so that their sum gives what we have judged to be the best fit on the experimental points. The proportions of the two absorbance spectra which contribute to a given composite curve are described in terms of the percentage of the total absorbance at 340 m $\mu$ , so that the percentage absorbance arising from the NADH to NAD transition can be estimated in each case. The percentages of absorbance at 340 m $\mu$  which corresponds to the NADH to NAD transition in the curves of plots (i) to (v) are respectively as follows: 25, 47, 75, 88, 100, corresponding to the state at 0.5, 1, 2, 4 and 10 sec after the oxygen pulse. It will be noted that the fit of the curves on the points is good except in the case of the 0.5 sec plot. This may have been affected by a stirring artefact in our system.

The data of Fig. 2 can be used to obtain the approximate time-course of the absorbance at 340 m $\mu$  corresponding to the “320 m $\mu$  pigment” and as a result of the transition from NADH to NAD as shown in Fig. 3, in

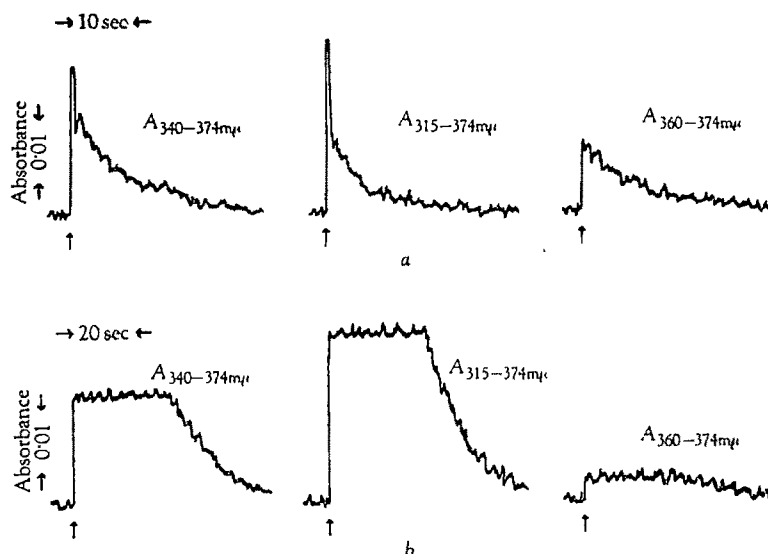


Fig. 1. Time-course of absorbance differences ( $A_{\lambda-374} \text{ m}\mu$ ) observed with an Aminco double beam spectrophotometer (cell light-path 1 cm). The mitochondria (32.0 mg protein) were suspended in a stirred anaerobic medium (18 ml.) containing 150 mmolar potassium chloride, 3.3 mmolar glycyl-glycine and 2 mmolar  $\beta$ -hydroxybutyrate at 20° C, with (b) or without (a) the addition of 25  $\mu\text{g}$  rotenone/g mitochondrial protein. At the time marked by the arrow, 23.5 mmg atom of oxygen was injected as air-saturated 150 mmolar potassium chloride, and the optical absorbance, shown in the figure, the pH and the oxygen tension were monitored simultaneously. The wavelength pair for which the absorbance difference was measured is indicated on each trace, and an upward deflexion denotes a decrease of absorbance.

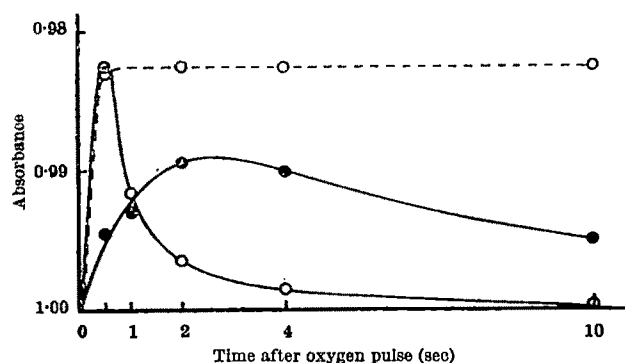


Fig. 3. Approximate time-course of oxidation and reduction of the "320  $m\mu$  pigment" (○) and of the NADH (●) in normal (—) and rotenone-treated (---) mitochondria shown in terms of the partial absorbance changes due to either pigment at 340  $m\mu$ .

the normal mitochondria and those treated with rotenone. Using the standard absorbance of NADH described by Fig. 2 (vii), a decrease of 0.01 on the absorbance scale of Fig. 3 can be shown to represent the oxidation of 0.55  $\mu$ mole NADH/g mitochondrial protein. In the mitochondria treated with rotenone the "320  $m\mu$  pigment" rapidly achieves a steady state of oxidation, and there is no change in absorbance corresponding to the transition from NADH to NAD. In the normal mitochondria, the peak of oxidation of the "320  $m\mu$  pigment" occurs about 0.5 sec after the oxygen pulse, and it decays rapidly; whereas, the NADH oxidation commences comparatively slowly, is maximal between 2 and 4 sec, and returns to about half the maximal value by 10 sec after the oxygen pulse. The amount of NADH oxidized at 2 sec in the normal mitochondria is 0.58  $\mu$ mole/g mitochondrial protein, and the curve appears to rise slightly above this value. The oxygen pulse in these experiments corresponded to 0.73  $\mu$ g atom of oxygen/g mitochondrial protein. Bearing in mind that the oxidation of NADH and its subsequent reduction do not occur as separate consecutive processes but overlap to an extent depending on the approach of the system to a steady state, the present results indicate a very satisfactory transfer of reducing equivalents through the respiratory chain from NADH (or  $\beta$ -hydroxybutyrate) to oxygen under the conditions of our routine experiments.

The time-course of the oxidation of NADH in Fig. 3 is in keeping with the fact that the liberation of protons in the outer (suspension medium) phase is virtually complete by 5 sec after the oxygen pulse under these conditions. The subsequent, slower reduction of the NAD by the  $\beta$ -hydroxybutyrate would not, according to the chemiosmotic hypothesis, influence the pH of the outer phase.

Slater *et al.*<sup>1</sup> have suggested that the apparent failure of the mitochondria to oxidize NADH under the conditions of their experiments is due to loss of various components during the preincubation period. We have no reason to suspect that any such functionally destructive changes occur during our experiments, even for anaerobic incubation times as long as 60 min.

It is, of course, difficult to copy experimental techniques in every detail, and it may be that, to some extent, the criticisms that Slater *et al.* have levelled at our work can be attributed to their failure to pay sufficient attention to certain technical points. For example, mitochondrial damage may result from insufficiently strict anaerobiosis during preincubation; and such damage might have impaired NADH oxidation in their experiments. On the other hand, it seems distinctly possible that NADH oxidation did occur in the experiments of Slater *et al.*<sup>1</sup>, but that they have so far failed to measure it.

The results described here provide strong support for our earlier interpretation that, using oxygen pulses of some 1  $\mu$ g atom oxygen/g mitochondrial protein, two of the six proton equivalents translocated during  $\beta$ -hydroxybutyrate oxidation by rat liver mitochondria are accounted for by a rotenone sensitive region (Site 1 or Loop 1) of the respiratory chain, associated with NADH oxidation, which is not utilized in the oxidation of succinate.

We thank Dr. D. E. Griffiths and the Science Research Council for the use of an Aminco double beam spectrophotometer.

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## Selecting Synchronous Populations of Mammalian Cells

by

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A method is described by which large synchronous cultures of mammalian cells can be obtained. Applied to HeLa cells, the method produces unperturbed populations of viable cells

SYNCHRONOUSLY growing populations of mammalian cells have been obtained by two general approaches. One involves the blocking and accumulation of cells in a particular stage of the division cycle, followed by release of this block<sup>1-12</sup>. Such a population has been termed, in the case of bacteria, a synchronized culture<sup>13,14</sup>. Low temperature

treatment<sup>1</sup>, serum starvation<sup>2</sup>, inhibition of DNA synthesis with ensuing accumulation of cells at the end of the pre-DNA synthesis ( $G_1$ ) phase by means of a variety of agents<sup>3-11</sup>, and temporary inhibition of mitosis by colchicine, with subsequent gathering of the inhibited cells<sup>12</sup>, have been used in this fashion. The normal physiology

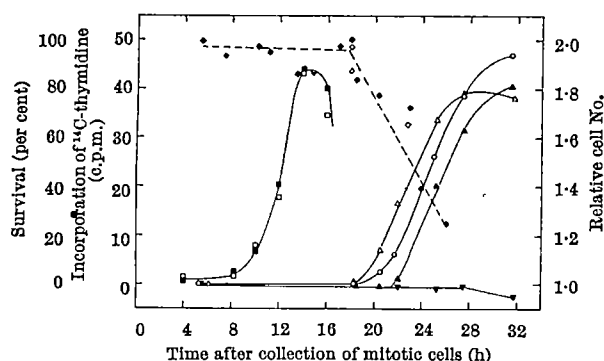


Fig. 1. Effect of 0.03 µg/ml. vinblastine on the progression (DNA synthesis and cell division) of synchronously growing HeLa cells. Zero h represents the time of collection of mitotic cells. Symbols represent: <sup>14</sup>C-thymidine incorporation into untreated control cells (○) and cells treated with vinblastine from 3 to 16 h (■); relative cell number (70 to 100 cells present at the first reading) for control cells (○), cells treated from 3 to 20.75 h (▲), and cells treated continuously from 3 h onward (▼); mitotic index in cells treated continuously from 3 h (Δ); and survival (four experiments) on treatment from 3 h until the time at which the point is plotted, for cells growing in N16FCF (◇) or N16HHF (◆).

of the cells, however, is affected to varying degrees during the process of synchronization by these methods (see refs. 3 and 4).

The second method of obtaining synchronously growing populations involves selection of a group of cells in or close to a particular stage of the division cycle, without altering their metabolic state. These populations have been termed synchronous<sup>13,14</sup>. For example, gentle shearing forces have been used to either collect<sup>15-17</sup> or remove<sup>18</sup> the more loosely attached mitotic cells from monolayer cultures, and high specific activity tritiated thymidine has been used selectively to kill cells synthesizing DNA so as to leave alive only a "window" of cells in the latter part of the *G*<sub>1</sub> phase of the division cycle<sup>19</sup>. These methods suffer from certain shortcomings: the mitotic cell collection method is limited by the relatively small fraction of cells that is in mitosis at any instant in a randomly dividing population; the mitotic removal method presumably requires frequent rinsing of the monolayer over a protracted period; and although the tritiated thymidine method can yield large populations, they are contaminated with dead or damaged cells, making most biochemical studies impossible<sup>19</sup>.

In this paper we present a method of obtaining large synchronous populations of mammalian cells that are free of dead cells. A population growing at random is exposed to the drug vinblastine sulphate<sup>20</sup>, a potent inhibitor of cellular proliferation that exerts its action through metaphase arrest<sup>21-23</sup>, at a concentration that completely blocks the passage of cells through mitosis. The drug is applied for a predetermined period of time, shorter than the doubling time of the culture. As a result, any desired fraction of the population can be accumulated in mitosis and subsequently washed away<sup>15</sup>, leaving behind cells in only the latter part of the division cycle. After a portion of the remaining cells is allowed to pass through mitosis in the absence of the drug, vinblastine is again added to arrest further division, the drug treatment being continued this time for a period less than that required for the first cells in the window to progress to the next mitosis. The vinblastine and cells accumulated after the second addition are then removed, the window of cells now being sharply defined on both the leading and trailing edges. It may be noted that the time at which the window is formed after the first addition of vinblastine may be selected at will.

Although it has been reported that vinblastine acts at the biochemical level as an inhibitor of the conversion of glutamic acid to both urea and citric acid cycle intermediates<sup>24</sup>, Bruchovsky *et al.*<sup>25</sup> have reported that in mouse *L* cell populations, only the mitotic cells are affected by the drug. Since the usefulness of the method depends

on this specificity for mitotic cells, experiments were first designed to confirm these results with HeLa S3 cells. This test with HeLa was considered important as the ever present possibility that a given agent will act differently on different cell lines has recently been confirmed; the agent hydroxyurea, which promptly kills Chinese hamster cells in the DNA-synthetic (*S*) phase of the division cycle<sup>10,26</sup>, is without toxic effect on HeLa cells in this phase<sup>27</sup>, even though it inhibits DNA synthesis equally well in the two cell lines.

HeLa S3 cells were maintained as monolayers in medium N16HHF by conventional methods<sup>28</sup>. Synchronous populations for studying the interphase effects of vinblastine were obtained by the mitotic cell selection procedure<sup>15</sup>, using modifications already discussed<sup>29</sup>. All experiments were carried out in medium N16FCF<sup>27</sup> unless otherwise stated. Small volumes (10–20 µl.) of <sup>14</sup>C-labelled thymidine (0.05 µc.; 33 mc./mmole), uridine (0.1 µc.; 230 mc./mmole), or leucine (0.2 µc.; 198 mc./mmole) were added to cultures at 37° C for pulse (10–30 min) labelling of DNA, RNA, or protein, respectively, in order to determine rates of macromolecular synthesis. At the end of the labelling period, the growth medium was withdrawn and the cells were prepared for measurement of incorporated radioactivity in either of two ways. (a) (Fig. 1, Table 1): The cultures were quickly rinsed twice with buffered saline and fixed with acetic acid-ethanol (1:3) for at least 30 min. Cultures labelled with <sup>14</sup>C-uridine were fixed in this way and then washed twice with 0.2 N perchloric acid for at least one hour per wash, all at 4° C. Finally, the culture dishes were rinsed with 70 per cent ethanol and air dried, and the bottoms were punched out for counting in a low-background gas-flow counter. (b) (Fig. 2): The cultures were rinsed twice with buffered saline at 37° C, and treated for 4 min with 0.03 per cent trypsin D1<sup>28</sup>. The trypsinized cells were washed into a tube with cold buffered saline, and an equal volume of cold 20 per cent trichloroacetic acid was added to the tubes. Microscopic examination of stained plates revealed quantitative removal of the monolayer cultures by this trypsinization procedure. After at least one hour at 4° C the cells were collected on 'Millipore' HA filters on which they were washed twice with cold 10 per cent trichloroacetic acid, twice with cold 70 per cent ethanol, and twice with cold diethyl ether, and then air dried for counting as in (a). Cell division was monitored by repeated microscopic observations of delineated fields<sup>30</sup>; only cells that had progressed past telophase were considered divided. Cell survival was determined by scoring colonies containing 50 or more cells after 10–14 days of incubation. Vinblastine sulphate was dissolved in double distilled water and stored at –20° C

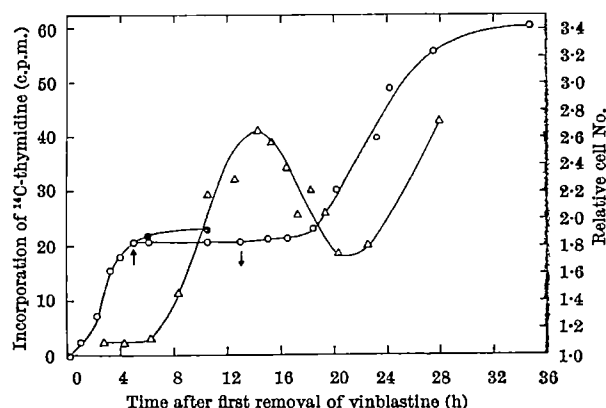


Fig. 2. Cell division and DNA synthesis in a synchronous population of HeLa cells selected by treatment with vinblastine. Zero time represents the first removal of the drug. The arrows indicate the readdition (↑) and subsequent removal (↓) of vinblastine. Open circles: relative cell number (124 cells present at the first reading) for cells treated from –17 to 0 h, and from 5 to 18 h; closed circles: total relative cell number, including cells arrested in mitosis after the readdition of vinblastine (see text); triangles: incorporation of <sup>14</sup>C-thymidine (30-min pulses).

until just before use. A calculated volume of 100-fold concentrated stock solution was added to a culture to obtain the concentration desired. To remove the drug, the medium containing vinblastine was withdrawn, the culture was rinsed once with fresh medium, and fresh, pre-conditioned medium was replaced.

Table 1. RATE OF PROTEIN AND RNA SYNTHESIS IN SYNCHRONOUS VINBLASTINE-TREATED AND CONTROL HELA CULTURES

Precursor	Time after addition of vinblastine (h)	Rate of incorporation (counts/min)	
		Control	Treated
<sup>14</sup> C-leucine	2	18	20
	10	23	26
	1	95	94
<sup>14</sup> C-uridine	3	104	100
	5	101	99

Vinblastine (0.03  $\mu$ g/ml.) was added to synchronous cultures 3.75 h after collection of mitotic cells. The rates of incorporation of radioactively labelled precursors into protein and RNA are expressed as c.p.m. incorporated per culture during a 20 (leucine) or 10 (uridine) min period of incubation. Each incorporation value represents the average of two or three plates.

Preliminary experiments indicated that vinblastine concentrations of 0.03  $\mu$ g/ml. completely blocked cells from passing through metaphase, while concentrations greater than 0.05  $\mu$ g/ml. caused crenellation of the cell membrane along with some loss of viability in cells exposed only during interphase. The lower concentration was therefore used in subsequent experiments.

Fig. 1 shows the effect of vinblastine treatment on DNA synthesis and on cell division. When added to synchronous cultures 3 h after mitosis, the kinetics of DNA synthesis is unaffected; the passage of cells into and through this phase is seen to be the same in the presence (closed squares) as in the absence (open squares) of the drug. When treatment was continued for 20.75 h, 10 per cent of the cells originally gathered had entered the next mitosis and had been arrested. These metaphase cells (as well as those in prophase and perhaps late  $G_2$ ) were only tenuously attached to the dish, and were easily removed from the culture, along with the vinblastine, by washing. After removing the drug, there was a lag of about 1.5 h in the treated culture before the number of cells (closed triangles) began to increase at the maximum rate. This was the time necessary for the first cells to progress through mitosis to cytokinesis. Thereafter, cell number increased in parallel with the control (open circles), levelling off when about 80 per cent of the cells had divided (compared with about 90 per cent division in the control culture). The effect of still longer treatment with vinblastine is also shown in Fig. 1: the mitotic index curve (open triangles) is seen to be parallel to, but 1.5 h ahead of the control cell number curve until about 27 h, while the number of cells (inverted triangles) remained constant. Thereafter, both cell number and mitotic index decreased as the arrested cells became so loosely attached that they spontaneously detached from the dish. It may be concluded that in the presence of concentrations of vinblastine that produce complete mitotic arrest, cells progress through interphase at the normal rate.

The ability of cells to form colonies after being exposed to vinblastine was also tested. The drug was added to cells 3 h after collection, and removed at various times thereafter; the cells were then incubated to allow formation of colonies. Viability (Fig. 1, diamonds) of cells exposed to vinblastine during interphase (3–18 h) is not decreased appreciably, if at all. The number of colony-formers is reduced only when cells are allowed to accumulate in mitosis before the drug is removed together with the accumulated cells.

Finally, the effect of vinblastine on the rates of protein and RNA synthesis in interphase cells was measured at various times after addition of the drug. The data in Table 1 show that at the drug concentration used here, these rates are not affected. A report<sup>21</sup> indicating that vinblastine inhibits RNA and protein synthesis in ascites tumour

cells *in vitro* involves drug concentrations greater by a factor of  $10^3$  to  $10^4$ . The relevance of those findings to the present experiments is therefore obscure.

These experiments with HeLa cells confirm the limitation of vinblastine action to the arrest of cellular progress at mitosis<sup>26</sup>. They show, in addition, that vinblastine does not affect the interphase rates of synthesis of protein or RNA. Because of uncertainty in their data, Bruckovsky *et al.*<sup>25</sup> cautiously stated that inhibition occurred "at or near" mitosis. The present work cannot eliminate this uncertainty as the resolution of the synchronous system is limited. This uncertainty in the precise point at which inhibition occurs does not, however, affect the usefulness of vinblastine for selecting synchronous populations.

Fig. 2 illustrates the application of vinblastine-induced inhibition of mitosis to obtain synchronous populations of HeLa cells. Randomly dividing populations growing in Petri dishes were treated with 0.03  $\mu$ g/ml. vinblastine for 17 h. The cells accumulated in mitosis were then washed away, and after rinsing the plate once with fresh medium to facilitate quantitative removal of the vinblastine and arrested cells, pre-conditioned medium was added to the plates. Incubation was continued for 5 h until most of the cells had divided, and then a second treatment with vinblastine was given in order to close the window. The drug was allowed to remain in the culture for 8 h this time, to trap the cells that only slowly progressed to mitosis. It was then removed, together with the laggards.

Fig. 2 shows that the first time the drug was removed the cell number increased sharply as the window of cells progressed through mitosis, 83 per cent of the original population dividing over the next 5 h (circles). The second treatment with vinblastine at this time caused cell division to cease. When the cells accumulated in mitosis after the second addition were counted as divisions and added to the cell number, it was found (closed circles) that cells continued to enter mitosis slowly over the next 8 h. A plateau was finally attained at a relative cell number of about 1.9. The cell number remained nearly constant for 18 h, when the second wave of division began. (The increase in relative cell number of 0.02 shown between 13 and 17 h, after removal of the second dose of the drug, is due to the initial division of a few very slow cells, not to the second division of rapidly progressing cells.)

The data in Fig. 2 serve to characterize further the synchronous population in several ways. First, the median doubling time, estimated from the time elapsed between the half-wave points of the cell number curve, was about 21 h. This is the doubling time of a randomly dividing population under these growth conditions. Second, DNA synthesis (triangles) underwent a 20-fold increase in rate subsequent to the very low level which obtained for 6 h immediately after removal of vinblastine. This increase, caused by cells entering  $S$ , is typical of the increase observed with mitotically synchronized cells<sup>14</sup>. The period from zero time to the onset of DNA synthesis at 6 h constitutes the minimum  $G_1$  time. Third, the degree of synchrony, calculated by the method of Engelberg<sup>32</sup>, was about 68 per cent for the first wave of division and approximately 39 per cent for the second wave. The decay in synchrony during this single cycle is in close agreement with that predicted by the theory of Engelberg (ref. 33, Table 4) which assumes that decay is due to random variation in generation time among the cells in the population. Analysis of time-lapse cinemicrographic data yields a standard deviation of generation times of 3.0 h (unpublished data).

This method of synchronization suffers from two principal limitations: it is restricted to monolayer cultures, and the degree of synchrony is less than can be obtained with a method in which the entire population is accumulated at essentially one point. Although the degree of synchrony can be improved by narrowing the window of cells, the size of the population is thereby



reduced, as it is directly proportional to the width of the window.

A potential third limitation, selection of the most slowly progressing cells, is easily overcome. The existence of such cells is shown by the slow attainment of a plateau in cell number after the vinblastine was removed (Fig. 2, closed circles). The last of these cells divided more than 30 h after the first addition of the drug (contrast with the mean doubling time of 21 h). In the method described here, nearly all of the slow cells are eliminated by a second application of the drug. Another solution would be to add high specific activity thymidine<sup>19</sup> at the time of removal of vinblastine, so as to kill cells still synthesizing DNA. This approach has been used successfully to purify a pre-mitotic ( $G_2$ ) population obtained originally from the mitotic cell collection method<sup>24,25</sup>. In general, however, secondary addition of vinblastine is to be favoured because the cells in question are thereby easily removed. It must be noted, finally, that neither of these approaches for eliminating cells which progress slowly can remove cells which do not progress at all. In this strain, plating efficiency is high (90–100 per cent), and non-progressing cells are not an important factor. They could be numerous enough to interfere, however, if the method were used with a cell strain in which an appreciable fraction of the population was constantly losing the ability to divide, but not to metabolize.

The method of synchronization described here has two principal advantages over the methods previously described. First, large populations can easily be obtained, the size being determined by that of the original culture of randomly dividing cells (which can be as large as desired, for example, Blake bottles with confluent layers of cells<sup>17</sup>) and the width of the window, which can also be adjusted to any size which is convenient. Second, the synchronous cells are relatively unperturbed, and are uncontaminated by dead, damaged, or metabolically altered cells. The method therefore is suitable for biochemical studies.

Certain of the survival data in Fig. 2 are from experi-

ments performed by Dr. B. Djordjevic<sup>24</sup>. This work was supported by a research grant from the U.S. Public Health Service. Mr. Pfeiffer is a trainee supported by a U.S. Public Health Service training grant.

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## Preleukaemic Syndrome in Germfree AKR Mice

by  
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Some mice of the AKR strain reared under germfree conditions become thin and "hunched up", with rough wet fur, depilation around the anus, shrunken thymus and enlarged lymph nodes. The histological appearance of the thymus and lymphoid tissue is similar to that in the early stages of mouse leukaemia

OVER the past 5 years we have maintained and propagated six strains of laboratory mice through numerous generations under germfree conditions. Apart from the anatomical and physiological alterations usually found in germfree mice, the reproductive rate, litter size, weight gain with maturation, life expectancy and general appearance of the germfree mice are similar to those of the conventional animals from which they are derived. In all the germfree mice which were examined, the caeca were enlarged and thin-walled; and the reticuloendothelial systems were small and relatively dormant<sup>1-4</sup>. The lymph nodes and spleens were small; but occasional germinal zones were detected in them. The intermediate zones<sup>5</sup> were relatively free from cells, and contained primarily reticulum cells. Serum globulin levels were quantitatively less than half those of the conventional counterparts<sup>6,7</sup>. The size and morphology of the thymus glands in the maturing "germfree" animal resembled those of their conventional counterparts<sup>8</sup>. Virus-like

particles have been found in thymic tissues of representative mice of our six germfree strains; and lymphatic leukaemia was induced in mice of all strains by repeated whole body exposures to small doses of X-rays<sup>9-11</sup>. In addition to the leukaemia syndrome induced by X-rays, some germfree *C3H* and *Balb/c* mice developed spontaneous mammary adenocarcinoma, in which "B" type virus particles were observed<sup>12</sup>. Therefore while germfree mice can be considered free of fungi, bacteria, and pleuropneumonia-like organisms (PPLO)<sup>12,13</sup>, they are not free from virus. The designation of the mice as "germfree" is admittedly a misnomer but it is used here to indicate the conditions under which they live.

In contrast to the six strains of germfree mice referred to here, a strain of germfree AKR mice was derived in this laboratory from animals provided by Dr. John Trentin of Houston, Texas. Germfree AKR mice also carried leukaemia-like virus particles in the thymic cells; and significant numbers of them developed lymphatic leuk-



aemia spontaneously between 4 and 11 months of age<sup>14</sup>. The leukaemic lesions in germfree and in conventional *AKR* control mice were indistinguishable. A peculiar syndrome was noted among some of the germfree *AKR* mice which might have relevance to the leukaemic disease which most of them were destined to develop.

In addition to the structural and physiological alterations which were usually associated with germfree status, and the development of spontaneous lymphatic leukaemia, some of the germfree *AKR* mice developed an additional syndrome (referred to as "puny" disease) which has not yet been observed among the conventional *AKR* mice, or in other strains of germfree mice. At an average age of 118 days (range 48-374 days), fifty-five of the germfree *AKR* mice appeared "hunched-up" and thin; they had rough wet fur, and some depilated and irritated areas around the anus. Eventually, such animals died. Each mouse which showed this syndrome was removed from the germfree enclosure, killed by ether anaesthesia, and subjected to thorough autopsy examinations. The thymus glands were reduced in size, usually to the extent that they were difficult to detect. The lymph nodes were enlarged at least two to four times, but the spleen sizes were usually within normal limits. The lymph nodes of disease-free *AKR* mice were usually enlarged at least twice compared with other strains of germfree mice. No other grossly visible lesions were noted. Tissue specimens were fixed in Bouin solution embedded in paraffin, sectioned and stained with haematoxylin and eosin for microscopic examinations. Control tissues were prepared from germfree mice without symptoms and from *AKR* mice which had been exposed to the microbial flora of the clean animal room.

The most prominent histological alterations were noted in the thymus and in the lymph node tissues: the thymuses in thirty-nine of forty-seven mice examined were very much reduced in size. The thymic cortex was shrunken and depleted of small lymphoid cells (thymocytes) and mitotic figures were not apparent (Fig. 1). The medullary areas contained epithelioid cells, fibrocyte-type cells, and Hassell corpuscles.

In all the fifty-five sick mice, the lymph nodes were larger than those usually encountered in mice of other germfree strains. The nodes contained extensive aggregates of lymphoid cells in the regions of the primary follicles in which germinal or reaction zones were seldom observed. The intermediate zones were enlarged and fused with the medullary areas which contained markedly increased numbers of plasma-like cells: distinct cells with clear, slightly basophilic cytoplasm in which the nuclei were located eccentrically. In many of the lymph nodes the solid cords of plasma cells caused distension of the medullary structures (Figs. 2 and 3).

The spleens of disease-free germfree mice contained small foci of lymphoid cells which were surrounded by

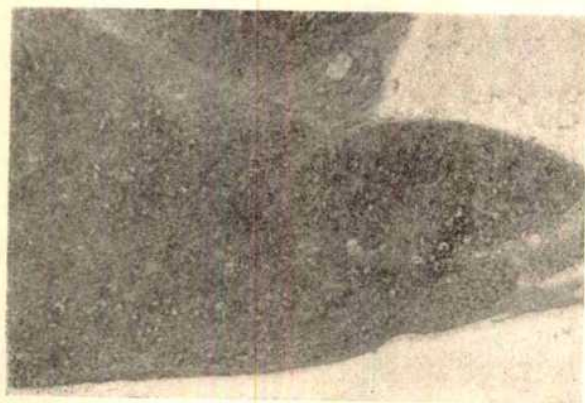


Fig. 1. Thymus gland from mouse with "puny" disease. The cortex is depleted of thymocytes. ( $\times 28.5$ .)

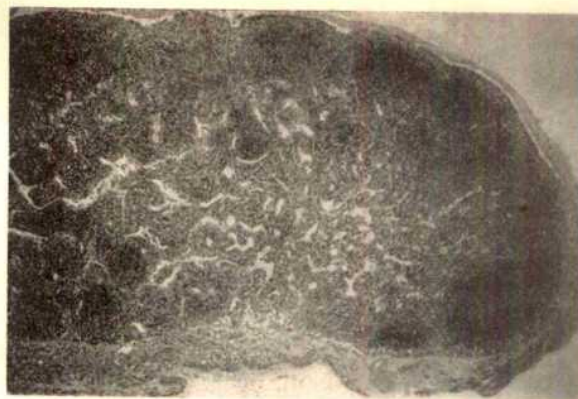


Fig. 2. Enlarged lymph node from mouse with "puny" disease. The intermediate and medullary zones are swollen with cells. ( $\times 18$ .)

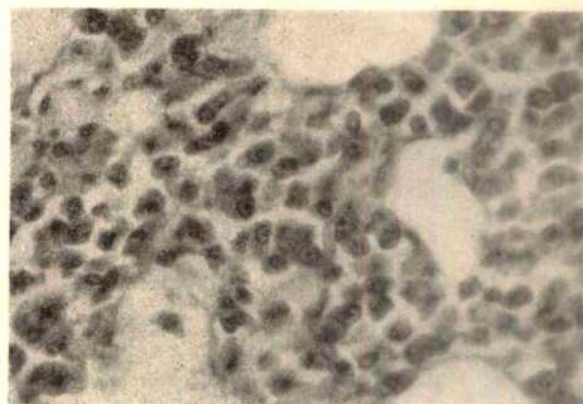


Fig. 3. Higher magnification of lymph node medulla of Fig. 2. Plasma-like cells distend this area. ( $\times 150$ .)

distinct perifollicular envelopes of reticulum-like cells, but the envelopes were thicker than those described by Krumbhaar<sup>15</sup> in the spleens of conventional rats. Germinal zones were rarely observed and the red pulp was clearly delineated from the white pulp. In the germfree "puny" *AKR* mice already described, the structure of the spleen was altered considerably. The lymphatic foci were enlarged. The white pulp was not clearly delineated from the red pulp, and the latter was infiltrated with large lymphoid, plasma and reticulum cells which appeared to have migrated from the perifollicular envelopes. The latter were no longer distinctly identifiable.

Haematocrit determinations were run on heparinized blood specimens which were collected in capillary tubes from the periorbital plexus. The red blood cell columns were within the normal limits of 30-40 per cent. The white blood cell counts of sick *AKR* animals were within normal limits; and microscopic examinations of stained blood smears from them revealed no abnormality.

The syndrome described here has not yet been observed in conventional *AKR* mice. Some of the "puny" germfree *AKR* mice were brought into contact with the laboratory flora, in order to determine whether they were suffering from a nutritional disease. Unfortunately, this was only done with sick mice showing severe symptoms of disease, otherwise we would have been uncertain of the original diagnosis. They all died.

The clinical appearance of the "puny" mice resembled that of runt disease; however, several significant conditions described in runt disease were not observed in the former. "Puny" mice did not show hepatomegaly with necrotic foci<sup>16,17</sup>. They did not show splenomegaly<sup>18</sup>, the lymph nodes were not depleted of cells<sup>18,19</sup>, and they were not anaemic<sup>17</sup>. Germfree *AKR* mice developed the "puny" syndrome at 2-3 months of age, whereas experi-



mentally induced "runting" usually appeared within 30 days of birth<sup>17</sup>.

The disease described here has been observed only in germfree *AKR* mice. We could not calculate the incidence of disease in the germfree colonies, as animals were withdrawn at frequent intervals for other experimental procedures. It occurred in all the five generations of *AKR* mice which have been propagated under germfree conditions. It is probably not attributable to dietary factors as six of the seven strains of germfree and conventional control mice in our laboratory have been on the same steam-sterilized diet (501° C) and 'San-i-cel' bedding for several years; and they were all maintained by the same procedures. Of all the seven germfree mouse strains examined, the *AKR* strain is the only one which has developed significant levels of lymphatic leukaemia spontaneously. One germfree Swiss-Webster mouse has developed spontaneous leukaemia during the past 5 years. The depletion of thymocytes observed in the "puny" *AKR* mice has been found in mice of all strains, after exposures to leukaemogenic doses of X-rays. The spontaneous thymic depletion may represent a stage in the development of leukaemia, which for some reason has not been clinically apparent in conventional *AKR* mice. The thymic tissues of four "puny" *AKR* mice were mostly shrunken and depleted of thymocytes; but the cortex contained some distinct hyperplastic areas of large lymphoblastic cells, with numerous mitotic figures among them. The latter areas resembled foci of leukaemic cells. In such animals the lymph nodes contained masses of plasma cells arranged as thick perivascular cuffs, and numerous well defined germinal zones. Siegler and Rich<sup>20</sup> described the early stages of leukaemia as atrophy of thymic tissues; and state that unilateral enlargement of the thymus is the initial lesion of leukaemia. Thymic atrophy was observed in "puny" mice, but at that stage of the disease unilateral enlargement of the thymus was not a remarkable occurrence. In germfree *C3H* mice, which had been inoculated with Gross virus at birth and which were thereafter examined at intervals, the leukaemic lesions developed in lymph nodes as irreversible hyperplasias of the germinal zones<sup>21</sup>,

and with increased numbers of plasma cells in the medullary areas. The plasma cells did not appear to be functional, as serum globulin levels remained low. Serum specimens from several germfree leukaemic *AKR* mice were examined by paper electrophoresis. The sera did not contain components in excess of those usually detected in sera from germfree mice. On the basis of accumulated observations leukaemia seems to be a manifestation of aberrant immunogenic function<sup>21,22</sup> and the syndrome described here in germfree *AKR* mice may be a reflexion of that condition. The depletion of thymic tissues and the hyperplasia of plasma cells in the lymph nodes may represent a prodromal stage of leukaemic disease. Histological evidence of transition from the depleted to the leukaemic thymus has been observed. We have no causative evidence for this spontaneous thymic depletion.

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## Mediators of Acute Inflammation in Leucocyte Lysosomes

by  
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It may be that a substance from polymorphonuclear leucocytes attacks mast cells and releases histamine into the area of inflammation

A CHIEF characteristic of inflammatory reactions is a sustained elevation in the permeability of small blood vessels which develops several hours after the initial increase in permeability has disappeared<sup>1-4</sup>. In certain forms of injury, this delayed phase is often accompanied by the appearance of emigrated polymorphonuclear (PMN) leucocytes in the tissues; nevertheless, the mechanisms of the permeability and cell responses are generally considered to be independent<sup>5-7</sup>.

We have already reported<sup>8-11</sup> the immediate increases in the permeability of small blood vessels in the rat mesentery, skin and cremaster muscle which appear after local injection or application of microgram quantities of an enzyme-free basic protein extracted from the lysosomes of exudate PMN leucocytes. In the rat the effect of this fraction on vascular permeability results, at least in part, from the fragmentation of tissue mast cells

by the PMN factor<sup>10</sup>. A basic protein isolated from eosinophilic leucocytes has recently been shown to exert similar effects<sup>12</sup>.

Although most previous work fails to support the suggestion that mast cell histamine or serotonin is responsible for the delayed change in permeability during inflammation<sup>13-15</sup>, or that a causal relationship exists between tissue leucocytosis and this phase of the reaction, the observed capacity of leucocytic cationic polypeptides to induce mast cell degranulation may have an *in vivo* counterpart in inflammation. It was therefore felt that the *in vitro* mastocytolytic action of this agent warranted further analysis.

We have compared the *in vitro* mastocytolytic action of lysosomal cationic polypeptides with that of compound 48/80, a known liberator of histamine, and with that of other naturally occurring and synthetic polycationic

polypeptides. In addition, we have compared the actions of these polycations on mast cells *in situ* with their effects on free mast cells.

PMN lysosomal polypeptide (LPP) was prepared as previously described<sup>9</sup> and stored in lyophilized form after dialysis against Ringer-Locke salt solution. Compound 48/80 (Lot No. NS 280) was obtained from the Burroughs-Wellcome Research Laboratory. Arginine-rich and lysine-rich preparations of calf thymus histones were purchased from Worthington Biochemical Corporation. Poly-L-lysine hydrobromide was obtained in two degrees of polymerization (molecular weights 2,600 and 230,000) and another sample was obtained with a molecular weight of 20,000.

*In vitro* assays of the effects of these substances on rat mast cells were carried out using morphological criteria of degranulation. Two types of mast cell preparation were used. Mesenteric mast cells were processed according to the method described by Norton<sup>16</sup>. Mesentery fragments were incubated for 30 min in Ringer-Locke glucose solution buffered with bicarbonate to pH 7.45 and containing known concentrations of test agents. Ringer-Locke alone was always included to provide a control test for spontaneous rupture of mast cells. Tissues were fixed in 10 per cent formalin containing 0.1 per cent toluidine blue, dehydrated in acetone, cleared in xylene, mounted in 'Permount' and examined under the microscope. Completely intact mast cells were counted as were those showing any degree of granule extrusion. In each experiment, the mean values of percentage disruption for each test agent were based on an examination of 1,500 cells. The alternative assay procedure involved incubating rat peritoneal mast cells with the test agents. In this case, free mast cells (along with other peritoneal leucocytes) were first deposited on 'Millipore' filter membranes with an average pore diameter of 5 $\mu$ , according to the technique of Lagunoff and Benditt<sup>17</sup>. The membranes were afterwards processed in exactly the same way as the mesentery fragments except that human serum albumin (Cohn Fraction V) was added to the Ringer-Locke in a concentration of 0.1 per cent to preserve the reactivity of the mast cells. In each experiment, the membranes were incubated in a solution containing 1  $\mu$ g/ml. of compound 48/80. This gave a further check on the responsiveness of the cells to degranulating agents. The second assay method, although less sensitive than the first, nevertheless has distinct advantages. First, all mast cells in any single experiment were obtained from one donor animal; and second, the test agents were free to act directly on the cells without any interference from components of the connective tissue ground substance.

Fig. 1 shows the log dose/response plots obtained when the percentages of mesenteric mast cells undergoing degranulation were determined for different concentrations of compound 48/80 and LPP. The results vary quite markedly over the middle range of doses but increase significantly at higher concentrations of the test agent. At intermediate doses, only a few granules are extruded and exact classification is difficult. At higher concentrations of degranulating agents, disruption of mast cells involves the extrusion of many granules and classification becomes easier. From such plots, the effective concentrations (or doses expressed as  $\mu$ g/ml.) required to produce degranulation in 50 per cent of the cells ( $ED_{50}$ ) can be calculated. The  $ED_{50}$  for compound 48/80 closely matches previously reported<sup>18</sup> values for this same lot (NS 280) and is about one-twelfth the  $ED_{50}$  of LPP on a simple weight/weight basis. Obviously, a more accurate comparison of the relative effectiveness of these two materials requires a pure preparation of the active PMN agent and more precise knowledge of its molecular size in order to make comparison with 48/80 on a molar weight basis. Preliminary information on the probable molecular weight of LPP has been obtained by gel-filtration chromatography. These experiments show that the mast cell active agent is completely retarded in 'Sephadex G-25'

and G-15 gels when chromatography is carried out in an acetate-water buffered solvent system at pH 4.0. Thus, our previous estimate of an approximate molecular weight of 10,000 for LPP<sup>10</sup>, based solely on the behaviour of this material during dialysis, was far too high. This aspect of the problem is discussed in greater detail elsewhere<sup>18</sup>.

Table 1 compares the degranulation of rat mesenteric mast cells produced by LPP with that produced by several other naturally occurring and synthetic polycations. It can be seen from the table that LPP is considerably more effective than calf thymus histones (both arginine-rich and lysine-rich preparations) as well as poly-L-lysine (especially polymers with molecular weights of 20,000 or more). Two possible explanations for this effect were considered in further detail.

One interpretation is that the relative effectiveness of these agents depends largely on their molecular weights and that in terms of molar concentrations the agents might prove more nearly equal in their effects. This interpretation is supported to some extent by Fig. 2, in which the reciprocals of the molecular weights and  $ED_{50}$  values of the agents tested are plotted logarithmically. The biologically active oligomer of compound 48/80 has a molecular weight of 600 (ref. 19). This is included in the comparison, but the histone preparations have been excluded because their molecular weights are not known exactly. We have assumed, on the basis of unpublished work, that the molecular weight of LPP is about 1,000. Fig. 2 shows that on the basis of these assumptions the logarithmic relationship between molecular weight and  $ED_{50}$  for these agents is, with one exception, reasonably linear.

An alternative explanation for the greater mastocytolytic activities of compound 48/80, LPP and the lower molecular weight poly-L-lysine could be based on the greater ability of these agents to penetrate the barrier of polyanionic substances (chondroitin sulphuric acid and

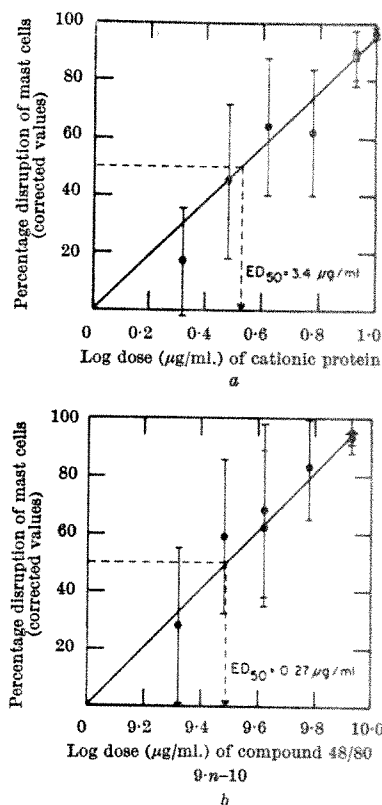


Fig. 1. Quantitative comparison of effects of LPP and 48/80 on rat mesenteric mast cells *in vitro*. Log dose/response plot for LPP (a) and compound 48/80 (b). Percentage degranulation at each concentration has been corrected for spontaneous cell breakage by subtracting the control value of corresponding experiment (Ringer-Locke value). Calculated  $ED_{50}$  values are shown in the figure.

Table 1. EFFECTS OF LPP, HISTONES, AND POLY-L-LYSINE ON RAT MESENTERIC MAST CELLS *in vitro*

Test agent	Percentage disruption of mast cells ( $\bar{X}$ )*															ED <sub>50</sub> value† ( $\mu$ g/ml.)	
	0	3	4	8	10	20	30	50	100	200	250	300	350	400	450		500
LPP	2	49	68	92	100												3-4
	4			94													
	4																
	4																
Arginine-rich histone	2				13		6	8	16								> 100
	2																
	3																
Lysine-rich histone	2				9		10	8	16								> 100
	2																
	2																
	3																
Poly-L-lysine (molecular weight, 2,600)	2		38	59	69	70											6
	2																
	2																
Poly-L-lysine (molecular weight, 20,000)	1				4	8		13	18	34		43		48		64	400-500
	2																
	2																
Poly-L-lysine (molecular weight, 230,000)	2				2	2		4	6		25	41	57	62	74		300-350
	2																
	2																
	2																

\* Percentage disruption is based on the number of mast cells showing any degree of granule extrusion ( $\times 640$ ). Each value represents 1,500 mast cells examined.

† Approximate effective dose disrupting 50 per cent of mast cells.

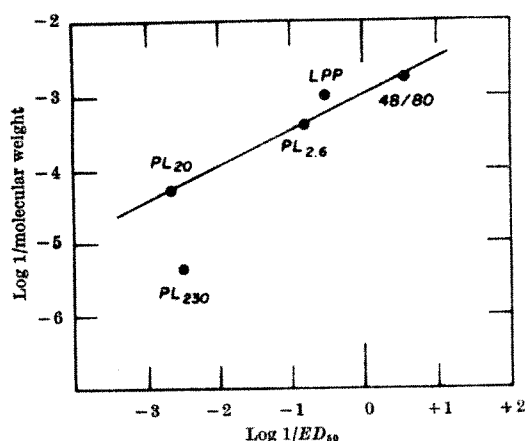


Fig. 2. Relationship between molecular weight of test agent and ED<sub>50</sub> value of the agent when tested on rat mesenteric mast cells *in vitro*. Individual agents are identified within the figure.

other acidic mucopolysaccharides) which surrounds the mesenteric mast cells *in situ*. According to this view, histones and larger molecular weight poly-L-lysines would be more readily trapped through the formation of charge-linked complexes with the acid polymers (as suggested by Dr. Ira Green) than would LPP or low molecular weight poly-L-lysine, while 48/80 would be least affected by this interaction. We carried out experiments to test this interpretation.

According to the latter hypothesis, calf thymus histones and high molecular weight poly-L-lysines ought to have effects more nearly equal to those of LPP when they are tested on mast cells which have been stripped of their surrounding connective tissue barriers. For this purpose, we used preparations of free peritoneal mast cells, washed and deposited on 'Millipore' filters. The data in part A of Fig. 3 show that such preparations did in fact respond to agents which had not affected mast cells *in situ*. Thus, poly-L-lysine (molecular weight 20,000), at a concentration which produced no degranulation of mesenteric mast cells (10  $\mu$ g/ml.), caused disruption of peritoneal mast cells equivalent to that produced by LPP at the same concentration. Arginine-rich histone (10  $\mu$ g/ml.) also exerted a moderate effect in this test system. Furthermore, as shown in part b of Fig. 3, the addition of 100  $\mu$ g/ml. soluble chondroitin sulphuric acid completely inhibited the mastocytolytic action of 10  $\mu$ g/ml. poly-L-lysine (molecular weight, 20,000); but, as expected, it did not significantly alter the effectiveness of LPP at the same concentration.

When chondroitin sulphuric acid was added to clear solutions of polycations, different degrees of turbidity

developed depending on the polycation used. Table 2 shows these results. Apart from polylysine of molecular weight 2,600, which also produced a significant turbidity, those polycations which had been effective degranulators of mesenteric mast cells, and which remained effective against free mast cells in the presence of chondroitin sulphuric acid, were not precipitated by the latter substance to the same degree as the high molecular weight polylysines. The concentration of polycation and chondroitin sulphuric acid was the same for all agents tested. To the extent that turbidity in this system reflects the formation of large, insoluble complexes between cationic and anionic polymers, these data support the interpretations put forward earlier.

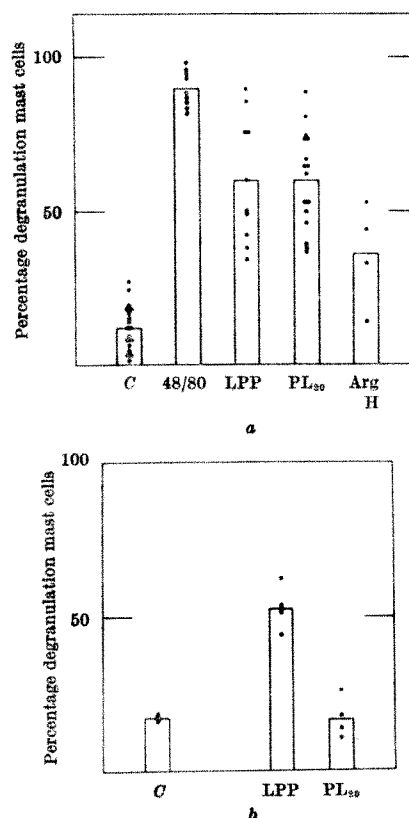


Fig. 3. Effects of 48/80 (1  $\mu$ g/ml.), LPP (10  $\mu$ g/ml.), poly-L-lysine (molecular weight 20,000) (10  $\mu$ g/ml.), and arginine-histone (10  $\mu$ g/ml.) on free rat peritoneal mast cells deposited on 'Millipore' membranes. Agents are identified in the figure. (C, Ringer-Locke control.) a, Agents tested alone; b, agents tested in the presence of chondroitin sulphate (100  $\mu$ g/ml.).

**Table 2. CHANGES IN OPTICAL DENSITY OF POLYCATION SOLUTIONS\* AFTER ADDITION OF CHONDROITIN SULPHATE† (O.D. OF ALL SOLUTIONS READ AT 520 mμ)**

Cation	48/80	LPP	Poly-L-lysine (molecular weight)		
			2,600	20,000	230,000
O.D. <sub>520</sub> ‡	0.003	0.022	0.186	0.194	0.263

\* 0.1 mg/ml. concentration.

† 1.0 mg/ml. concentration.

‡ O.D.<sub>520</sub> against water of cation or of chondroitin sulphate alone is zero.

These results lead us to suggest that the effectiveness of LPP against mast cells *in situ* may to some extent be due to its relatively low molecular weight (and therefore correspondingly high molar concentration at standard test doses). A further basis for its activity clearly lies in its failure to form insoluble or irreversible complexes with chondroitin sulphate and presumably other acid mucopolysaccharides of the connective tissue ground substance. Thus, LPP has the physical-chemical properties required for a high degree of biological activity as a mastocytolytic agent in connective tissue, in which inflammation actually proceeds. This component of exudative PMN leucocytes deserves further consideration as a potential endogenous liberator of histamine, although it remains to be shown that it is a natural mediator of inflammation.

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## Morphological Changes in the Adrenal Glands of Wild Rabbits

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The physiological condition of Australian wild rabbits, *Oryctolagus cuniculus*, is accompanied by changes in the adrenal glands which may, in particular, be helpful in studying "the pathology of overpopulation"

Christian<sup>1,2</sup>, Chitty<sup>3,4</sup> and Calhoun<sup>5,6</sup> have stimulated many ecologists to examine critically what Christian calls "the pathology of overpopulation". The activity of the adrenal glands is a prominent factor in this pathology, and as part of a comprehensive programme of research into the ecology of the wild rabbit, intensive observations are consequently being carried out on changes in the adrenal glands and other organs in relation to density, sex, age, season, social status, reproductive condition and health, in four natural populations of rabbits in widely different climatic regions, and in experimental populations confined within large enclosures and managed under conditions similar to those described earlier<sup>7</sup>.

Sampling will continue until 1966 when more than four thousand rabbits will have been examined. The analysis of the data will not be completed until 1967. In the light of modern interest in this field it seems appropriate to describe briefly the type of information that has been collected from a study of the adrenals taken from the first fifteen hundred animals.

**Seasonal Changes in Adrenal Weight.** There is a well marked and significant seasonal rhythm in adrenal weight and size, with a peak in the winter and a trough in the summer. The fluctuations occur in both sexes and in all regions and bear no direct relationship to age, breeding or behaviour. They may be of metabolic significance. The seasonal fluctuations in weight of rabbit adrenals are of the order of 30–50 per cent, and appear to be restricted to variations in the amount of cortical tissue.

The mean weights of adrenal glands from sub-tropical and sub-alpine areas are 30 per cent greater than those taken in hot, arid areas and warm, temperate regions.

**Histological Changes.** Changes in histology and size occur without relation to changes in adrenal weight. Histological changes accompany reproduction, stress, ill-health, mineral deficiencies and certain other as yet undefined stimuli.

The "resting" adrenal gland of healthy sub-adult rabbits consists of a narrow glomerular zone, usually 20–30 per cent of the cortex and devoid of lipid; a broad dense fasciculate zone with narrow radial strands two cells wide and containing very abundant lipid; and a narrow lipid-filled reticulate zone of packed clumps of cells arranged in alveolar manner about the medulla. Strands of reticulate zone cells often project into the medulla. The inner parts of the radial fasciculate strands merge imperceptibly into the clumped cells of the reticulate zone (Fig. 1).

Sexual maturity is marked by an almost total loss of lipid from the clumps of cells surrounding the medulla, and a partial loss from the inner third of the fasciculate zone, probably caused by the onset of sex hormone secretion.

**Reproduction.** With the start of the breeding season, the cells in the fasciculate strands abutting the reticulate zone hypertrophy and become clumped and indistinguishable from those in the reticulate zone proper, except for the higher lipid content of the latter.

In females, as the breeding season progresses, and apparently with successive pregnancies, the interface between reticulate and fasciculate zone moves progressively outwards, with the fasciculate cells clumping up in advance of the wave. Degeneration of the clumped cells follows and the lipid coalesces to form large extracellular masses of lipid or ceroid tissue occupying large vacuoles (fatty vacuolation<sup>8</sup>; spontaneous vacuolar degeneration<sup>9</sup>; possibly akin to ceroidogenesis<sup>10</sup>) (Fig. 2).

Masses of new cells now appear at each end of the medulla, apparently associated with connective tissue components, and the degenerating tissue appears to be obliterated by an active phase of regeneration which also moves outwards behind the active fasciculata-reticularis interface.

In young females (<25 months) large extracellular vacuoles rarely occur, for the lipid disappears quickly after



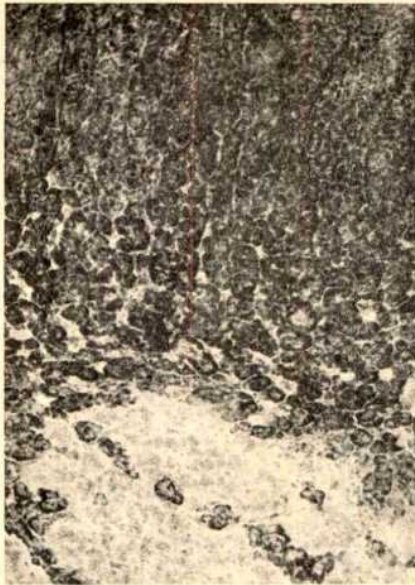


Fig. 1. Section of adrenal gland from a sub-adult male rabbit showing medulla, and reticulate and inner fasciculate zones. Lipid is present in abundance in both cortical zones ( $\times 100$ ).

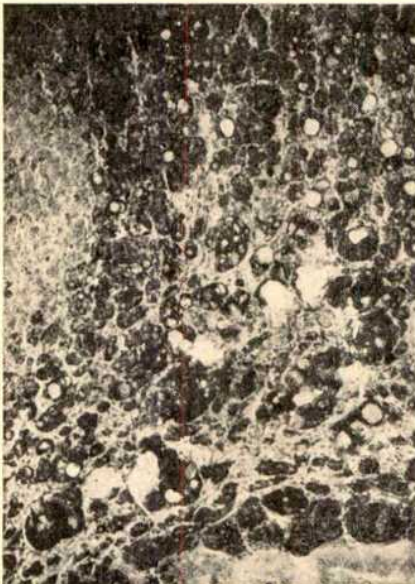


Fig. 2. Section of adrenal gland of adult female rabbit (26-33 months old) during breeding season, showing large masses of lipid surrounding the medulla (lower right) and large extracellular vacuoles containing orange-coloured lipids along the old fasciculate strands. The fasciculata-reticularis interface is at the top of the photograph ( $\times 100$ ).

formation. In older females, on the other hand, extracellular lipid is common and remains for longer periods of time. In very old females ( $> 33$  months) the vacuoles become very large and masses of lipid often surround the medulla, almost cutting it off from the cortex. It is also common in old females to find islands of old cortical tissue near the medulla which have been isolated by irregular patterns of regeneration, sometimes well within the fasciculate zone.

In males fatty vacuolation is less spectacular and the fasciculata-reticularis interface does not move far into the fasciculate zone. Moderately severe cases of fatty vacuolation are seen only in very old males.

The whole process appears to form a regular cycle of regeneration of adrenal tissue and is intimately connected with reproduction.

**Psychological Stress.** The rabbit is not easily stressed physically<sup>11</sup>. Adrenal weight decreases during starvation,

together with most other organs of the body. There is a marked adrenal response, however, to psychological stresses, occasioned by increased friction, which occurs as population density increases<sup>12</sup>.

Adrenal glands from rabbits in experimental populations of high density show a typical histological picture, with minor variations related to social status and health. The clumps of cells in the reticulate zone hypertrophy and become compact, and the clumping process moves outwards along each strand of the fasciculate zone, often reaching the fasciculata-glomerulosa interface, each strand taking on a ropy appearance (Fig. 3).

The glomerular zone becomes narrow, forming only 5 per cent of the cortex, and lipid accumulates along the capillaries between the glomerular whorls giving the interface a ragged appearance. In extreme cases lipid increases in the glomerular zone, the individual glomerulae



Fig. 3. Section of adrenal gland of adult male rabbit from a high density population showing fasciculate strands completely composed of clumped cells. Medulla is situated to lower left ( $\times 100$ ).



Fig. 4. Section of adrenal gland from adult female rabbit from sub-alpine region showing large glomerular zone occupying almost half the cortex, and commencement of nodular hyperplasia (see Fig. 7) at glomerulosa-fasciculata interface ( $\times 40$ ).



become clumped and the fasciculate zone breaks through to the capsule, apparently by reorganization of glomerular tissue. The glomerular zone is then left as festoons hanging from the capsule.

Unlike the changes described for reproduction the clumps of cells do not break down and extracellular vacuoles full of lipid are not formed. Reconstitution of the glomerular zone appears to occur by cell division from elements next to the capsule.

**Disease.** Adrenal glands from rabbits suffering from diseases of bacterial and viral origin (pasteurellosis, myxomatosis) show a lack of zonation, and narrow dense lipid-filled fasciculate strands fill the cortex from capsule to medulla. There is no clumping of cells.

**Mineral Deficiencies.** The glomerulosa-fasciculata interface is one of dynamic interplay between the two regions. The apparent ease with which the fasciculate zone replaces the glomerular tissue under conditions of stress and disease

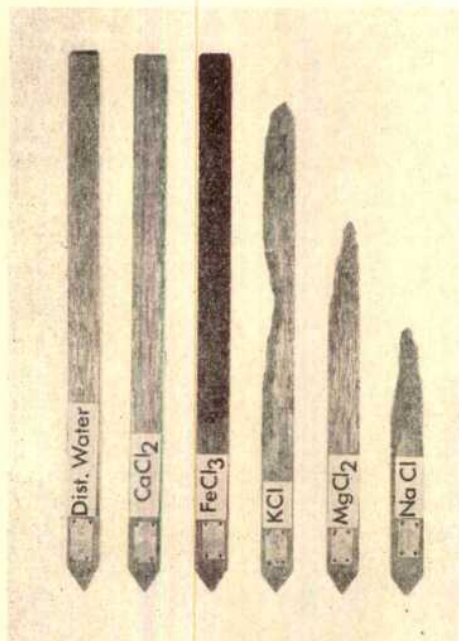


Fig. 5. Soft wooden pegs presented to wild rabbits in the sub-alpine region at a time when adrenal glands of sampled rabbits display a hypertrophied glomerular zone. Rabbits avidly attack pegs soaked in NaCl and MgCl<sub>2</sub> solutions, lightly attack pegs soaked in KCl and hardly touch pegs soaked in FeCl<sub>3</sub>, CaCl<sub>2</sub> and distilled H<sub>2</sub>O. Pegs are 0.75 in. square.

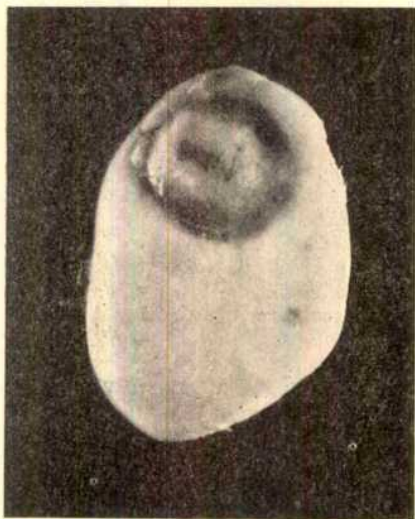


Fig. 6. Adrenal gland of adult male rabbit from sub-tropical region showing large cortical nodule on surface of gland ( $\times 8$ ).

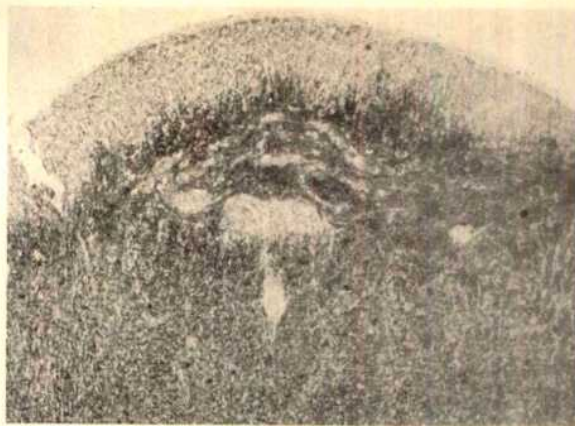


Fig. 7. Adrenal gland from adult male rabbit from sub-tropical region showing nodular hyperplasia at glomerulosa-fasciculata interface. Note normal narrow glomerular zone, almost devoid of lipid ( $\times 40$ ).

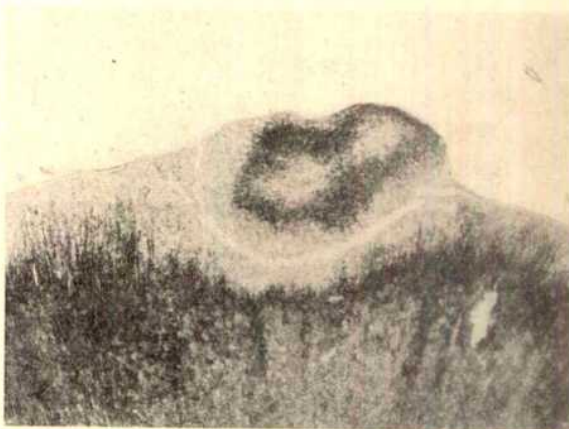


Fig. 8. Adrenal gland from adult male rabbit from sub-tropical region showing cortical nodule isolated from main adrenal by ingrowth of capsular tissue bordered each side by glomerular zone ( $\times 40$ ).

has already been mentioned. The glomerular zone also invades the fasciculate zone under certain conditions. This kind of activity is preceded by an unusual loss of lipid from the outer parts of the fasciculate zone and by an apparently heightened cell division along the interface, indicated by the appearance of numerous, small, darkly staining nuclei, and the presence of mitotic figures. In sub-alpine areas, adrenal glands in which the glomerular zone forms half the cortex are common at times (Fig. 4), and in all other regions studied—except the arid zone—dramatic changes in glomerular width occur. The main causes appear to be mineral deficiencies in pastures occasionally superimposed on the physiological drain of repeated lactations and possibly low temperatures<sup>13</sup>. The presentation at these times of soft wooden pegs permeated by different salt solutions leads to ravenous attack on those soaked in Na<sup>+</sup>, Mg<sup>++</sup> and K<sup>+</sup> salts (Fig. 5), particularly sodium chloride and sodium bicarbonate. Similar findings have been reported for ruminants<sup>14</sup>.

**Unknown Stimuli.** Another condition of equal interest is the formation of cortical nodules (Fig. 6). These have been found on adrenal glands of rabbits from all regions. They form at the interface of glomerular and fasciculate zones and are seen in sections as large hyperplasias forcing the two zones apart (Fig. 7). Further growth causes the adenoma to lift the surface of the adrenal, and capsular tissue grows inwards and beneath the nodule to cut it off from the adrenal body. Glomerular cells proliferate along each side of the invading capsular tissue and the accessory body, when isolated, consists of a small adrenal without medulla (Fig. 8). The accessory body may be thrown



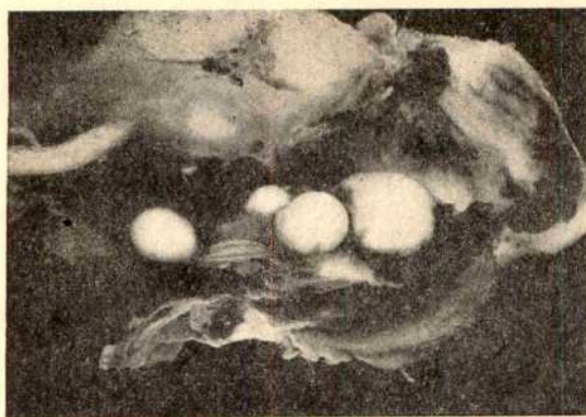


Fig. 9. Adrenal gland from adult male rabbit from sub-tropical region showing three large accessory adrenals lying free in the body cavity. Each accessory adrenal consists of fasciculate and glomerular tissue only ( $\times 5$ ).

off to lie free in the mesenteries of the body cavity. The accessory adrenal, the surface of which is covered by a finely anastomosing pattern of blood vessels, is clearly a secreting gland when formed. In some cases several bodies may be formed simultaneously (Fig. 9). The data being collected suggest that after the stimuli causing their formation have passed, the size of accessory bodies

rapidly decreases. Three mass occurrences of the formation of accessory cortical bodies have been observed. In each case they appear to have resulted from sudden and excessive stimulation of both the glomerular and fasciculate zones at the same time. In a recent experiment a significant enlargement of the zona glomerulosa was produced in rabbits maintained on a sodium poor diet. Incipient nodulation occurred in three of these rabbits challenged with ACTH.

The observations described above suggest that the adrenal gland is a sensitive indicator of several physiological conditions in the wild rabbit and is a valuable organ for the ecologist to use in population analysis.

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## Early Stimulation and Maternal Behaviour

by

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Stimulating young mice by various methods can accelerate their development. Some of the changes evidently result from the direct action of the stimulation on the young, but some seem to be due to changes in the behaviour of the mother toward her offspring

COLONIES of several strains of laboratory mice (*Mus musculus*) can breed indefinitely in a room kept at  $-3^{\circ}\text{C}$ . They require only a standard diet, blocks of ice (which they lick, for water), and bedding such as cotton wool. Both inbred and genetically heterogeneous mice maintained in this way have progressively improved in reproductive performance over a number of generations. The improvement has been especially evident in a substantial decline in the death rate between birth and weaning<sup>1</sup>. There is no way evident of accounting for this by a genetical change in the inbred strains<sup>2</sup>; maternal effects, which might contribute to it, are therefore being sought.

By a "maternal effect" we mean any non-genetical influence of a female on her offspring. The development of a mammal reared in a cold environment may be influenced by (i) the direct action of the cold; (ii) the effects of the cold on (a) the uterine environment provided by the mother, (b) her milk secretion, (c) her behaviour in the nest—for instance, tending the young and nest building<sup>3</sup>. The way in which these factors operate is influenced by the genotype of both mother and young<sup>4</sup>. This article describes an exploratory study of the effects of (i) exposure to cold in early life, and (ii) alteration in maternal behaviour, on the development of mice.

### Experiments on Maturation and Breeding

Mice of the highly inbred strain, *A2G/Tb*, were kept in permanently mated pairs in a room at  $21^{\circ}\text{C}$  (ref. 5). Second to fourth litters were used, after reduction to five at the age of 6 days. One mouse in each litter was left in the nest from the sixth to the eleventh day inclusive,

and received no ear mark (control). One mouse had a hole punched in one ear, but was left also in the nest. The remaining mice were ear-marked and exposed daily, for 90 min/day, from day six to day eleven, either at  $34^{\circ}\text{C}$  (warm-exposed) or  $21^{\circ}\text{C}$  (cold-exposed). At the beginning of each period of exposure, all mice from each litter were placed by hand in a plastic box. From this they were lifted back to the nest or to their exposure boxes. At the end of the period, all mice were again put in the box before they were finally returned to their nest. Hence all the mice were handled to the same extent.

Mice of all classes were weighed at 6, 11 and 21 days, and females also at 35 days. Mice from each class were each subjected to the following tests. (i) From day eight all mice were tested for the Preyer (startle) reflex by exposure to a short, loud sound (handclap at 10 cm), and the day of its appearance was recorded. (ii) The first day on which both eyes were open was also recorded. (iii) Females were examined each day after weaning at 3 weeks, and the day of vaginal opening was noted. (iv) At 22 days males were transferred to a room kept at  $-3^{\circ}\text{C}$ , where they were put separately in metal cages with cotton wool bedding, and ice cubes instead of water.

At 35 days, females were put each with an adult male which had not been used for experiment. The reproductive performance of these mated pairs was recorded to include all litters born within 112 days of the birth of the mother. Each litter was removed from its parents at the age of 21 days.

The observations during this first group of experiments concern (a) indices of maturation, (b) reproductive performance.



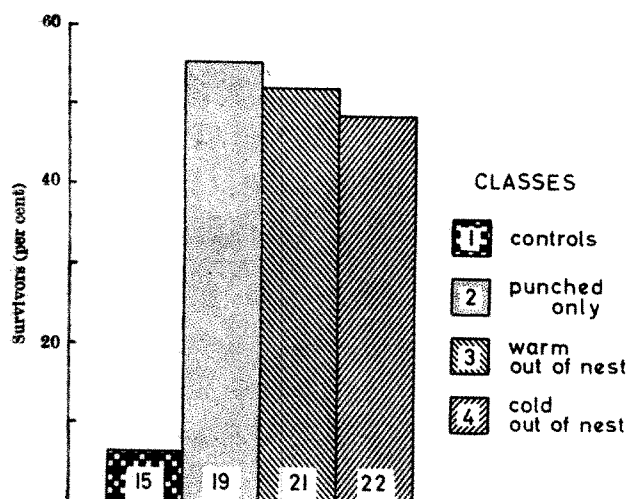


Fig. 1. Percentages of male mice which survived exposure, alone, with bedding, to  $-3^{\circ}\text{C}$ , for 7 days from the age of 22 days. Numbers of mice exposed are shown in the rectangles.

There was no evidence of an effect on body weight of any of the treatments. Table 1 and Fig. 1 summarize the results of other tests of development. The effects of exposure of young mice to cold are included here, because the chance of survival in the cold is a function of age<sup>6</sup>.

Exposure outside the nest, whether to a warm or to a cold environment, had no effect on any of these four developmental features. None of the slight differences between the experimental classes (ear-punched only, warm-exposed and cold-exposed, respectively) even approached statistical significance. There was, however, in each case, evidence of an effect of ear-punching. The appearance of the Preyer reflex, and the opening of the eyes and the vagina, were each earlier in all the experimental groups than in the controls (that is, the only group whose ears had not been punched). A more marked difference was in survival on exposure to cold at the age of 3 weeks (Fig. 1): there was no evidence of an effect of previous exposure outside the nest; but few of the controls survived, while about half the experimental mice did so. Hence maturation was not, in these experiments, influenced by exposure to cold in early life.

By contrast, reproductive performance was substantially affected. This was most clearly shown in the survival, between birth and weaning, of the young of the cold-exposed females (Tables 2 and 3). The proportion of litters wholly lost by cold-exposed females was lower than that of any other group. Of the weaned litters, the largest at weaning were those of the cold-exposed females.

### Maternal Behaviour

Mated pairs similar to those of the first group of experiments were used. When a litter was 6 days old it was reduced to even numbers of each sex. The young were then divided into four groups as follows: group A had no ear mark; group B had two marks in the left ear; group C had one mark in the right ear; group D had one mark in the left ear. All the mice in any one litter were either of groups A and B, or of groups C and D. After day 6, mice of groups A and B were left undisturbed in their cages until weaning at 21 days; those of group D were placed alone in a box kept at  $34^{\circ}\text{C}$ , for 90 min/day from

Table 1. INDICES OF DEVELOPMENT, FEMALES: MEAN AGES, DAYS, WITH STANDARD ERRORS

	No. of mice	Preyer reflex	Eyes open	Vagina open
Controls	15	$13.1 \pm 0.19$	$13.3 \pm 0.17$	$26.4 \pm 0.33$
Ear punched only	13	$12.5 \pm 0.25^*$	$12.6 \pm 0.22^*$	$25.5 \pm 0.38^*$
Out of nest: warm	18	$12.5 \pm 0.20^*$	$12.6 \pm 0.17^*$	$25.6 \pm 0.33^*$
Out of nest: cold	18	$12.4 \pm 0.23^*$	$12.7 \pm 0.24^*$	$25.4 \pm 0.45^*$

\* Significantly different from controls ( $P < 0.05$ ).

Table 2. LOSSES OF WHOLE LITTERS

	No. of mated pairs	No. of litters	No. lost	Percentage lost
Controls	10	25	6	24
Ear-punched only	11	25	5	20
Out of nest: warm	17	45	10	22
Out of nest: cold	16	40	6	15*

\* Significantly different from controls ( $P < 0.05$ ).

Table 3. NUMBER OF YOUNG IN EACH WEANED LITTER: MEANS WITH STANDARD ERRORS

	No. of litters	Young/litter
Controls	19	$5.0 \pm 0.49$
Ear-punched only	20	$5.3 \pm 0.50$
Out of nest: warm	35	$5.3 \pm 0.29$
Out of nest: cold	34	$6.0 \pm 0.33^*$

\* Significantly different from controls ( $P < 0.05$ ). (Numbers of mated pairs as in Table 2.)

Table 4. INCIDENCE OF PARENTAL CARE. MEANS WITH STANDARD ERRORS

Class	No. of observation periods	Care: sec/5 min
A, Controls	10	$4.8 \pm 0.62$
B, Ear-punched only	10	$14.0 \pm 1.94^*$
C, Handled	7	$8.9 \pm 2.21$
D, Out of nest	7	$20.3 \pm 5.92^{\dagger}$

\* Significantly different from controls ( $P < 0.001$ ) and from handled ( $P < 0.05$ ).

$\dagger$  Significantly different from handled ( $P < 0.05$ ).

day 6 to day 11; mice of group C were handled as much as those of group D, but were returned to the nest instead of being exposed.

Parental behaviour was observed for periods of 5 min. The amount of contact (licking, nuzzling, biting and carrying) received from either parent by the two classes of young was recorded. These observations were made on days 6–10, inclusive, from birth.

The most important results of the experiments on maternal behaviour are summarized in Table 4. A nestling with a hole punched in its ear, but not otherwise different from a control, was likely to receive about three times as much parental attention as a control. Handling by an experimenter had no significant effect. The greatest amount of parental attention was given to nestlings which had had their ears punched and had also been exposed outside the nest.

Our observations illustrate the multiple effects of early stimulation already described by other workers. These may be put into four classes.

(i) Earlier maturation has been described in a number of investigations. Early exposure of infant rats or mice to cold can accelerate the development of thermo-regulation<sup>7</sup>; handling in early life increases the rate of deposition of cholesterol in the brain<sup>8</sup>, promotes earlier eye opening<sup>9</sup> and results in earlier development of the adult pattern of loss of adrenal ascorbic acid in response to adverse conditions<sup>10</sup>. (ii) Superior resistance to various adverse conditions has been reported in adults which had been stimulated in infancy. The "stressors" used have included immobilization<sup>11</sup> and deprivation of food and water<sup>12</sup>. Handling, too, has increased the survival rate of adult rats on exposure to stressful conditions<sup>13</sup>. (iii) Diverse changes in adult behaviour have been believed to result from early stimulation: in particular, handled animals have been said to be less "emotional", and more exploratory, than controls<sup>14</sup>. (iv) Finally, our own experiments give an example of an effect of early exposure to cold on the reproductive efficiency of females.

Not all the results of experiments have been consistent. Cowley and Widdowson<sup>15</sup>, for instance, in careful experiments on rats, failed to find the increase in body weight which had been believed to result from handling. Our own observations on gain in weight resemble theirs. Similarly, McMichael<sup>16</sup> did not observe an expected increased resistance to unfavourable conditions in animals handled in infancy; nor did Tobach and Schneirla<sup>9</sup> find an effect on "emotionality". Other conflicting evidence on behaviour has been given by Levine<sup>14</sup>, Weininger<sup>11</sup> and Eells<sup>17</sup>.

Some of the apparent contradictions may be due to differences of method. The kinds of stimulation used

have included handling, exposure to cold, shaking and electric shock. The age at which the stimulation is given is important; a recent example is given by Bell<sup>18</sup>.

Despite these difficulties, attempts have been made to find a unitary explanation of the effects of early disturbance. One possibly fruitful notion is that handling, exposure outside the nest and other treatments all constitute mild "stressors"<sup>14</sup>; that is, they activate the pituitary and adrenal glands in such a way that, it is supposed, this system becomes more efficient, and confers greater resistance in later life. Some workers have emphasized the role of the central nervous system. Bovard<sup>19</sup> has suggested that the primary effect of early handling should be sought in the cerebral cortex; the cortex, he supposes, acts on the hypothalamus and hence on the pituitary and other endocrine organs. Another sort of hypothesis is that early disturbance induces a generalized arousal through the reticular formation<sup>20</sup>; and Lát<sup>21</sup> has emphasized the possible role of early stimulation in permanently altering nervous function in general.

Our own observations, reported here, do not make possible any decisive contribution to a unitary theory. They lead us to emphasize the need for close scrutiny of the details of the conditions of each experiment. In particular, when nestlings are being investigated, the exact behaviour of the parents toward them needs to be recorded. Young<sup>22</sup> has come to a similar conclusion on the basis of observations on rats, and Ressler<sup>23</sup> from a study of fostered mice. There is already one notable example of the influence of maternal care on the development of the nervous system. Reyniers and Ervin<sup>24</sup> found it necessary to stimulate the skin of neonatal rats reared without females, if the reflexes of elimination were to develop; this cutaneous stimulation is normally provided when the mother licks her young just after birth. Čapek and his colleagues have confirmed this observation on mice<sup>25</sup>.

We believe that the marked effects of ear-punching in our experiments were due, not to any direct arousal or

stress, but to stimulation of the mother by the abnormal state of the ear. Ear-punching itself is over in a few seconds; but the mothers persistently, during several days, gave their ear-clipped young more attention than they gave the controls. Mere handling of the young, in our experiments, did not have this effect to a significant extent, though it may have had some influence on maternal behaviour. In fact, the incidence of parental care, directly observed, paralleled the effects we recorded, in the first series of experiments, on maturation. Perhaps there is a general influence of maternal attention on the development of the nervous system.

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## Green Gully Burial

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Human bones and implements in the Keilor Terrace of the Maribyrnong River, near Melbourne, provide new evidence of the antiquity of man in Australia

IN August 1965, Mr. Donald Mahon uncovered some bones while digging through consolidated river terrace silts in the Maribyrnong River Valley, near Melbourne. He considered this to be of interest and importance, and left the bones in place in the near vertical quarry face. One of us (T. A. D.) immediately visited the site, and collected a fragment of a frontal bone which consisted of a portion of the orbital plates, supraorbital margins, glabella, superciliary eminences and a few centimetres of the frontal squame. It was said to have been broken off by the front-end loader, and it was positively identified as human. Some portions of the face, thigh and leg bones were not evident, having been dislodged and perhaps damaged by the bucket of the loader, but most other bones appeared to be represented and in approximately normal relationship.

The skeletal remains were 4 ft. below the surface of a river terrace (at approximate river level (R. L.) 58 ft. 6 in.) and 10 ft. above the present floodplain (R. L. 48 ft.). The terrace forms part of the Keilor terrace in which a human cranium was found in 1940. The new discovery is approximately 2 miles south of this site, and is on the right bank of the Maribyrnong River near the junction

with Green Gully tributary (lat. 37° 44' south, long. 44° 50' east) and 1 mile south of the township of Keilor (Fig. 1).

Measures were immediately taken to safeguard the site, and, to ensure recognition of the authenticity of the discovery, scientists from Melbourne and Canberra were invited to inspect the bones in position before any excavations commenced. All agreed that the remains were overlain by undisturbed terrace deposits and accepted their authenticity.

The Director of the National Museum of Victoria initiated the recovery of the remains, to establish their stratigraphical and environmental context and to assess the archaeological significance of the site. We were invited to take responsibility for these aspects of the investigation, and other specialists were invited to contribute to the investigation of particular problems. A Keilor Project Committee was set up by the Trustees of the National Museum of Victoria to co-ordinate work in the area for which financial support has been provided by the Ian Potter Foundation and the Australian and New Zealand Bank.

Immediate excavation of the site of the remains confirmed that the upper 3 ft. of terrace sediment was un-

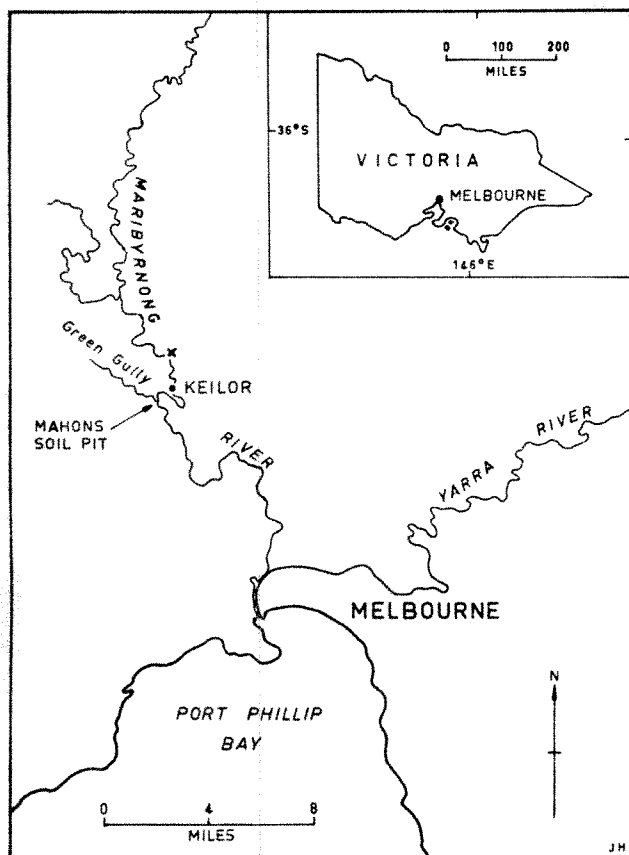


Fig. 1. Diagram showing the location of Mahon's soil pit near the junction of Green Gully and the Maribyrnong River near Keilor. The site of the cranium found in 1940, in the same terrace as the present skeleton, is marked by a cross.

disturbed, but a zone of soil disturbance immediately around and above the bones indicated that they had been buried in a shallow grave, about 12 in. deep. The appearance of the bones suggested a skeleton deposited on its left side with the legs semi-flexed at the hips and knees. Later work suggested that the bones could have been desiccated at the time of burial. The bones were fragile and fractured and no attempt was made to separate them *in situ*. As the earth was removed from above them they were painted with a strengthening solution of 'Bedacryl' in acetone and covered with several layers of tissue paper pasted on to protect them from the plaster of Paris used for reinforcement. The remains were removed in three blocks—the head, the trunk and limbs, and the feet. These three blocks were undercut, lifted and removed to the Museum. They were later transferred to the University of Sydney where a detailed investigation is being undertaken by Prof. N. W. G. Macintosh.

There are four terrace levels in this part of the valley, but only the two intermediate levels are present in the soil pit. The oldest (highest) and the youngest (lowest) of the four are not represented in the pit and are therefore not discussed here. The environmental history of the valley immediately before and after the burial has been reconstructed from detailed stratigraphic data. In this way the human bones and the burial horizon are seen as part of a complex sequence of events, a tentative outline of which is given below (see Fig. 2).

Deposition of gravels with medium to fine sands took place at the base of the Keilor terrace (zone A). A pause in aggradation followed with the development of a surface (near R.L. 42 ft.) with signs of extensive burning probably caused by bush or grass fires. Further deposition to 44 ft. occurred, followed by a longer pause with the development of a weak soil profile now shown by remnant

prismatic cleavage. Aggradation continued with deposition of fine sands and silts to near 60 ft. in the Keilor terrace. A long period of non-deposition followed, with intensive organic activity and soil profile development. The buried soil below 60 ft. is characterized by abundant infilled and now fossilized worm tubes, a high degree of organization of carbonate into vertical concretions, and a well developed soil structure (strongly developed vertical cleavage with differentiated soil units breaking down to good crumb). A period of stream incision followed, and part of the terrace sediment was eroded to form a steep cliff which delineated the edge of the river channel which existed at that time.

Accumulation of eroded sediment and soil occurred along the base of the cliff and worm tubes and carbonate concretions from the upper soil were reworked, to form zone B. Many trees grew along the steep aggrading river bank and these were burnt at intervals leaving massive charcoal deposits in zones of pink oxidized sediment and soil. Some of this was redistributed to produce bands of pink ochreous pellets through the steeply dipping marginal deposit (zone B) along the edge of the channel. Organic activity, similar to that in the Keilor terrace proper (below 60 ft.), continued in this zone and some weak reorganization of carbonate occurred to produce vertical concretions in areas previously burned by fires.

The burial of the Green Gully human remains occurred near a zone of burnt earth in Keilor terrace sediment. The material used to cover the body shows evidence of its disturbance in the intermingling of burnt and unburnt pellets of earth in random association, and in the loss of soil structure when compared with similar soil immediately below and around the walls of the shallow grave in the undisturbed zone.

Aggradation recommenced in the area, and the result was the deposition of 10 ft. of medium sands on the lower terrace (zone D channel sands) simultaneously with 3 ft. of finer overbank clayey sands on top of the Keilor terrace forming zone E. A later phase of stream incision occurred, after which deposition on the terrace surfaces ceased and soil profiles developed to their present condition. There is no evidence of intensive organic activity or of significant carbonate accumulation in material deposited after the burial of the Green Gully remains.

The grave was located close to the terrace contact and close to the steep slope which, at the time of the burial, formed the edge of the river channel. Evidence of the precise position of this slope was destroyed by the bulldozer, but reconstruction from the preserved stratigraphic detail shows that the grave was only a few feet west of the slope. There was charcoal and pink oxidized earth around and below the burial, burnt remains of trees which had grown along this slope. Soft pink oxidized earth had been partially used to infill the grave.

Radiocarbon dates have been obtained from two samples of charcoal collected from near the bones. These samples were from 3 ft. 6 in. below the body in the zone of charcoal associated with the burnt tree root, and from 1 ft. above the body, from charcoal redistributed along a horizontal bedding surface overlying the burial. Both samples were dated in the Institute of Applied Science at Melbourne and gave dates of 8,155 ( $\pm 130$ ) B.P. in both cases. (A thorough check of laboratory procedure has failed to provide an explanation for this remarkable coincidence of dates other than pure chance. Samples dated were collected by different workers on different days, to eliminate the possibility of mixing or incorrect labelling. The dates reported are the averages of three runs on each sample after rounding to the nearest 5 yr.)

The dates, consistent with the environment reconstructed above, represent the ages of charcoal from trees which grew on the same steeply sloping ground surface: the lower sample is from an *in situ* root and the upper sample is from charcoal redeposited by overbank deposition but derived from trees growing along the edge



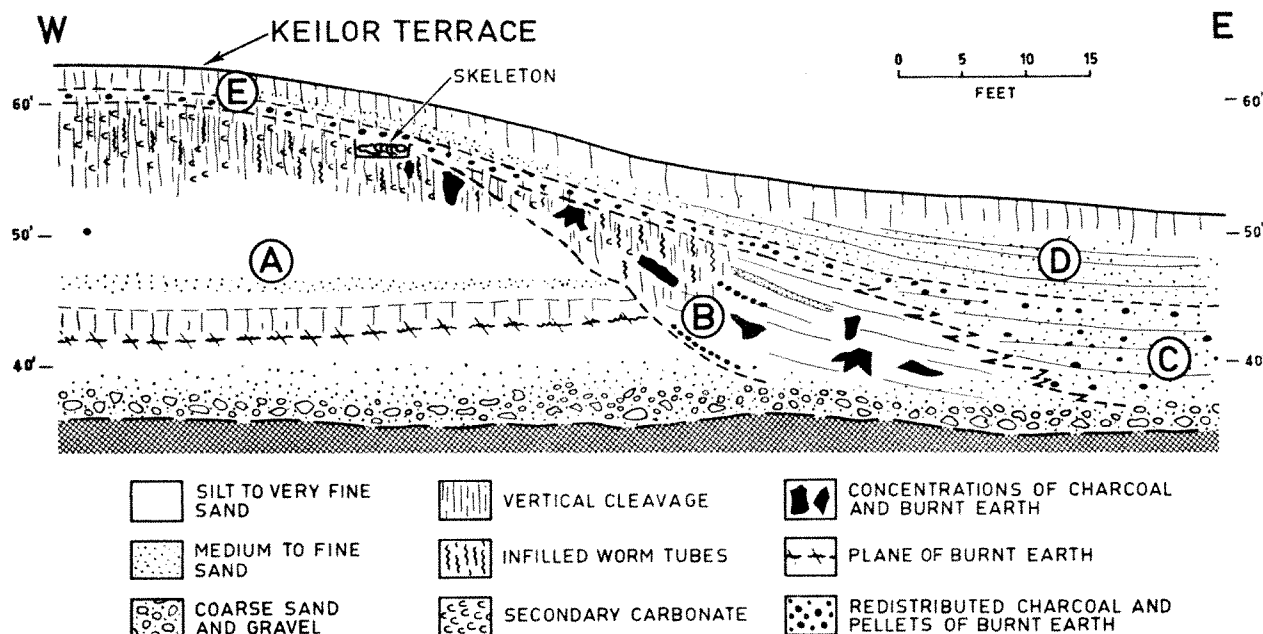


Fig. 2. West to east composite section through Mahon's soil pit showing the stratigraphic interpretation of the area and the relative position of the skeleton. For detailed description see text.

of the channel. The postulated sequence of depositional events suggests that the skeleton may be a little younger than the carbon dates, but the coincidence of dates is a clear indication of the approximate age of the ground surface on which the trees grew and on which the bones were buried. Additional samples from representative zones through this sequence have been submitted for determination. These results, together with comprehensive laboratory analyses of soil and sediments, will throw more light on the stratigraphic and environmental history of the site.

Irrespective of the significance of the skeletal remains, the site is of considerable archaeological interest. Evidence for human activities is stratified sporadically through a depth of 25 ft. of the Keilor terrace and throughout the younger terraces. Several archaeological excavations have been carried out in different areas of the pit since August, under our supervision, and another was directed by R. V. S. Wright of the University of Sydney. The area immediately surrounding the grave has been trenched on three sides, the deepest trench reaching 14 ft. below the bones. Approximately 220 stone primary flakes and cores, mostly of quartzite, were recovered, over half of them coming from below the ground surface of the time of burial; in addition, ten flake artefacts were found bearing traces of secondary trimming and shaping. From other excavations nearby a large collection of stone flakes was recovered, particularly at depths from R.L. 61 ft. to R.L. 57 ft. The deepest sounding reached the base of the terrace, 27 ft. below the bones. The oldest artefacts consist of two quartzite flakes with distinct bulbs of percussion, at R.L. 44 ft. 6 in. and a possible quartzite core at R.L. 40 ft. 6 in. The lowest trimmed implement was found, however, at R.L. 55 ft. 6 in. It is important to observe that none of these artefacts was derived from elsewhere—flakes and cores have sharp edges.

Several arrangements of basalt pebbles, possibly hearths, were uncovered. Some contained faunal remains, although the total sample of animal bones from the excavations is meagre. The lowest presumed hearth was only 5 ft. from the grave, on an earlier occupation surface, just below R.L. 57 ft.

The trimmed artefacts found stratigraphically below the burial in all areas with the exception of Wright's trench include some which are merely retouched in nondescript fashion, but the majority are classifiable

under the genus "scraper". Varieties represented include side, end, concave and discoidal; no other implement types are represented. In the overlying deposit, the top-most 2 ft. contain small blades which are characteristic of a microlithic industry, although no actual backed blades were found at the locus of the bones. Elsewhere in the pit, however, microlithic blade cores, a geometric trapezoid microlith and broken blades with steep blunting retouch were located *in situ* in this upper deposit, together with three unifacially trimmed pebbles. These latter are fine specimens of a type well known in Australia, reminiscent of the Hoabhinian pebble tools of south-east Asia.

Wright's excavation also uncovered several scrapers, and, between R.L. depths 60 ft. and 57 ft., he also found delicately trimmed "thumbnail" scrapers and implements known in Australia as flake fabricators, many of which were made from white quartz. Current age expectations for these tools, based on evidence from New South Wales, would predicate a more recent date.

Archaeological evidence, with the exception of the qualifications introduced by Wright's work, therefore suggests that at Keilor until less than 8,000 yr ago flake implements of the "scraper" type were preferred. This is consistent with evidence from other parts of Australia. Broadly comparable types apparently constituted the sole stone tool kit used at Kenniff Cave, Queensland, during a period extending from 5,000 yr ago in Pleistocene times. The later appearance of microlithic techniques and uniface pebble tools in the Keilor sequence is also in conformity with carbon-14 dates for those industries elsewhere. The earliest dated occurrence of both industries is in north-eastern New South Wales, where they belong to the fourth and fifth millennia B.C., respectively. The cultural sequence at Keilor may, indeed, serve as an independent chronological indicator to confirm the stratigraphic inferences. Further excavations are projected.

Despite their poor state of preservation, these fragmented human bones presumably constitute, by some 2,000 yr, the oldest dated post-cranial skeletal remains of *Homo sapiens* known in the Australasian region. The Green Gully discoveries offer a unique opportunity for scientific collaboration, to ascertain the environmental conditions and cultural status of human occupation in late Pleistocene and in early post-glacial times.

## An Early Miocene Member of Hominidae

by

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Hitherto, the earliest fossils recognized as belonging to the Hominidae, from Fort Ternan and the Siwaliks, have been dated to the Mio-Pliocene. Discoveries in early Miocene deposits at Songhor and Rusinga, Kenya, have made it possible to carry the date of the separation of the true Hominidae from the Pongidae back to the Lower—or possibly Early Middle—Miocene

IN 1934, G. Edward Lewis<sup>1</sup> described a new genus of fossil primate from the Siwalik deposits in India under the generic name of *Ramapithecus*, with *brevirostis* as the specific name of the genotype. He emphasized that *Ramapithecus* possessed some hominid characters but classified it, in his official diagnosis, as a member of the family Simiidae, cautiously adding "(Hominidae?)". In 1938, Gregory and Hellman, in a joint paper with Lewis<sup>2</sup>, remarked about this genus: "While the Siwalik genus *Ramapithecus* and the South African *Australopithecus* are still apes, by definition, they are almost on the human threshold in their known anatomical characters".

In 1961, Elwyn Simons<sup>3</sup> stated that he believed that *Ramapithecus* might be a member of the Hominidae, but he did not make a definite claim to this effect. He regarded its geological age to be "within the Nagri zone, which is of Pliocene—early Middle Siwalik—age".

In 1961, I published<sup>4</sup> a preliminary note on what I considered to be a new genus and species of primate from Fort Ternan, Kenya. I named this *Kenyapithecus wickeri* and provisionally gave the geological age as Early Pliocene (Pontian in the English sense). Because the fossil fauna from the same site and level has become better known, I have more recently referred it to the Upper Miocene<sup>5</sup>. The fauna of Fort Ternan is rather more primitive than the Early Pontian fauna from such sites as Pikermi, Samos, etc. In my paper of 1961, I noted similarities between *Kenyapithecus wickeri* and *Ramapithecus brevirostis*, and mentioned its resemblance to the Hominidae. I left the familial position open, however, and described it as "Family, *incertae sedis*".

During the past few years, most authorities have come to agree that *Kenyapithecus wickeri* and *Ramapithecus brevirostis* should both be regarded as early representatives of the Hominidae. It is also widely accepted that both belong, geologically, to the closing stages of the Miocene and/or to the very early Pliocene. Evernden and Curtis<sup>6</sup> have indicated a potassium-argon date for the Fort Ternan deposits, which yielded *Kenyapithecus wickeri*, of about 14 million years. A glass fission track date can be expected soon.

Simons and Pilbeam<sup>7</sup>, as well as Simons<sup>8</sup>, have recently advanced the opinion that *Kenyapithecus wickeri* should be regarded as a synonym of *Ramapithecus brevirostis*. At the same time they have suggested that Pilgrim's *Dryopithecus punjabicus* is also identical. Because the name of this species has priority, they have proposed that the Mio-Pliocene representatives of the Hominidae should be known as *Ramapithecus punjabicus* (Pilgrim). In their view (p. 136), moreover, *Dryopithecus fontani* (partim), *Bramapithecus thorpei* and *Bramapithecus sivalensis*, as well as Woo's far eastern *Dryopithecus keyuanensis*, should now, also, be regarded as synonyms of *Ramapithecus punjabicus* (Pilgrim). This seems to be a somewhat extreme example of taxonomic lumping. Although it is possible—but by no means certain—that all the Asiatic

representatives listed may belong to the single genus *Ramapithecus*, the generic distinctiveness of the East African *Kenyapithecus* will be demonstrated in this article.

In my view, the species *Kenyapithecus wickeri* differs from the Asiatic forms at the generic as well as the specific level.

It must also be noted that although the original type of Lewis's *Ramapithecus brevirostis* came from the Nagri zone, which is usually regarded as of early Pliocene age, Pilgrim's *Dryopithecus punjabicus* was listed as from the Chinji formation, which is usually regarded as Uppermost Miocene rather than Early Pliocene. On the evidence of the palaeontological data which are so far available, both are probably geologically slightly younger than *Kenyapithecus wickeri* of Kenya.

In 1951 Le Gros Clark and Leakey<sup>9</sup> described a small maxillary fragment of a primate from site R.106 on Rusinga Island, Kenya, tentatively placing it in the genus *Sivapithecus* with *africanus* as a specific name. It was shown to differ markedly from any of the material representing the various species of *Proconsul* from Kenya, while it seemed to share certain features in common with some of the specimens which were described as *Sivapithecus sivalensis*. It was also regarded as differing from the genus *Dryopithecus* of Europe and Asia as described in the literature. This was a view based only on the small maxilla fragment which formed the Type, which came from deposits usually regarded as of Lower Miocene age.

In 1961 I suggested that when the time came to classify the primate which I had named *Kenyapithecus wickeri* into a zoological family it would have to be joined in that same family by *Sivapithecus africanus*. This view was supported at one time by Simons, for he wrote in 1963: "*Sivapithecus* apparently crosses the Mio-Pliocene boundary but is not easily separated from *Ramapithecus*, a conclusion indicated by Leakey's report". In his most recent work with Pilbeam, however, Simons has withdrawn from this view. He now treats all known *Sivapithecus* material as falling within the genus *Dryopithecus* with the name "*Sivapithecus*" reduced to sub-generic rank. He even goes so far as to claim that Le Gros Clark and Leakey's *Sivapithecus africanus* is both generically and specifically "identical" with *Sivapithecus sivalensis* of India. I shall endeavour to show that this cannot be the case.

There is thus a growing consensus of opinion that *Ramapithecus* and *Kenyapithecus* both represent primitive members of the Hominidae; if this is correct and as they were already present in India and East Africa respectively, in Mio-Pliocene times we should expect to find still earlier ancestral members of the family Hominidae either in Middle or even in Lower Miocene deposits. This could be either in Africa or in Asia or in both continents, and the origin of the family Hominidae may even be found to extend back to the Oligocene.

In consequence of pondering this idea, I recently began a detailed re-examination and re-evaluation of the large collection of Lower Miocene primates from Rusinga, Songhor, Koru, etc., which is in Nairobi. This resulted in an examination of some specimens which had been collected since the last study and others which had been overlooked previously, as well as a review of certain others which might have been wrongly identified in earlier publications. The report which follows deals with the recognition, within this collection, of a number of specimens which seem to me to be unquestionably representatives of the family Hominidae. All but one are, moreover, on geological as well as faunal evidence, older than both *Ramapithecus brevirostis* and *Kenyapithecus wickeri*. The specimens to be discussed come from deposits which are usually regarded as of Lower Miocene age, and they include the type specimen of what was formerly called *Sivapithecus africanus*. There is also one specimen from Maboko Island which is regarded as representing *Kenyapithecus sp. indet.*

### The Genus *Kenyapithecus*

FAMILY Hominidae  
GENUS *Kenyapithecus* Leakey 1961

My original diagnosis of the genus *Kenyapithecus* was as follows: "A genus within the Superfamily HOMINOIDEAE, with low crowned molars and premolars; the upper canines are small and set vertically in their sockets. There is a well defined canine fossa and the root of the malar element of the malar-maxillary process is set just above the first molar".

For purposes of comparison with both my 1961 diagnosis of *Kenyapithecus* and also with the revised diagnosis of the genus, which will be given later, Lewis's generic diagnosis of *Ramapithecus* is included in full. Similarly Simons and Pilbeam's new diagnosis of *Ramapithecus* will also be quoted in full.

Lewis's original diagnosis of *Ramapithecus* read as follows:

"Simiidae (Hominidae?) in which the dentition parallels the hominid type in its broader aspects. The dental arcade of the upper jaw is parabolic rather than "U" shaped as in recent Simiidae, and hence the palate broadens posteriorly. The cheek teeth of opposite sides of the jaw are more widely separated posteriorly than anteriorly rather than approximately equidistant from  $M^2$  to  $P^3$ . The face is very slightly prognathous, as contrasted with recent Simiidae. There are no diastemata in the dental series. The canine is small, not an antero-posteriorly elongated trenchant tusk, but of hominid type with a transverse dimension exceeding the antero-posterior dimensions."

In place of this generic diagnosis, Simons and Pilbeam have recently suggested the following:

*Ramapithecus* "Differs from *Australopithecus* and members of the *Dryopithecus* group in the following general features:

Slightly smaller overall size (except for *Dryopithecus africanus*) [by which they mean *Proconsul africanus* of other authors], shallower mandible, less complex patterns of tooth crenulation, little or no evidence of cingula or Carabelli's cusps and shorter face. Incisors and canines reduced in relation to cheek-tooth size when compared to *Dryopithecus* but not as markedly as in *Australopithecus*; incisor procumbency intermediate. Differs from *Dryopithecus* and other apes in showing more widely spaced and much lower molar cusps, so that the central or occlusal fovea of the molars covers more of the crown surface of the tooth (even so, these features show some variability in *Ramapithecus* and *Dryopithecus* as well as in modern *Homo* and *Pan*); sides of the upper molars, particularly, are more vertical; also differs from *Dryopithecus* in showing a larger and lower canine fossa, an arched palate, arcuate tooth row and a much shorter rostrum."

Although this generic definition of *Ramapithecus* by Simons and Pilbeam is much lengthier than that of Lewis, it cannot be regarded as satisfactory for the following reasons:

(a) It makes size a generic character in that it states "slightly smaller overall size than *Dryopithecus* and *Australopithecus*", but immediately refers to an exception. Moreover, should a larger species of the genus be discovered, it would automatically be excluded by this diagnosis. It must, therefore, be emphasized that size is never really valid as a generic character.

(b) It states that the mandible is "shallower" than in *Australopithecus* and *Dryopithecus*. It does not say whether this is in relation to the overall size, or whether it is shallower only relative to the length of a tooth row. This feature again makes size, for example, "shallower", a generic character, which is taxonomically unsatisfactory.

(c) It goes on to say "shorter face", but because no complete face of *Dryopithecus* (other than in the *Proconsul* group) has been found and certainly no complete face from gnathion to nasion in *Ramapithecus*, this seems to be a most unwise diagnostic character to attribute to the genus, in the present state of our knowledge.

(d) The character "incisors and canines reduced in relation to cheek teeth when compared with *Dryopithecus*" may be valid for Asia but it is not true for specimens from East Africa which Simons and Pilbeam wish to include in *Ramapithecus*. While it may be true in respect of *Ramapithecus punjabicus* (*brevirostis*) (if indeed incisors of this genus and species are known in Asia, of which I am not aware) when compared with the more classical *Dryopithecus* species, it is certainly not true (see below) in respect of comparisons of the incisors as between *Kenyapithecus* and the *Proconsul* group, which Simons and Pilbeam insist on including in the genus. One thing is clear: neither the original East African species *Kenyapithecus wickeri* nor the new species of *Kenyapithecus*, which is to be described below, can be accommodated within the genus *Ramapithecus*, as it is now diagnosed by Simons and Pilbeam. *Kenyapithecus* must, therefore, on their own showing, be retained as a distinct and separate genus.

### Revised Diagnosis

The following new diagnosis for the genus *Kenyapithecus* is now proposed. It is the result of examining not only the original type as well as other more recently recovered additional Fort Ternan specimens, but also the specimens which are now to be placed in a second and geologically rather older species of *Kenyapithecus*, from Songhor and Rusinga.

Hominidae in which the dentition closely resembles that of *Homo* in the broader aspects, but not in the crown structure of the canines, nor that of the upper 3rd premolars. The upper canines have short crowns with compressed and relatively short roots, the crowns are not of the *Homo* type, but more primitive. The upper incisors are shovel-shaped, and closely resemble those of some members of the genus *Homo*. The dental arcade of the maxilla is arcuate, not U-shaped as in the Pongidae, while the alignment of the cheek teeth in the maxillae and also in the mandible diverges posteriorly. The face is only slightly prognathous. There is no diastema in the upper or lower dental series. The premolars and molars, including both upper and lower, either have no cingulum, or else a very reduced one. The lower molar cusps are widely spaced and crowns of the teeth lower relative to crown size than in Pongidae. A true fossa canina is present and is morphologically somewhat of the general type seen in *Homo sapiens*. In this character, *Kenyapithecus* differs very markedly from the Pongidae, but resembles *Ramapithecus*. The cross-section through the symphysis of the mandible resembles that of primitive species of the genus *Homo*, and is quite unlike that to be seen in *Dryopithecus*, *Sivapithecus* or *Proconsul*, or in modern Pongidae.



*Kenyapithecus wickeri*, Leakey

The first species of *Kenyapithecus* to be described is *Kenyapithecus wickeri*, Leakey. It comes from the Upper Miocene deposits of Fort Ternan, Kenya, and was announced in 1961. Since then an additional upper central incisor has been found from the same site and level as the original material. The description and measurements of this incisor are as follows:

Crown width	10 mm
Crown height (labial)	10-25 mm
Crown height (lingual)	10-25 mm
Diameters of root at junction with crown	
Labio-lingual	6.5 mm
Bilateral	7.5 mm

This left upper central incisor of *Kenyapithecus wickeri* from Fort Ternan was recovered after the original type specimen had been described. It was found the following year a few feet further into the cliff at the same horizon. It is very well preserved except for the root, the distal end of which is missing.

This tooth is remarkably like that of *Homo* and differs in a number of characters from the corresponding teeth of *Proconsul* (Fig. 1). Moreover, it has characters in common with the australopithecines, in particular in the cross-section of the root where it meets the crown. In the upper central incisors of *Proconsul*, the root at the junction with the crown is approximately trihedral, and has a greater diameter from the labial to the lingual aspect than from side to side. In the *Kenyapithecus wickeri* specimen, the cross-section is not trihedral but much more oval with the maximum diameter from side to side, while the labial-lingual diameter is reduced. The height of the crown of this tooth, which is practically unworn, is less than the width, and it is more compressed labio-lingually compared with corresponding teeth of *Proconsul*. The region near the cutting edge is very thin providing a fine chisel edge very different from the upper incisors of

*Proconsul*, where a marked medial thickening extends almost to the top of the crown.

The upper central incisors of *Proconsul* are not known for the species *Proconsul major*, but we have well preserved examples of both *Proconsul africanus* and *Proconsul nyanzae*, some of them found in direct association with the rest of the upper dentition, so that identification is certain (see Fig. 1). MacInnes<sup>10</sup> described the upper central incisors of a specimen found in 1932-33 on Rusinga Island. At that time, *Proconsul nyanzae* had not been recognized as distinct, so that he provisionally attributed the specimen to Hopwood's *Proconsul africanus*. Subsequently, Le Gros Clark and Leakey<sup>8</sup> showed that it belonged to the species *Proconsul nyanzae*. These authors accepted MacInnes's description of the incisors as adequate, and did not, therefore, elaborate on them in their own report. MacInnes's description is reproduced here for convenience. It reads as follows:

"The root and base of the crown of the first incisor is roughly trihedral in section, with a flat surface to the front. The apex of the crown is sharply constricted from front to back, the anterior surface being gently convex from above downwards, while the posterior surface is rather sharply concave, producing a flat chisel edge. The median surface shows a pressure facet produced by contact with the first incisor of the opposite side, which lies at right angles to the cutting edge of the tooth, while the outer angle of the cutting edge is more rounded. From each of these two angles a very distinct crest curves downwards and backwards and inwards, the two uniting posteriorly. From the middle front of the posterior surface a massive enamel buttress extends from the base of the crown to a point about half-way to the cutting edge. The enamel is considerably wrinkled, particularly on the posterior surface."

New *Kenyapithecus*

The second and new species of the genus *Kenyapithecus* has, as its Type, the maxilla provisionally described in 1951 by Le Gros Clark and Leakey as *Sivapithecus africanus*.

*Kenyapithecus africanus* (Le Gros Clark and Leakey)

The *africanus* species of the genus *Kenyapithecus* differs from the genotype (*wickeri*) in the following characters: the canine fossae are present, but are less developed; the upper 3rd premolars exhibit two strongly developed and divergent labial roots; their crowns are markedly wider labially than lingually, giving a triangular outline when viewed from the occlusal surface. Traces of anterior internal cinguli are present in the molars and premolars; the upper canines are larger relative to the premolars and molars than in the species *wickeri*. The following additional characters can be seen in the mandible. The corpus in the region of the 4th premolar and 1st molar is deep and very slender, in marked contrast to all Pongidae, including *Proconsul* and *Dryopithecus*; the anterior face of the mandible has a rounded contour, which projects forward in front of the gnathion; the cross-section through the symphysis is markedly hominid in shape, and quite unlike *Proconsul*, *Dryopithecus* or *Sivapithecus*. The available evidence suggests marked sexual dimorphism.

*Type.* The type specimen is CMH 6, Nairobi, from Rusinga site R. 106. 1948. It is in the British Museum (Natural History), London.

This specimen was originally described in some detail, and therefore only a few additional features need to be commented on. In the first place, definite evidence of the presence of a canine fossa can be seen on the maxilla. Second, the root of the malar-maxillary process is set forward over the 1st upper molar as in *Kenyapithecus wickeri* and in *Ramapithecus*, not as in the Pongidae or *Proconsul*. Both these features seem to be characteristic of the early Hominidae and are missing in *Dryopithecus*

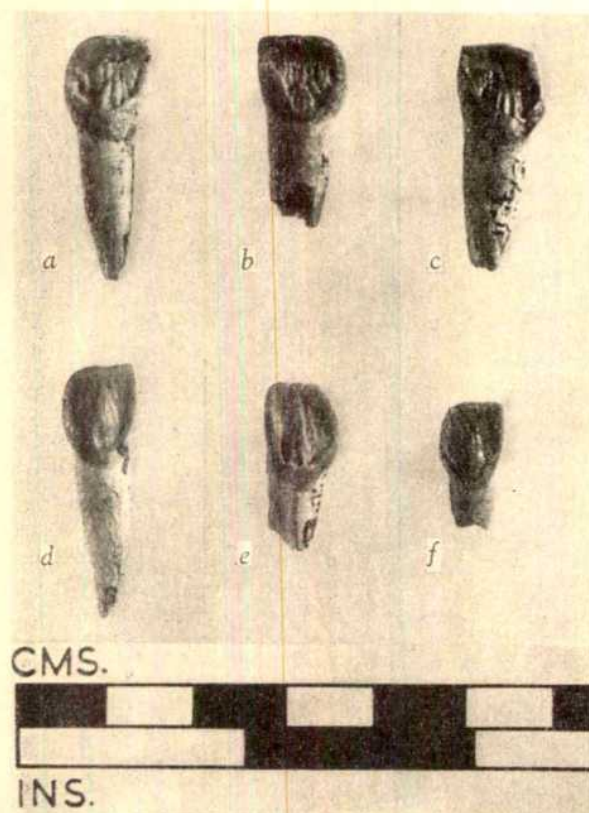


Fig. 1. Comparisons of upper central incisors. a, *Kenyapithecus africanus*; b, *Kenyapithecus wickeri*; c, *Kenyapithecus* sp. indet.; d, e and f, *Proconsul nyanzae*.



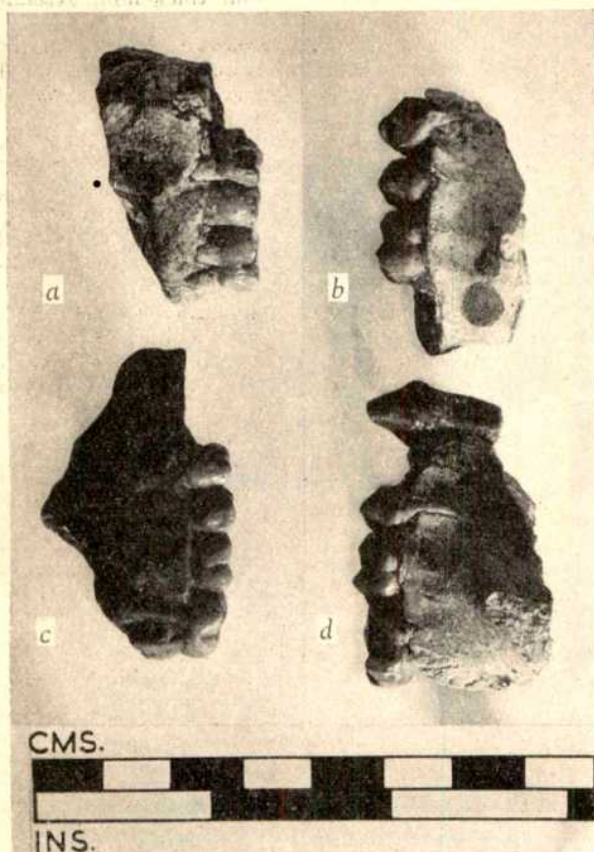


Fig. 2. Comparisons of buccal view of maxillae. *a* and *b*, *Kenyapithecus africanus*; *c*, *Ramapithecus brevivostis*; *d*, *Kenyapithecus wickeri*.

and *Proconsul*. The upper 3rd premolar has two pronounced labial roots (Fig. 2).

### Additional Material

The following seven specimens which are in the collections in the Centre for Prehistory and Palaeontology, Nairobi, are now referred to *Kenyapithecus africanus*. (1) Part of the right side of a mandible No. CMH 142 from Rusinga, site 106. (2) Part of a mandible from Rusinga found on the surface in 1948—specimen No. 276. (3) Two incomplete maxillae of a single individual, from Songhor, specimen Nos. Sgr. 52 and Sgr. 111 of 1948. (4) A fragment of a mandible from Songhor, specimen Sgr. 417 of 1951. (5) An incisor from Rusinga, site R.106. (6) An isolated right upper 2nd molar from Songhor, specimen 404 of 1948. (7) A maxilla fragment from Songhor, No. 748 of 1962. (8) A maxilla fragment from Songhor No. 1377 of 1962. (9) Part of a clavicle from Rusinga, site R. 106 of 1947.

#### (1) Mandible fragment, specimen No. CMH 142

Specimen No. CMH 142 comes from the same site (R. 106) as the Type, but was found on the surface nearby. It consists of part of the right corpus of a large mandible which is broken anteriorly in the region of the root of the right lateral incisor, and posteriorly through the roots of the 1st molar. All the crowns of the teeth are missing, but the corpus is well preserved from the alveolar margin to the lower border (Fig. 3).

The mandible fragment was provisionally referred by Le Gros Clark and Leakey to *Proconsul major* on account of the very great depth of the corpus, which measures 39.5 mm in the region of the lower 4th premolar, compared

with 41 mm in the large mandible which is the paratype of the *Proconsul major*. The crowns of the teeth were all missing and, in view of the very scant knowledge of the fauna in 1951, it therefore seemed preferable at that time to refer this specimen to the largest of the *Proconsul* species. The most important differences which can now be clearly distinguished are:

(a) At the level of  $P_4M_1$  the depth of the corpus is only very slightly less than that seen in *Proconsul major*, but the thickness of the mandibular, at this point, is totally different; the corresponding measurements at  $P_4M_1$  are:

<i>Proconsul major</i>	Depth 41 mm	Thickness 22 mm
<i>Kenyapithecus africanus</i>	Depth 39.5 mm	Thickness 12 mm

(b) In the known mandibles of *Proconsul major* there is a clearly defined diastema between the lower 3rd premolar and the lower canine; in this specimen the anterior lingual root of the lower 3rd premolar is set far forward and extends well beyond the posterior rim of the alveolus of the canine, and there is no diastema (Fig. 4).

(c) In *Proconsul major* (as also in both the other species of the genus) the furthest posterior projection of the symphyseal region is set high in the mid-line and extends well back, so that its limit is almost in line with the lower 4th premolar. In this specimen the most backward part of the symphysis is not further back than the level of the front of the 3rd premolar, and may be even further forward.

(d) In *Proconsul major* the inner wall of the corpus, in the region of the premolars, slopes slightly inwards, and the corpus itself is very thick. In this specimen the outer wall is at first straight, then turns slightly outwards.

(e) The area of the root of the canine in *Proconsul major* is marked by a strong surface swelling of the anterior face of the mandible, giving a clear line of demarcation between the "chin" region and the lateral wall of the corpus. In this specimen, there is no such swelling and the root of the canine is much less massive.

(f) The roots of the lower canines are more laterally compressed than in any *Proconsul*, and are orientated more antero-posteriorly and less transversely than in any species of *Proconsul* or Pongid.

(g) The whole mandibular structure is gracile even though the corpus is very deep (39.5 mm).

(h) The mental foramen lies relatively low on the corpus, and is situated beneath the 4th premolar, instead of below the 3rd premolar as in *Proconsul major*.

#### (2) Mandible fragment, specimen No. 276

The second specimen now referred to *Kenyapithecus africanus* is the anterior part of another mandible. It is somewhat weathered and was found on the surface. As such, it was not considered of much importance when the 1951 report was prepared. Its morphology, however, is of such a nature that it now becomes clear that it cannot represent the genus *Proconsul*. It displays a number of features, especially in the cross-section of the symphysis and on the anterior face of the "chin" region, which indicate a remarkably hominid structure (Fig. 3).

Because it clearly does not represent a *Proconsul*, and because it shares so many features in common with other specimens now referred to *Kenyapithecus africanus*, it seems wise to consider this specimen as representing the latter genus and species. This decision is reinforced by the fact that a wholly comparable, but rather smaller, fragment of mandible (see later) has been found at Songhor, close to the point where two maxillae specimens of this same species had been discovered (see following section).

In this second referred specimen, all the crowns of the teeth are missing. The preserved part extends from the anterior edge of the alveolus of the 4th premolar on the right side round to the alveolus of the left 4th premolar. While both the 4th premolars are only represented by



parts of the roots, the lower 3rd premolars, both canines and all four incisors, have their roots intact. There is no trace of a diastema between the lower 3rd premolars and the canines. This is a marked contrast to the conditions to be seen in the various *Proconsul* species. Instead of a diastema, we find that the anterior root of the 3rd premolar is pushed well forward beyond the posterior rim of the alveolus of the canines (see Fig. 4).

The arrangement of the incisors in the alveolus is clearly to be seen along the alveolar margin, and differs very markedly indeed from the crowded condition to be seen in any mandible of any species of *Proconsul*. The four incisor teeth were set more or less in a straight line between the canines instead of with the two central incisors being more forwardly placed (Fig. 4). In this character there is close resemblance both with *Homo* and with *Australopithecus*, but not with *Proconsul* or *Dryopithecus*. The roots are set vertically in the mandible and are not procumbent. The preserved roots of the canines indicate somewhat robust, but not very large, teeth. They are strongly compressed bilaterally, and are placed more antero-posteriorly than in *Proconsul*. Viewed from the front the chin region differs most markedly from that of any species of *Proconsul*, in all of which the anterior region suggests an inverted triangle with the base of the triangle along the alveolar margin of the incisors and the apex of the triangle near the middle point of the lower border of the symphysis. In *Proconsul* there is also a marked backward slope of the anterior face of the mandible in the chin region. In the specimen now referred to *Kenyanthropus africanus* the morphology of the anterior part of the mandible is remarkably like that in a primitive *Homo*, such as the Mauer jaw. The most forward projecting part lies below the alveolus of the central incisors. The whole shape of this region is, moreover, well rounded and filled out (Fig. 3), quite unlike any *Proconsul* or *Dryopithecus*. The cross-section through the symphysis is also different (Fig. 5) and recalls that of the more primitive members of the genus *Homo* and of some australopithecines.

### (3) Incomplete maxillae, specimens

Nos. Sgr. 52 and Sgr. 111

Two parts of a palate became separated before they were embedded in the deposit in which they were found. The fracture of one premolar is, however, subsequent to the specimen having been eroded from the deposits. Specimen No. 52 consists of a part of the right maxilla. It contains both premolars and the 1st

Two parts of a palate became separated before they were embedded in the deposit in which they were found. The fracture of one premolar is, however, subsequent to the specimen having been eroded from the deposits. Specimen No. 52 consists of a part of the right maxilla. It contains both premolars and the 1st

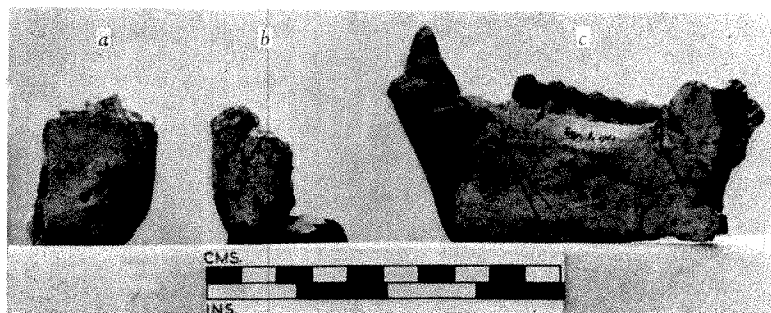


Fig. 3. Comparisons of anterior slope of mandibles. a and b, *Kenyanthropus africanus*; c, *Proconsul major*.



Fig. 4. Comparison of occlusal views of mandibles. a and b, *Kenyanthropus africanus* (males?); c, *Kenyanthropus africanus* (female?); d, *Proconsul nyanzae*; e, *Proconsul major*.

molar. The root of the canine is preserved in the alveolar wall, but the crown has broken off. The specimen has also been broken away some 15 mm above the alveolar margin, in an irregular fracture. In this region, a cross-section of the canine root near the tip is exposed. Above the upper 4th premolar and the upper 1st molar, the maxillary sinus can be seen, filled with matrix. On the palatal aspect the fracture is just short of the palatal suture, which is not preserved. The two cross-sections through the root of the canine (one near the tip and one at the alveolus) reveal that this tooth was orientated in the maxilla with its long axis in much the same direction as that of the molar-premolar series. The labio-lingual width is almost equal to the antero-posterior measurement. At the fracture near the alveolar margin the measurements of the root are 11 mm (antero-posteriorly) and 10.5 mm (bucco-lingually). At the fracture near the tip of the root, about 15 mm above the alveolar margin, the corresponding figures are 8 mm and 6 mm, respectively.

The 3rd premolar has a crown which is roughly triangular in outline when viewed from the occlusal surface—it is very much wider on the labial than on the lingual aspect. The greater labial width is linked with the presence of a very robust anterior labial root and with a rather pronounced anterior cingulum (Figs. 2 and 6). A somewhat similar morphology can be seen in certain specimens from Asia which are classified as *Sivapithecus sivalensis*, and is also present in the Type specimen of *Kenyanthropus africanus*. The single lingual root of the 3rd premolar is very robust and is directed at a wide angle into the palatal area, instead of nearly vertically into the alveolar margin as in *Proconsul*. The two labial roots of this tooth are strongly developed as in the Type specimen of *Kenyanthropus africanus*. The contact area between the crowns of the upper 3rd and 4th premolars is large and flat, as a result of considerable antero-

posterior compression of the teeth, which in turn is correlated with the marked facial shortening. In this character, there is a strong resemblance to the position to be seen in the other and geologically younger species of the genus—*Kenyapithecus wickeri*, from Fort Ternan. The crown area of this upper 3rd premolar is unfortunately rather worn so that the details of the cusp pattern cannot be clearly studied. The measurements of this tooth are: bucco-lingual width 10.25 mm, antero-posterior length 9 mm.

The 4th premolar is narrow antero-posteriorly (both on the lingual and labial aspects) and is wide bucco-lingually. Like the upper 3rd premolar, it has two labial and one lingual root. The labial enamel surface of the crown is very convex antero-posteriorly, and there is no trace whatever of a cingulum. The contact facet of the crown with the upper 1st molar is also large and flat.

The 1st molar, while roughly rectangular in outline viewed from the occlusal surface, is slightly less wide labially than lingually. All four cusps are worn, but the two on the lingual aspect much more so than those on the labial side. No trace of a cingulum is visible. The cusp pattern of this molar is entirely different from that of any species of *Proconsul*. On the labial face of the crown, moreover, there is a clear valley which separates the anterior from the posterior cusp area. This valley extends half-way down the side of the crown.

The second maxilla fragment, *Sgr.* 111, comes from the left side of the same individual. Only the 4th premolar is intact; the 3rd premolar is represented by its roots and by a small portion of the crown. The canine is represented by a broken root which is still in the socket. This root is clearly set in a much more vertical position in the maxilla than in any species of *Proconsul*, or for that matter in any member of the Pongidae. The length of this canine root can be estimated to have been about 24 mm. It has a similar cross-section to that which has been described in the other maxillary specimen.

An interesting feature of *Sgr.* 111 is the presence of a well marked *fossa canina* on the maxillary surface, above the 3rd and 4th premolars. This does not exhibit quite the same depth, or morphology, as in *Kenyapithecus wickeri*, where the position is nearly like that in *Homo*. Nevertheless, it is much more developed and more man-like than in any fossil or living pongid. A trace of a similar canine fossa occurs in the type specimen, but unfortunately the main part of the relevant area is broken away. On this second maxilla fragment the fracture of the palate is at or near the palatal suture and the palate is seen to have been rather flat and shallow.

Table 1

	<i>M</i> <sup>1</sup>		<i>P</i> <sup>4</sup>		<i>P</i> <sup>3</sup>	
	<i>L</i> <sup>*</sup>	<i>B</i> <sup>*</sup>	<i>L</i>	<i>B</i>	<i>L</i>	<i>B</i>
<i>Kenyapithecus africanus</i>	10	11	7.5	10.5	9.5	11.5
<i>Kenyapithecus wickeri</i>	10.5	10.5	6	10.25		
<i>Ramapithecus brevirostris</i> (cast)	9.2	10.9	6.7	10.0	6.9	10.3
<i>Sivapithecus sivalensis</i> (cast)	10.5	11.5	7.0	11.0	9	10.5

All measurements are in mm.

Note. Measurements made on casts may not be very reliable because the degree of accuracy of the cast is unknown.

\* *L*, Antero-posterior length; *B*, bucco-lingual width.

Table 1 sets out the measurements of the teeth of the type of *Kenyapithecus africanus* compared with the type of *Kenyapithecus wickeri*, as well as the type of Lewis's *Ramapithecus brevirostris* and a comparable specimen of *Sivapithecus sivalensis*.

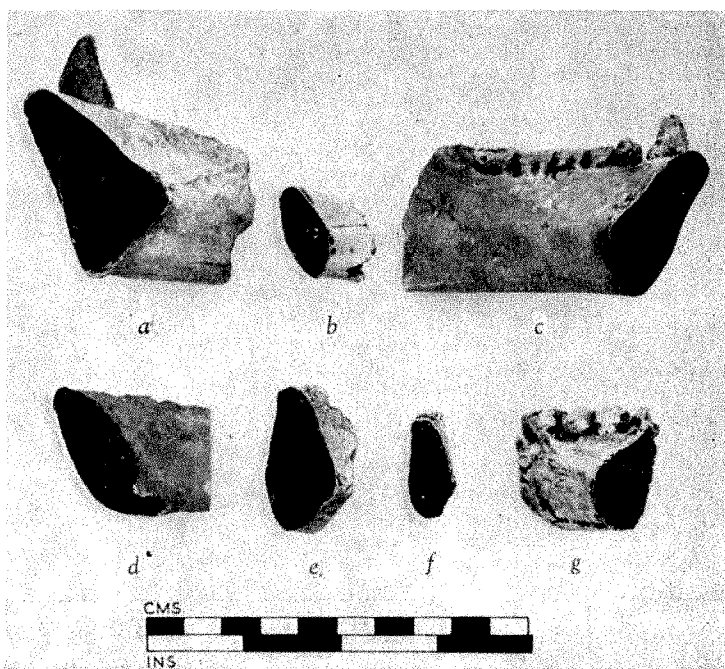


Fig. 5. Comparisons of cross-section of mandibles at symphysis. *a*, *Proconsul major*; *b*, *Proconsul africanus*; *c*, *Proconsul nyanzae*; *d*, *Pan satyrus*; *e*, *Kenyapithecus africanus* (male?); *f*, *Kenyapithecus africanus* (female?); *g*, *Homo habilis*.

#### (4) Mandible fragment, specimen No. *Sgr.* 417

The fourth referred specimen is a mandibular fragment which was found at Songhor near the maxillae described above. It may perhaps represent the same individual. It clearly comes from a mandible that must have been considerably smaller than the first and second referred specimens, and this may, perhaps, be regarded as evidence of sexual dimorphism. It has been noted that the Songhor maxillae fragments found nearby are also somewhat smaller than the type specimen from Rusinga site *R.* 106. This specimen consists of the anterior segment of a mandible of small size, embracing the broken right canine, the roots of all four incisors and the fractured root of the left canine. The canine roots are very small. The whole depth at the symphysis is preserved and the inferior border is intact. One of the most striking characters, and one which clearly places this specimen outside the genera *Proconsul*, *Limnopithecus* and *Dryopithecus*, is the cross-section through the symphyseal region (Fig. 5) together with the shape and fullness of the anterior face of the mandible in the mid-line. In this character the present specimen duplicates what has already been described in the second referred specimen.

The nature of the cross-section through the symphysis of this specimen is very similar to that in the second referred specimen of the *Kenyapithecus africanus* mandible. The roots of the canines and of the incisors are placed nearly vertically in the corpus, and the incisor roots are noticeably less crowded together than in a *Proconsul* of comparable size.

#### (5) Upper central incisor, specimen No. *CMH* 9

In 1951 Le Gros Clark and Leakey figured an upper central incisor from Rusinga Island as a possible incisor of *Proconsul nyanzae*, but more detailed study shows that it does not agree in its overall morphology with specimens which can be shown to belong to *Proconsul*. On the other hand, it agrees remarkably, in structure, with the upper central incisor of *Kenyapithecus wickeri* (see Fig. 2). It is slightly less like a shovel-shaped incisor of the genus *Homo* than the corresponding tooth of *Kenyapithecus*

*wickeri*, but we have already seen that in other characters also *Kenyapithecus africanus* is somewhat less evolved in the hominid direction than the geologically younger species *wickeri* from Fort Ternan. The tooth is large, with a maximum crown diameter of 10 mm and a labial-lingual diameter at the base of the crown of 7.25 mm. It exhibits only a small degree of wear on the crown, and the dentine is not exposed. The lingual surface of the crown is rather concave in longitudinal section and slightly so in transverse section. The cutting edge is straight, and on the median side the lateral edge is almost at right angles at first, and then curves inwards to the neck. The lateral edge, in contrast to the median edge, descends in a smooth curve outwards before turning inwards to the neck of the tooth, at the commencement of the root. In these characters the crown parallels the upper central incisor of *Kenyapithecus wickeri* to a remarkable degree (Fig. 1). The root is rather less compressed labio-lingually than in *Kenyapithecus wickeri* and is more nearly trihedral. In this single respect it resembles the roots of *Proconsul* incisors but the labial face of the root, near the neck, is slightly convex from side to side. The measurements of the root at the neck are labio-lingual width 7 mm, bilateral width 7 mm.

The morphology of the lingual aspect of the crown is perhaps the most interesting character, and here the resemblance is wholly with *Kenyapithecus wickeri*, and not at all with *Proconsul nyanzae* or *africanus*. The typical structure of the labial aspect of the upper central incisors of *Proconsul* as described by MacInnes<sup>10</sup> has already been quoted.

There are now six additional *Proconsul nyanzae* upper central incisors available for study in the Nairobi collection. Although they show minor points of variation, they all conform to the same morphological pattern, which has at its most marked feature a convex buttress running centrally from the base of the crown almost to the tip on the lingual face.

**(6) Upper right second molar, specimen No. 404** Closely resembling the 1st upper molar of specimen *Sgr.* 52 is an upper right second molar from Songhor. Nevertheless the condition of wear on the occlusal surface and examination of the contact facets on either side indicate that it belongs to another individual. It is probably female. The measurements of this tooth are bucco-lingual diameter 11.5 mm, antero-posterior diameter 10 mm.

**(7) Maxilla fragment, specimen No. 748** This maxilla fragment is from the right side. It contains the 3rd and 4th upper premolars, which are beautifully preserved and only very slightly worn. As in the other specimens of *Kenyapithecus africanus*, the width of the 3rd premolar is much greater on the labial than on the lingual aspect so that the tooth viewed from the occlusal aspect is slightly triangular in outline. There are two labial roots to this tooth and, as in the type specimen from Rusinga site *R.* 106, the anterior one is very massive. The contact with the 4th premolar is large and flat. The 4th premolar is wide bucco-lingually and narrow antero-posteriorly, the respective measurements being 11.5 mm and 7 mm. In size and morphology these two teeth conform closely with the corresponding teeth in the type specimen and probably represent a male.

**(8) Maxilla fragment, specimen No. 1377** The maxilla fragment No. 1377 from Songhor is from the left side and, like No. 7, also contains only the two premolars. It is distinctly smaller than No. 7 and the teeth are comparable in size, as well as in morphology, with the corresponding two teeth in *Sgr.* 52 of 1948 (see section 3). It is presumed to be female.

The measurements of these two teeth are as follows:

*P*<sup>3</sup>—Antero-posterior length 8.5 mm; bucco-lingual width 10 mm.  
*P*<sup>4</sup>—Antero-posterior length 6 mm; bucco-lingual width 10 mm.

Besides the specimens listed, which certainly represent *Kenyapithecus africanus*, there are some eight other specimens which, possibly, should also be referred to this genus and species, but the evidence is less certain. When more complete material has been discovered it may be possible to diagnose these specimens further.

**(9) Part of a clavicle, specimen No. 604, 1947**

Le Gros Clark and Leakey<sup>9</sup> briefly noted a part of a clavicle from Rusinga site *R.* 106, No. 604. It was found close to the type specimen and to specimens 1–5 here, and may belong to the same individual.

### *Kenyapithecus*, species indet.

In 1951 Le Gros Clark and Leakey provisionally referred an upper first molar found on Maboko Island to what they then called *Sivapithecus africanus*. The question therefore arises as to whether this specimen represents the genus *Kenyapithecus* and, if so, which species.

The deposits at Maboko Island are no longer regarded to be of the same age as those of Rusinga, Songhor and Koru. They are usually now either referred to the uppermost Miocene, or to the early Pliocene, and are considered to be of roughly the same age as those of the Fort Ternan site.

The molar tooth in question conforms in all respects to molars of *Kenyapithecus* and must certainly be regarded as representing that genus. For the moment it seems better not to assign it to any particular species. It was illustrated in Plate VI, Fig. 44, of Le Gros Clark and Leakey.

During the current study an upper left central incisor from Maboko Island (Fig. 1) was discovered. It clearly also represents the genus *Kenyapithecus* and is also, for the moment, treated as species indet. The measurements of this incisor tooth *MB.* 142 (*CMH* 11) are as follows: labio-lingual width at base of crown 7.25 mm; transverse diameter of crown 9.75 mm; anterior height of crown 11.00 mm; internal height 11.5 mm.

## Discussion

### Status of *Kenyapithecus africanus*

Although in 1963 Simons supported the view that what Le Gros Clark and Leakey called *Sivapithecus africanus* would prove to stand very close to *Kenyapithecus wickeri*, he abandoned that idea in 1965. He and Pilbeam have now jointly suggested that specimens which they group together as the "Sivapithecus" group of Primates are no more than a sub-genus of *Dryopithecus*. They suggest that there are two distinct species of this "sub-genus"; one *Sivapithecus indicus* and the other *Sivapithecus sivalensis*.

I consider that it would be much wiser to retain the generic name *Sivapithecus* for the truly Asiatic members of Dryopithecinae, reserving the generic name *Dryopithecus* for the European forms belonging to this sub-family. This is purely a matter of definition and it is never easy to decide just what should constitute a genus or what a sub-genus. It is, however, important to bear in mind that the differences between *Sivapithecus indicus* and *Sivapithecus sivalensis* appear to be marked, and that what is known as *Sivapithecus sivalensis* is possibly not a *Sivapithecus* at all. There seems to be no doubt that the species *Sivapithecus sivalensis* in India may stand much closer to the genus *Ramapithecus* than it does either to *Sivapithecus indicus* or to the true European *Dryopithecus* specimens.



or even to the East African *Proconsul* stock. This can be clearly established by examining the morphology of the molar and premolar teeth.

Simons and Pilbeam have expressed their opinion that the specimen which Le Gros Clark and Leakey originally called *Sivapithecus africanus* is indistinguishable "both generically and specifically" from the Asiatic *Sivapithecus sivalensis*. They also maintain that *Kenya-pithecus wickeri* is identical with *Ramapithecus punjabicus*. In the light of the foregoing study, I strongly reject both these opinions as having no adequate foundation in the light of the data which are now available. The following are two of the principal reasons for the rejection of Simons and Pilbeam's views on this matter:

(a) The diagnosis of *Ramapithecus* which has been given by Simons and Pilbeam states that, in the genus *Ramapithecus*, the mandible is "shallower" than in *Dryopithecus* and *Australopithecus*. This is certainly not true of *Kenya-pithecus africanus*, where we have a mandibular fragment which is 39.5 mm deep at the level of the 4th premolar.

(b) They further state that the incisors and canines are "reduced in relation to the cheek teeth when compared with those of *Dryopithecus*". I do not know what incisor teeth of the Asiatic *Ramapithecus* they have examined as a basis for this statement, but it is emphatically not the case when the size of the upper central incisor of *Kenya-pithecus wickeri* or of *Kenya-pithecus africanus* is compared with the premolars and molars of the same individual.

Table 2

	Upper central incisors		Upper 4th premolars		Upper 1st molars	
	Crown bilateral width	Bucco-lingual width at base of crown	Antero-posterior diameter	Bucco-lingual width	Antero-posterior diameter	Bucco-lingual width
<i>K. wickeri</i>	10	6.5	6	10.25	10.5	10.5
<i>K. africanus</i>	10	7.5	7.5	11.5	10.6	11.3
<i>P. nyanzae</i>	7	8.0	7.0	10.5	10	11.0
<i>P. africanus</i>	6	6.4	7.5	9.7	7.9	9.6

The corresponding modules comparing central incisors with 4th premolars are 8.25-8.125 and 8.625-9.65 in *Kenya-pithecus* and 7.5-8.75 and 6.2-8.6 in the *Proconsuls*. Similarly the modules comparing measurements of the first molars are 8.25-10.5 and 8.625-10.9 in *Kenya-pithecus* and 7.5-10.5 and 6.2-8.75 in the *Proconsuls*.

Table 2 sets out the relative measurements of the upper central incisors, upper 4th premolars and upper 1st molars (in each case in a single individual) from specimens representing *Kenya-pithecus wickeri* and *Kenya-pithecus africanus* (Hominidae) and *Proconsul nyanzae* and *Proconsul africanus*. Reference to the upper 3rd premolars is omitted because we do not have this tooth in *Kenya-pithecus wickeri*. Table 2 shows that in *Kenya-pithecus* the upper central incisors are not reduced relative to premolars and molars, but are large relative to them when compared with the *Proconsul* group.

While it is readily agreed that *Ramapithecus* should be regarded as a member of the Hominidae—an Asiatic member—the new evidence suggests that it should not—at least at present—be treated as identical to *Kenya-pithecus*. Good specimens of the symphyseal region of the mandible of *Ramapithecus* are required, as well as information about its upper incisors, before we can be certain.

It has been proposed in this article that the former "*Sivapithecus africanus*" should now be regarded as a Lower Miocene representative of the genus *Kenya-pithecus*, and called *Kenya-pithecus africanus*, because it clearly stands close to *Kenya-pithecus wickeri*. It has among other things (a) an incipient *fossa canina* of *Homo* type, (b) a very thin mandibular ramus relative to depth, (c) no diastema in the lower dental series, (d) transverse section through the symphysis of hominid type, and (e) an upper central incisor of hominid structure. Until *Sivapithecus sivalensis* can be shown to share all these features, identity cannot be accepted.

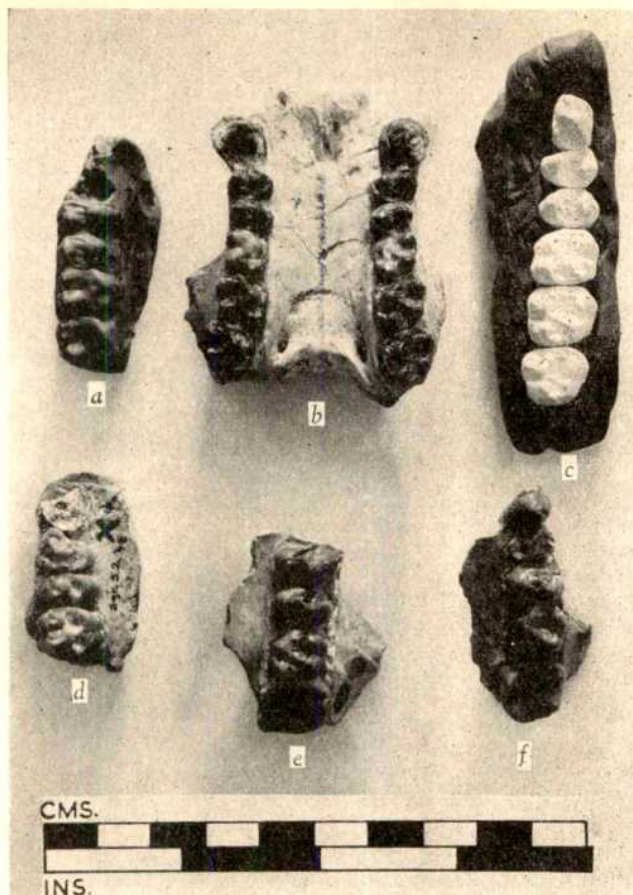


Fig. 6. Comparison of upper dentition from occlusal view. a, *Ramapithecus brevivostis* (cast); b, *Proconsul africanus*; c, *Sivapithecus sivalensis* (cast); d and e, *Kenya-pithecus africanus*; f, *Kenya-pithecus wickeri*.

## Geological Age

The geological age of the Rusinga deposits and also of those at Songhor is usually regarded as Lower Miocene. The fauna of even the youngest part of the series is regarded as at least two "faunal stages" older than the fauna of Fort Ternan<sup>11</sup>, which is considered to be Upper Miocene. The Fort Ternan fauna includes primitive Bovidae with well developed horn cores, while even in the youngest deposits of Rusinga and Songhor there are no true Bovidae with horns. Ungulates are represented, instead, by several hornless members of the Tragulidae.

While dealing with the question of age, it is necessary to consider briefly certain suggestions that have been made that the fauna of Rusinga, Songhor and Koru, etc., ought not to be regarded as of Lower Miocene age, but as somewhat younger. The most serious argument which has been advanced against a Lower Miocene age for the Rusinga formation has come from Evernden, Curtis and Savage, and this must, therefore, be critically examined. They have, in effect, expressed the view that an age of about  $15 \times 10^6$  yr, which was obtained by the potassium-argon method of dating on a specimen collected in the R. 106 region of Rusinga, near the foot of the Kiahera Hill, should be regarded as the date for the whole Rusinga series. Kiahera Hill consists mainly of rocks which belong to Shackleton's "Kiahera Series"<sup>12</sup> which he believes to represent the oldest part of the Rusinga sequence. Curtis and Evernden's sample certainly came from "near the foot of Kiahera Hill" and it has been assumed that it must, therefore, belong to a low level in the Kiahera series.



This is not necessarily the case. There are a number of faults and unconformities in the area, and the Kathwanga series, which Shackleton regarded as possibly the youngest part of the Rusinga rock sequence, rests against Kiahera Hill in several places. The sample that was used to obtain a potassium-argon age of about  $15 \times 10^6$  yr may perhaps have come from the Kathwanga series.

Of greater significance than this single potassium-argon figure, for the purpose of accurate dating, is the fact that the fauna of even the youngest part of the Rusinga Miocene series (as already stated) differs markedly from the Upper Miocene fauna of Fort Ternan. It differs, in fact, by at least two "faunal stages". If the Fort Ternan deposits are correctly dated by potassium-argon dating, as about  $14 \times 10^6$  yr, then the age of  $15 \times 10^6$  yr given for the deposits at Rusinga, near to the base of Kiahera Hill, must be wrong. The possibility cannot, however, be wholly excluded that the uppermost part of the Rusinga sequence, the Kathwanga series, may be of Middle Miocene age, but even this seems to me somewhat unlikely, on the basis of the faunal evidence.

The real question, for the purpose of this present article, is not only the question of the age of the Rusinga Island deposits but also those at Songhor, whence some of the specimens now referred to *Kenyanthropus africanus* have come. At the present time the Songhor deposits are usually regarded to be of the same general age as the main Rusinga series. It must, however, be noted that there are distinct differences in the faunal assemblages between those from Songhor and those from Rusinga. These differences have usually been explained by suggesting that the two sites represent different ecological settings, rather than that they are due to a real difference in geological age because there are certain well defined faunal elements in common. It is, however, possible that the Songhor beds may only represent the upper part of the Rusinga series, and that both may be of Middle Miocene age, leaving the main Rusinga deposits in the Lower Miocene.

Another reason which has been put forward in support of a later age for the Rusinga formation was that advanced by Savage in 1964. The argument runs as follows: *Pliohiprax*, *Mesopithecus*, *Sivapithecus*, together with members of the Tenrecidae, occur in the faunal assemblage from Rusinga and these prove that the age is younger than Lower Miocene, because they are creatures which occur in Middle and Upper Miocene beds elsewhere. This view overlooked the following points:

(1) The identification of *Pliohiprax* has been shown by Whitworth<sup>13</sup> to have been incorrect and the material formerly referred to under that name is, in reality, a *Megalohyrax*.

(2) The specimen which MacInnes very tentatively identified in 1943 as "*Mesopithecus*" was a mandible which came from the site on Maboko Island, and not from Rusinga. This site has since been shown by Simpson to be younger than the Rusinga series, and is probably of Pliocene age. In any event the specimen is not a "*Mesopithecus*". This specimen, therefore, has no bearing whatever on the age of the Rusinga deposits.

(3) The specimen which Le Gros Clark and Leakey called "*Sivapithecus*" was only very tentatively placed in that genus, and it has now been shown not to be a *Sivapithecus*.

(4) The presence of Tenrecidae supports rather than contradicts a Lower Miocene age, because this family no longer exists on the African mainland but only on Madagascar Island, which was cut off from Africa during Lower Miocene times, or even earlier.

While the available evidence suggests, therefore, that *Kenyanthropus africanus* carries the Hominidae back to the Lower Miocene, the possibility that it might be of Middle Miocene age cannot be wholly ruled out, but it is emphatically not of Upper Miocene age. It is, therefore,

distinctly older than *Ramapithecus* and *Kenyanthropus wickeri*, and is the oldest member of the family Hominidae known at the present time.

One of the results of the discovery of remains of a genus of the Hominidae, *Kenyanthropus africanus*, at Songhor and on Rusinga Island, is that it is no longer possible to treat certain of the post-cranial skeletal material which was formerly attributed to *Proconsul* as necessarily belonging to that genus. As Le Gros Clark and Leakey observed on p. 87 of their 1951 report: "A few isolated limb bones attributable to some of the large Hominidae have come to light. Although they are from sites which have also yielded teeth and jaws of *Proconsul*, none of those to be described in this section was found in such close association with those other remains as to permit any assurance that it belonged to the same individual."

The talus and calcaneum (CMH 145 and 146) from Songhor and a very similar talus from Rusinga (CMH 147) were assumed to belong to *Proconsul* and referred provisionally to the species *nyanzae* (on account of their size), but it was recognized in 1951, and must be again emphasized here, that these specimens could just as well belong to some other primate.

The incomplete femora and the shaft of the humerus from the Maboko Island site were also assumed to belong to *Proconsul*, but we now know that the Maboko deposits are younger than the main Rusinga series, and are probably of an Upper Miocene or Early Pliocene age. While *Proconsul* (species indet.) is known to persist as late as the Upper Miocene in East Africa (compare with Fort Ternan and Maboko), it is also clear that at least one incisor tooth of *Kenyanthropus* has been recorded from the Maboko beds, while a primate related to *Oreopithecus* is also present at both sites.

A tibia fragment from Rusinga was also described by Le Gros Clark in 1952 (ref. 14). This, too, can no longer be regarded as necessarily representing the genus *Proconsul*.

Two incomplete clavicles were reported by Le Gros Clark and Leakey; one of them was from Maboko Island, the other was found on Rusinga at site R. 106, which is the site also yielding the mandible fragment and the type maxilla fragment of *Kenyanthropus africanus*, and an incisor of this species. Of these two clavicle fragments, the specimen from site R. 106 is particularly interesting, in view of its close association with material representing *Kenyanthropus africanus*. It has been provisionally referred to that genus. It will be sent to a specialist for study and report.

The work on Rusinga Island and at Songhor was carried out with the aid of grants from the Royal Society (1948), the Boise Fund, and the Wenner-Gren Foundation, while the Fort Ternan site was studied under the auspices of the National Geographic Society. The help of all these bodies is gratefully acknowledged.

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<sup>2</sup> Gregory, W. K., Hellman, M., and Lewis, G. E., *Fossil Anthropoids of the Yale-Cambridge India Expedition of 1935* (Carnegie Institute of Washington, 1938).

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<sup>8</sup> Simons, E. L., *Proc. U.S. Nat. Acad. Sci.* (1964).

<sup>9</sup> Le Gros Clark, W. E., and Leakey, L. S. B., *The Miocene Hominoids of East Africa, Fossil Mammals of Africa No. 1* (Brit. Mus. Nat. Hist., 1951).

<sup>10</sup> MacInnes, D., *J. East African and Uganda Nat. Hist. Soc.*, **17**, Nos. 3 and 4 (November, 1943).

<sup>11</sup> Simpson, G. G., *Amer. J. Sci.*, **263** (1965).

<sup>12</sup> Shackleton, R. M., *Quart. J. Geol. Soc. Lond.*, **106** (1951).

<sup>13</sup> Whitworth, T., *The Miocene Hyracoids of East Africa, Fossil Mammals of Africa, No. 7* (Brit. Mus. Nat. Hist., 1954).

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Relation between Optical and Radio Properties of Quasars

THE purpose of this communication is to point out some interesting relationships between  $U$ ,  $B$ ,  $V$  colours of quasars<sup>1,2</sup> and their radio polarization properties<sup>3-6</sup>.

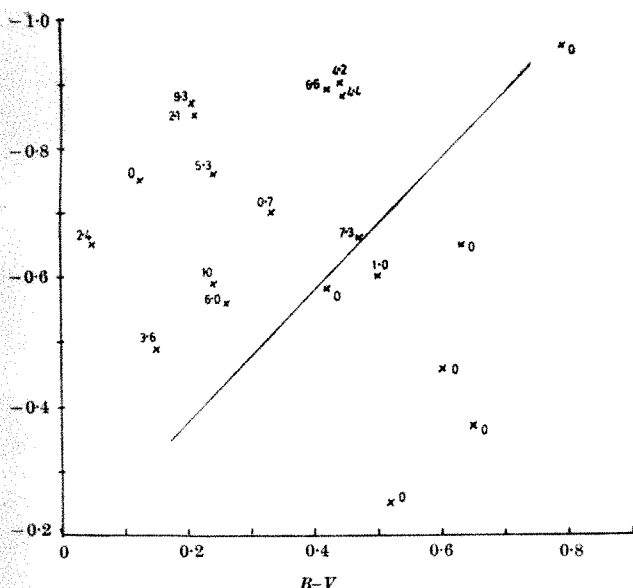


Fig. 1. Percentage polarization at 21 cm wavelength for quasars with measured  $U-B$  and  $B-V$ .

In Fig. 1 the percentage polarization at 21 cm wavelength has been indicated on a  $U-B$  against  $B-V$  plot for the quasars for which all three parameters are known. There is a remarkable division: zero polarization in the lower right-hand corner and the higher polarizations in the upper left-hand corner. Values of zero percentage polarization have been allocated to those sources the polarizations of which were less than 1.0 per cent and the quoted errors of which were of the same order as the polarization. Fig. 2 shows a plot of radio rotation measured against the ratio  $U-B/B-V$ . Again a significant relationship is apparent, although further experimental points are needed, such as would be provided by an extension of polarization measurements to lower radio frequencies and weaker sources.

If the  $U$ ,  $B$ ,  $V$  colours are intrinsic source properties, then the relationship between these and the polarization properties is significant. In particular, Fig. 2 indicates that a considerable part of the rotation measures may well be intrinsic to the sources and hence these cannot be used to make any precise models of the galactic magnetic field<sup>4,5</sup>.

Selection effects may be important in such a small sample of data. It is possible, for example, that objects with large  $z$  are only observed if they have a large intrinsic luminosity, which might itself be related both with colour and with radio polarization properties. On the other hand, there is no apparent dependence of the radio polarization properties on  $z$ . Such correlations are harder to explain if the colour or the polarization is determined

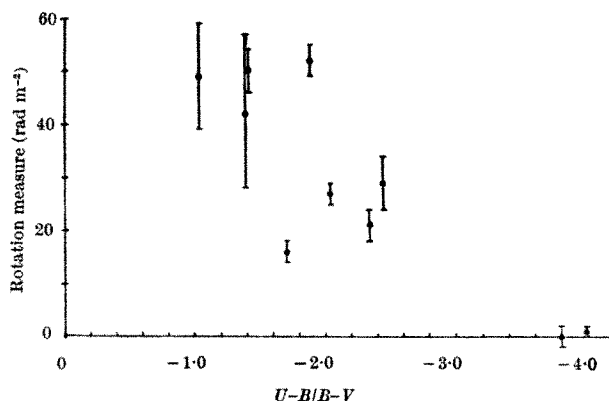


Fig. 2. Radio rotation measure versus  $U-B/B-V$  for all quasars for which these parameters are known to date.

by the interstellar medium, but further observations might exclude this possibility. In the case of 3C 138 ( $b'' = -11^\circ$ ), Sandage<sup>1</sup> has corrected the colours for interstellar reddening, but the corrections are uncertain and the source has not been included in the data.

With these reservations in mind, the following comments may be made on the two diagrams. Fig. 1 shows that the objects that are most highly polarized are those with relatively large ultra-violet excess, while the redder objects are unpolarized at 20 cm. Fig. 2 shows that the redder objects also have the largest rotation measures. This could be an evolutionary effect. Initially, the source consists mainly of synchrotron electrons emitting polarized radiation, and as it evolves an increasing number of these electrons decay to thermal velocities, producing an increase in the observed rotation measures. The associated decrease in polarization could be due to differential Faraday rotation, combined with a possible increase in the tangled nature of the magnetic field structure within the source. When a significant fraction of the synchrotron electrons has been thermalized the effect on the spectrum of the source might be detectable.

If we do conclude that the polarization properties are intrinsic to the sources, then the rotation measures should be multiplied by  $(1+z)^2$  before we can compare them closely with other source properties. Extension of polarization measurements to lower frequencies would enable red-shift corrections to be applied to the observed degrees of polarization, so that Fig. 1 could be redrawn showing the intrinsic source polarization at 21 cm, rather than the observed values.

Observations of  $U$ ,  $B$ ,  $V$  colours of radio galaxies would clearly be valuable for comparison with the radio polarization data in the same way.

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## PLANETARY SCIENCE

**B and C "Sources" of Jovian Decametric Radiation**

A STUDY has been described of very short duration (less than 50 msec) pulses observed in the decametre-wave radiation from Jupiter<sup>1</sup>. For the period of the observations (November 21, 1965–March 17, 1966) it seemed that this type of radiation was associated with the subsidiary *B* and *C* "sources"<sup>2</sup> on Jupiter rather than with the main source *A*. It is the purpose of this communication to point out a similar tendency in certain polarization observations as well as in the lower frequency observations of Jupiter.

During the 1965–66 apparition a detailed investigation was made at the Florida State University Radio Observatory of the polarization of the radiation from Jupiter. These observations have been reported briefly<sup>3</sup> and a more detailed account will be published later. Left- and right-hand components were compared at 16, 18 and 22 Mc/s using very short time constants and several different chart-speeds as a part of the millisecond pulse experiment already mentioned. The polarization was observed as one of several criteria for establishing that the short pulses did indeed originate from Jupiter.

During the course of the apparition a more elaborate study was also developed at 18 Mc/s. Based on the system described by Cohen<sup>4</sup>, a polarimeter was constructed which measures left- and right-hand components and also the phase and correlation between these components so that all the polarization parameters can be determined. Some correlation measurements previously made by Sherrill<sup>5</sup>, using a longer time constant system, indicated a fairly high degree of polarization, usually greater than 80 per cent. This is confirmed in general for the events analysed so far, although fairly rapid variations sometimes occur

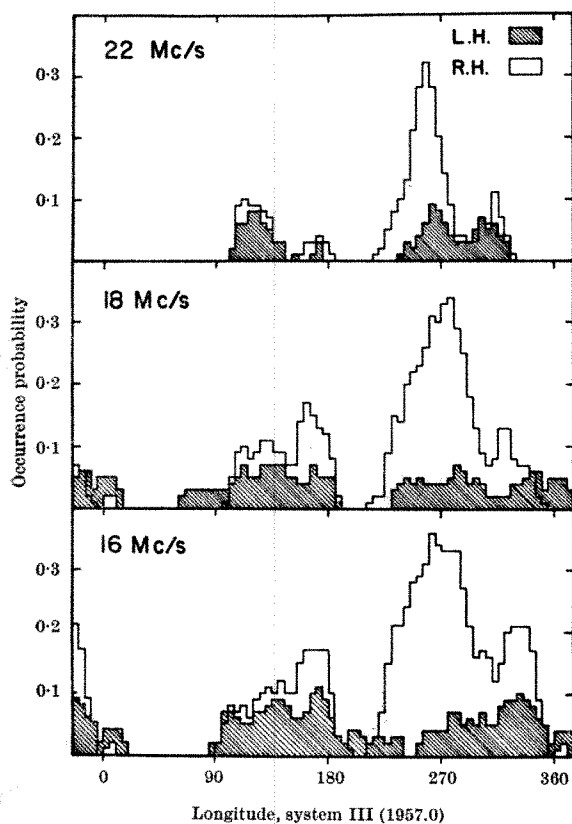


Fig. 1. Histograms of left- and right-hand polarized Jupiter activity, November 1965–March 1966.

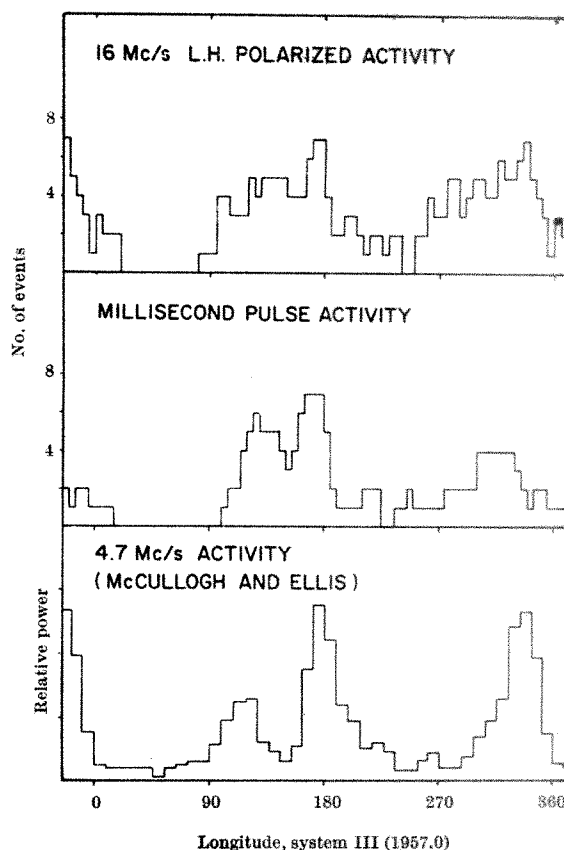


Fig. 2. Comparison of different observations in which the main source *A* histogram peak is absent.

which would not have been detectable with Sherrill's polarimeter. While it is unlikely that a detailed analysis of all the observations will be completed for some time, the general trend of the polarization studies can be seen from histograms of periods of predominantly left- and right-handed activity at each frequency.

These histograms are shown in Fig. 1. It can be seen that, as in previous investigations, right-handed polarization predominates at all frequencies, while the incidence of left-handed polarization increases somewhat towards the lower frequencies. In Fig. 2, the histogram of left-handed polarized activity at 16 Mc/s is compared with the histogram of millisecond pulses observed during the same period<sup>1</sup>. The profile of relative power obtained by McCulloch and Ellis<sup>6</sup> at very low frequency is shown in the same diagram. It can be seen that there is a general similarity in the longitude profiles for the millisecond pulse events, the left-handed polarized events and the relative power at 4.7 Mc/s. In particular, the main source *A* peak is not present, while the *B* and *C* sources peaks and the *D* null region are as well defined as in most general observations. Similar tendencies have been found to some extent in earlier investigations by other workers. Dowden<sup>7</sup>, for example, found that the source *A* peak was absent from left-handed polarized bursts observed at 10.1 Mc/s in 1962. Sherrill and Castles<sup>8</sup> and Barrow<sup>9</sup> found some evidence of a left-handed peak corresponding to source *C* at 17.2 and 16 Mc/s in 1962 and 1963.

In the present series of observations, although the millisecond pulses all showed a definite polarization, there did not appear to be any systematic tendency for either left-handed or right-handed pulses to predominate. In other words, the millisecond-type pulses and the left-handed polarization may be independent characteristics of the *B* and *C* sources.

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### Crater Diameter-Depth Relationship from Ranger Lunar Photographs

IN 1965, Baldwin<sup>1</sup> investigated the relationship between diameter and depths for some lunar craters from *Ranger VII* photographs. He used the values from the *Ranger Lunar Charts* (RLC) published by the Aeronautical Chart and Information Center. Baldwin found that the diameter-depth relationship for these craters may be represented by the equation

$$D = 0.0256d^2 + d + 0.6300 \quad (1)$$

where  $D$  and  $d$  are the logarithmic diameter and depth of craters in feet.

Recently, I measured the cross sections of some primary craters from the *Ranger VII*, *VIII* and *IX* photographs<sup>2</sup>. Fig. 1 shows the measured values of logarithmic diameter ( $D$ ) and depth ( $d$ ) in metres. There is no difference between the diameter-depth relationship for craters, photographed by the three different *Ranger* missiles

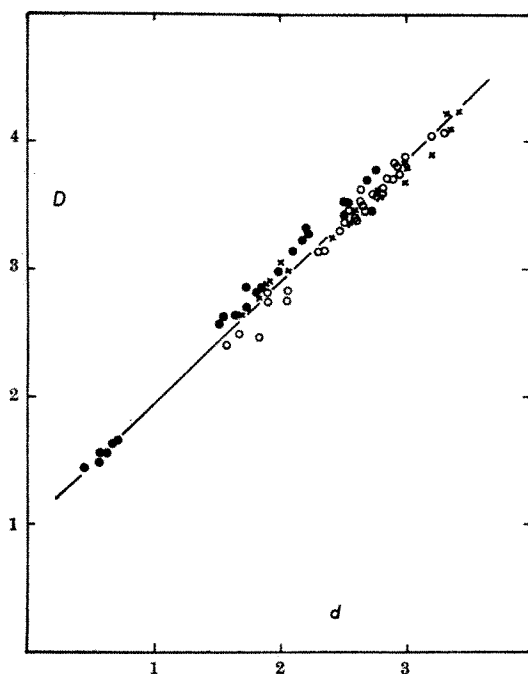


Fig. 1. Logarithmic diameter  $D$  versus logarithmic depth  $d$  (both in m) from *Ranger VII* (○), *Ranger VIII* (×) and *Ranger IX* (●) photographs.

in different lunar regions. The crater diameter-depth relationship, shown in Fig. 1, may be represented by the equation

$$D = 0.96d + 0.98 \quad (2)$$

where  $D$  and  $d$  are the logarithmic diameter and depth of craters in meters. The relationship between  $D$  and  $d$  is practically linear for craters, diameters of which are between 29 and 17,100 m. This fact shows that the well known Ebert's rule is not valid for small lunar craters.

The results obtained are not a contradiction of the meteoritic-impact theory of the origin of lunar craters and it seems that most of the small craters are of meteoritic origin. I thank Professor H. Haffner, director of the Hamburg Observatory, for the hospitality of this institution and for many informative discussions.

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Received December 12, 1966.

<sup>1</sup> Baldwin, R. B., *Astron. J.*, **70**, 545 (1965).

<sup>2</sup> "Ranger" VII Photographs of the Moon, Part II, Camera "B" Series (Photographic Edition) (Jet Prop. Lab., Calif. Inst. of Technology, 1964). "Ranger" VIII Photographs of the Moon (Photographic Edition, Camera "B") (1965). "Ranger" IX Photographs of the Moon (Photographic Edition, Camera "B") (1965).

### Kamacite-Taenite Relationships in Iron Meteorites

A NUMBER of investigators<sup>1-3</sup> have used the method of electron probe X-ray microanalysis in the examination of metallic meteorites. For those simple octahedrites which are free of visible thermal metamorphism there is general agreement that the various morphological forms of taenite are distinctly non-homogeneous with respect to nickel, whereas the bulk of the kamacite is of more uniform nickel content, although there is a decrease of nickel within the kamacite where it comes into contact with taenite<sup>1,3</sup>. Extensive measurements of this effect have been made by Agrell, Long and Ogilvie<sup>3</sup> on a sample of the Canyon Diablo meteorite, for which they found a nickel content of  $6.8 \pm 0.2$  per cent for the bulk kamacite as compared with about 5.7 at the interface. In the machine used by Agrell, Long and Ogilvie the X-rays could be collected in a direction lying in the plane which contained the normal to the specimen surface and the intersection of the kamacite-taenite boundary with the surface. This simple geometry minimizes the anomalous effects which can arise if X-rays generated in one phase are absorbed by passage through another phase.

In the present work an A.E.I. SEM2 microprobe analyser was used. This is a fully focusing machine of different geometry from that used by Agrell. The meteorite specimens were cut in such a way that the kamacite-taenite interfaces under examination were perpendicular to the surface of the specimen; the traverse of the probe was perpendicular to the interface and also coincided with the Rowland circle of the spectrometer.

Some specimens of the Canyon Diablo meteorite contain crystals of the carbide phase, cohenite, whereas other specimens are free of cohenite and it is not clear which type of material was examined by Agrell. In the present work samples of both cohenite free (CF) and cohenite rich (CR) Canyon Diablo material were examined. In addition, samples of artificially shock hardened CF material were available at 138 and 228 kbar shock wave pressures respectively. Both samples showed the acicular kamacite structure which is characteristic of cosmically shock hardened meteorites and both samples were subject to microprobe examination. Finally, samples of the cosmically shock hardened meteorite Trenton were examined. In all cases the microprobe examination was conducted on unetched specimens after a detailed metallographic

Table 1

Meteorite	Matrix bulk (per cent nickel content)	Kamacite- taenite inter- face (per cent nickel)	Taenite width ( $\mu$ )	Maximum per cent nickel at taenite interface
1 Canyon Diablo CR	6.8 ± 0.1	6.1 ± 0.2	28	42 ± 1
2 Canyon Diablo CR	6.7 ± 0.1	5.7 ± 0.1	34	42 ± 1
3 Canyon Diablo CF	6.9 ± 0.1	5.8 ± 0.2	32	38 ± 1
4 Canyon Diablo CF	6.8 ± 0.2	6.0 ± 0.2	43	42 ± 1
5 Canyon Diablo CF	6.8 ± 0.2	6.1 ± 0.2	Plessite	43 ± 1
6 138 kbar CF	6.8 ± 0.1	6.1 ± 0.2	34	40 ± 1
7 228 kbar CF	6.7 ± 0.1	5.9 ± 0.1	34	40 ± 1
8 228 kbar CF	7.0 ± 0.1	6.1 ± 0.2	50	44 ± 2
Overall mean	6.81	5.97		
9 Trenton	7.4 ± 0.1	6.9 ± 0.1	Plessite	32 ± 3
10 Trenton	7.3 ± 0.1	7.0 ± 0.1	Plessite	35 ± 1
11 Trenton	7.5 ± 0.1	7.0 ± 0.1	Plessite	34 ± 2
Overall mean	7.4	7.0		

examination of the etched specimen outside the microprobe analyser. The results are laid out in Table 1, from which it appears that there is satisfactory agreement between the present measurements on Canyon Diablo meteorite and the results previously obtained by Agrell, Long and Ogilvie using a different machine.

In Table 1 measurements 1-4 and 6-8 relate to bands of taenite, and the tabulated values are the means of values measured on either side of the bands. Measurement 5 relates to the interface between massive kamacite and the outer, nickel rich, taenite which surrounds a plessite region. The metallographic evidence suggests that all such interfaces form under the same conditions and this is supported by the similarity of interface nickel values. All measurements on Trenton were made on the outer rims of plessite areas.

It appears from Table 1 that the conditions at the kamacite-taenite interfaces are identical for both cohenite free and cohenite rich samples of Canyon Diablo. This suggests that both types of material formed under similar conditions and are, in fact, portions of the same large body.

The kamacite-taenite interface relationship is not altered by artificially induced shock wave transformation, at least up to shock pressures of 228 kbar, and it can be presumed that interface composition relationships are similarly undisturbed by the intervention of cosmically induced shock transformation in this pressure range. The kamacite of Trenton has a microhardness (249) similar to that of Canyon Diablo after 228 kbar shock (245) and has a very similar microstructure; thus the interface relationships obtained for Canyon Diablo can be compared directly with those obtained for the cosmically shock hardened material of the Trenton meteorite.

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### Trace Element Content of Antarctic Lakes

THE Taylor and Wright Valleys, McMurdo Sound, Antarctica, contain a series of lakes of particular geochemical interest. Several of these, including Lakes Bonney, Joyce, Fryxell and Hoare in the Taylor Valley and Lake Vanda in the Wright Valley, are, although ice covered, not completely frozen. They are also saline and strongly density stratified, with the waters at the bottom of the lakes containing large amounts of dissolved solids<sup>1-7</sup>. A recent survey of the concentrations of a number of trace elements in the water of the above lakes has been undertaken with the intention of elucidating the origin of the salts present in them<sup>8</sup>. This has shown that it is possible that Lake Bonney has had a sea water origin and

that Lake Fryxell may also have contained some sea water in the past, but that it is unlikely that the other lakes considered here were of marine origin.

Neglecting a sea water origin for the lakes, the salts present could have come from three possible sources:

(1) *Atmospheric recirculation*. It is well known that rain water at considerable distances from the sea contains appreciable quantities of several inorganic ions<sup>9</sup> and it is possible that the salts in the lakes are in part obtained from melted snow. Salts from blown sea spray have also been suggested as contributing to the dissolved material in Lake Fryxell<sup>2</sup>.

(2) *Thermal springs*. These have been suggested as the source of heat in Lakes Vanda, Bonney and Fryxell<sup>3,10</sup> but the evidence is not conclusive<sup>5,6,11</sup>.

(3) *The surrounding rocks*. The Antarctic environment causes widespread alteration of rocks and minerals converting those which are normally stable into readily soluble forms<sup>12</sup>. Elements which are thereby released can be collected into lakes and pools if periodic flushing of the soil occurs, as in the annual thaw. Melt waters of glaciers have been shown to contain small amounts of dissolved rocks<sup>13</sup>. Since all these lakes are fed by the melt waters of glaciers, it is reasonable to expect that elements extracted from the rocks by the glaciers and contained in the melt waters would contribute to the salts in the lakes.

Assuming that the last source is the most probable origin of the elements present in the lakes (there is no definite proof for the existence of thermal springs and the contribution from atmospheric precipitation should be negligible), the relative concentrations of trace elements in the lakes with a probable non-marine origin should provide an indication of possible regions of mineralization in the area.

It has been shown<sup>14,15</sup> that about 1,000 yr ago Lake Vanda had lost most of its water by evaporation (the lake has no outflow), after which the climate changed and fresh water flowed in on top of the strong salt solution and since then the salts have been diffusing upwards. A similar mechanism is proposed for the formation of density gradients in the other lakes. The ions present in the bottom waters are therefore much more concentrated than they were in the original water entering the lakes.

Provided there has been no precipitation of dissolved material during the period in which the lakes are assumed to have evaporated to low bulk, the ratio of trace element concentration to that of total dissolved solids in the lakes now will be the same as in the water originally present in the lakes. If this water was originally supplied by glacial-melt and other surface waters only, any anomalously high relative concentrations of trace elements in the lakes should provide a guide to mineralization in the area.

Table 1. CONCENTRATION OF CERTAIN ELEMENTS IN THE BOTTOM WATERS OF LAKES OF MCMURDO OASIS, ANTARCTICA (DATA FOR RIVER WATER INCLUDED FOR COMPARISON)

Lakes	Concentration ( $\mu\text{g/l.}$ )					
	Zinc	Lead	Bismuth	Iron	Manganese	Molybdenum
Vanda	5,400	< 30	6.1	490	44	2.1
Bonney	150	< 30	7.1	640	23	8.7
Fryxell	28	42	< 2	14	2.9	0.23
Joyce	38	340	3.8	500	27	7.0
Hoare (East Lobe)	31	83	< 2	130	11	1.0
Hoare (West Lobe)	20	91	< 2	500	18	3.1
River water <sup>17</sup>	—	4	—	300	20	0.35

Table 2. RATIO OF THE CONCENTRATION OF CERTAIN ELEMENTS TO THE CONCENTRATION OF TOTAL DISSOLVED SOLIDS IN THE BOTTOM WATERS OF LAKES OF MCMURDO OASIS, ANTARCTICA

Lakes	Ratio ( $\times 10^3$ )					
	Zinc	Lead	Bismuth	Iron	Manganese	Molybdenum
Vanda	31	< 0.18	0.035	2.8	0.25	0.011
Bonney	0.38	< 0.077	0.018	1.6	0.059	0.022
Fryxell	2.9	4.3	< 0.21	1.4	0.80	0.024
Joyce	7.6	68	0.76	100	5.4	1.4
Hoare (East Lobe)	31	83	< 2	130	11	1.0
Hoare (West Lobe)	18	83	< 2	450	17	2.6

The absolute concentrations of certain trace elements in these lakes are given in Table 1 while the concentrations relative to the total dissolved material in the lakes are given in Table 2. The elements were determined by a combination of solvent extraction with cyclohexanone and emission spectroscopy. The method is fully reported elsewhere<sup>8</sup>. Data for Lake Bonney are included for the sake of completeness though they are not discussed in this paper to any extent.

The most significant high relative concentrations of zinc are in Lakes Vanda and Joyce. Lake Hoare is fed by the Canada Glacier on the Asgaard Range, which separates the Taylor and Wright Valleys, while Lake Vanda is fed by melt waters of the Wilson-Piedmont Glacier, the Clarke Glacier, and subsidiary glaciers flowing into the Wright Valley from the Asgaard Range. Accordingly, it is probable that the region of the Asgaard Range near the Canada Glacier may contain significant zinc deposits. This is supported by the occurrence of zinc in Lake Fryxell which is fed by the Canada and Commonwealth Glaciers, both of which are on the Asgaard Range.

The lakes containing the highest relative concentrations of lead are Joyce and Hoare. The former is fed principally by waters from a local glacier with some melt water from the Taylor Glacier. Lead was not detected in Lake Bonney so that the presence of this element in the material in the Taylor Glacier is unlikely. It is therefore probable that lead occurs in the area of the local glacier supplying Joyce and also in the area surrounding Hoare. The relative concentration of lead in Lake Fryxell is considerably less than in Hoare, a similar trend to that shown for zinc. This would seem to imply that the area containing relatively high amounts of zinc and lead in the region of the Canada Glacier does not extend to the Commonwealth Glacier.

Although lead has been shown to be among the most common minor elements in river water<sup>16</sup>, so that it would be expected to be found in waters as concentrated as those considered here (assuming a glacial origin), the fact that it is undetectable in the lakes having the highest concentration of total dissolved material (Bonney and Vanda) makes its relatively high concentration in the others significant.

The high relative concentration of bismuth in Lake Joyce implies a probable deposit of bismuth in this area. This chalcophilic element is often found in galena and it is interesting to note that this lake also contains a high concentration of lead.

Iron constitutes a major fraction of most igneous rocks and would therefore be expected to be present in reasonable concentrations in all lake waters. Although the relative concentration of iron varies appreciably between the lakes, the absolute value, with the exception of Lake Fryxell, remains relatively constant. The concentration therefore seems to be independent of the age or composition of the lake, suggesting a chemical factor influencing the solubility of the iron.

Manganese is also relatively common in igneous rocks and is readily leached from soils and rocks. It is present in river waters in concentrations ranging from 0 to 185 µg/l. with a medium value of 20 µg/l.<sup>17</sup>. As with iron, the variation in absolute concentration is not large, with the exception of Lake Fryxell, and the concentrations of iron and manganese in the lake waters are within the range found in river waters for these elements.

Molybdenum is present in igneous rocks in trace amounts and tends to concentrate in granites, which have been shown to be present in this region<sup>18,19</sup>. It is also commonly found in river waters in concentrations ranging from 0 to 6.9 µg/l.<sup>16</sup>. With the exception of the western lobe of Lake Hoare, the concentration of molybdenum in the lakes lies very close to this range and the variation is therefore most probably not significant.

The above geochemical reconnaissance suggests that the two most likely areas for mineralization in the McMurdo Oasis area of Antarctica are the regions surrounding and

feeding water to Lake Hoare and the region surrounding Lake Joyce. Zinc deposits may also be present in the area between the Lower Wright Valley and Lake Hoare.

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## Meteorology and the Great Fire of London, 1666

THE tercentenary of the Great Fire of London (September 2-5, 1666) has given rise to discussion of the incidental meteorological factors. Atallah<sup>1</sup> compares the London fire with that at Hamburg during the Second World War and with the Chicago fire of 1871. He states that the low velocity ground winds at Hamburg and Chicago were ideal for the generation of fire-storms (that is, convection columns in which super-adiabatic atmospheric temperature lapse rates lead to vortices of hurricane force) but that there was no indication of such conditions in the London fire. The purpose of this communication is to consider the inherent (natural) stability or instability of the air mass as a further relevant factor in the manner and intensity of the development of the London fire.

Meteorological evidence for 1666 is limited and any conclusions about the relationship of the fire and the weather must necessarily be somewhat speculative. The summer of 1666 is classified by Shepherd<sup>2</sup> as fine and described by Short<sup>3</sup> as very hot. Samuel Pepys refers, on September 2, 1666, to a long drought, "it being brave dry and moonshine and warm weather . . . and everything, after so long a drought, proving combustible, even the very stones of churches".

The prevailing easterly winds and persistent fine weather<sup>4</sup>, at this time of the year 1666, suggest that atmospheric instability may not have been extreme. Evelyn<sup>4</sup> gives corroborative evidence that smoke from the Great Fire trailed 50 miles in the form of a "column": even allowing for any exaggeration on the part of the diarist, this picture is not what would be expected with a fair wind speed and a steep lapse rate. On the fourth day of the fire (Wednesday, September 5), following a decrease of wind during the previous night, the fire for the first time "lost its capacity for large destruction", and smoke, "having no wind to disperse it, hovered low".

It might be argued that there could have been in September 1666 some persistence of the exceptional instability of the earlier part of the summer and the



preceding spring. There were reports<sup>5</sup> of phenomenal hailstorms on July 17, 1666, in East Anglia, with stones "full as bigg as turkey's eggs" and "one hailstone 12 inches about", and a "great tempest of thunder" and raining fish just before Easter 1666 at Cranstead, Kent. Both the areas mentioned are particularly exposed to winds between easterly and northerly, on the east side of England, and the maximum symptoms of instability were apparently at some distance from the coast; thus the reported phenomena may well be a further manifestation of a period of relaxed westerlies in mid-1666. Again, this state of affairs would agree with the report that 1666 was "a very droughty year"<sup>3</sup>.

The strong instability of spring and early summer might thus be explained by the evidently relaxed westerlies allowing a more marked difference between the heating of land and sea, thus leading to a more intense European monsoon effect. Such instability, however intense, would not necessarily or indeed normally extend into late summer, and all the available evidence suggests that it did not persist in the London area during early September 1666. This assessment of the weather sequence is further supported by the estimate<sup>6</sup> that 1666 was a year of sunspot minimum and by the observed association of a sunspot minimum year with similar unstable weather of transient type<sup>7</sup>.

On the other hand, at Hamburg at 1700 G.M.T. on July 27, 1943 (the day before the night of the fire), an aircraft sounding shows a sharp lapse rate, from 1,000 to 12,000 ft., of 4.8° F (ref. 8); and on the 0700 G.M.T. synoptic weather chart for this day, Germany is situated in a col pressure area (1,015 mbar) between lows over the north-east Atlantic and Russia.

Similarly, in the Chicago region, between October 7 and 10, 1871, the decreasing atmospheric pressure (about 1,005 mbar on October 10 at Detroit) and increasing southerly or south-westerly winds spreading across the area from the west (reaching 34 miles/h on the tenth at Detroit) and changes in pressure, wind and weather generally<sup>9</sup>, reflect a type of disturbed weather which could well be associated with fairly steep lapse rates up to medium levels.

Furthermore, it is not certain that the increasing winds of the Chicago fire were entirely due to a "fire-storm", for there were atmospheric pressure changes which could be expected to lead to an increasing pressure gradient and increasing wind velocities over a wide area. For example, during the 24 h following 0735 h on October 7, 1871, the wind speed at Chicago is reported to have increased from 10 to 19 miles/h, but there were corresponding increases of from 0 to 6 miles/h at Toledo, more than 200 miles to the east, and from 10 to 16 miles/h at Davenport, more than 100 miles to the west<sup>9</sup>.

It is therefore suggested that at the time of the Great Fire of London in 1666 the natural instability of the air mass around London could well have differed substantially from that of the Hamburg and Chicago fires, and atmospheric instability may have been an important differentiating factor in the manner and intensity of fire development.

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## THE SOLID STATE

### Methods for interpreting Electron Diffraction Patterns of Thin Alloy Films

THE electron diffraction patterns of thin films of alloys sometimes show fine detail that is not fully understood<sup>1</sup>. Empirical methods of solution are not easy to apply in view of the large number of possible models. We are therefore investigating the possibility of using optical diffraction methods<sup>2</sup>; the diffraction pattern of a mask of punched holes representing an atomic arrangement can be directly compared with the observed electron diffraction pattern.

As an example, we have considered the relatively simple pattern (Fig. 1) given by the alloy Pd<sub>3</sub>Mn (ref. 3). The structure is assumed to be based on a perfect lattice containing anti-phase domains, the unit being the structure postulated by Schubert *et al.*<sup>4</sup>. To simplify the punching of the mask we have represented a palladium atom by a hole and the manganese atom by absence of a hole (Fig. 2); the fine detail of the diffraction pattern is not affected by this procedure, and its intensity is enhanced with respect to the main orders of diffraction. Thus the pattern is clearer than if we had attempted to represent the relative scattering factor of the two atoms.

Because the mask is rather small, we have attempted to incorporate only two types of domain; the complete structure was simulated by making a double exposure of the diffraction pattern with the mask turned through 90°

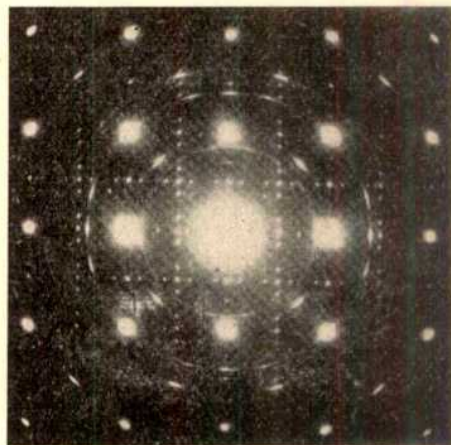


Fig. 1. Electron diffraction pattern from a thin film of Pd<sub>3</sub>Mn, containing about 25 per cent manganese, annealed from 650° C to 500° C in 28 h. (Originally published in *Trans. Jap. Inst. Met.* **3** (4), 234 (1962).)

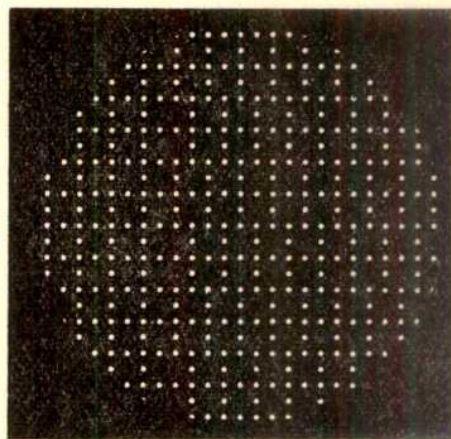


Fig. 2. Mask representing atomic positions in Pd<sub>3</sub>Mn.



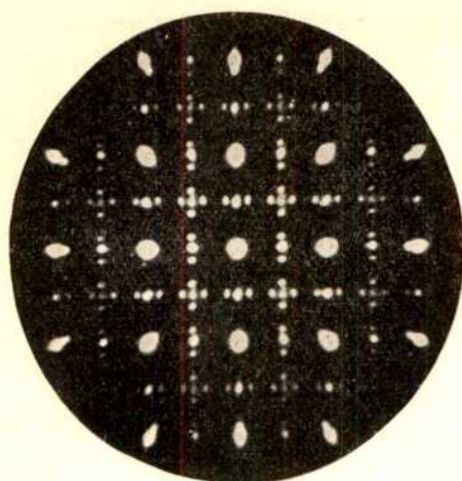


Fig. 3. Optical diffraction pattern obtained from mask shown in Fig. 2.

in its own plane. The resulting diffraction pattern (Fig. 3) shows excellent agreement with the electron diffraction pattern.

We hope to extend this work to more complicated problems. The method cannot, of course, be complete in itself, as it is only two-dimensional, but we hope that it can provide models that can be tested by more conventional methods. To this end we are proposing to use an optical diffractometer with lenses of 35 cm diameter and 10 m focal length so that far more detail can be inserted in the masks than is possible with the present diffractometer.

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### Influence of Heating Rate on Transformations in an 18 per cent Nickel Maraging Steel

SOME observations were made of the effect of heating rate on the martensite (M)→austenite (γ) reversion in a 300-grade 18 per cent nickel maraging steel. These are discussed here with reference to dilatometry, although the conclusions drawn are based also on other sources. Heating rates varied from 0.0037° to 1,200° F/sec, and covered a much wider range than has been reported for alloys of this type<sup>1</sup>. The composition of the material in wt per cent is as follows: 18.40 nickel, 8.86 cobalt, 0.60 titanium, 0.09 aluminium, 0.02 carbon, 0.04 silicon, 0.05 chromium, 0.06 vanadium, 0.008 sulphur, 0.007 phosphorus, 0.016 zirconium, 0.003 boron, 0.05 calcium (added). All specimens were initially in the annealed-martensitic condition.

Heating curves for annealed wire and cylindrical specimens are shown in Figs. 1 and 2, respectively. The inflexion points, denoted by  $T_A$ ,  $T_B$ ,  $T_C$ , and  $T_E$ , are plotted as a function of heating rate in Fig. 3. Up to 40° F/sec these points are displaced to higher temperatures with higher heating rates, which suggests diffusion control. Plots of the logarithm of heating rate, up to 40° F/sec, against  $1/T$  for  $T_A$ ,  $T_B$ , and  $T_C$  give activation energies of about 80 kcal/mole, consistent with values reported for substitutional solute diffusion<sup>2-6</sup> in both α and γ iron.

The dip between  $T_A$  and  $T_B$ , present up to 40° F/sec in the curves of Figs. 1 and 2, is attributed to precipitation.

Precipitates rich in molybdenum, titanium and nickel have been identified as forming during ageing of this alloy<sup>7-9</sup> and serve as nucleation sites for austenite formation. We therefore feel that the large contraction between  $T_B$  and  $T_C$  is due to the M→γ reversion at precipitates, and is referred to as precipitate reversion. Since the γ that is formed would be relatively rich in molybdenum, titanium

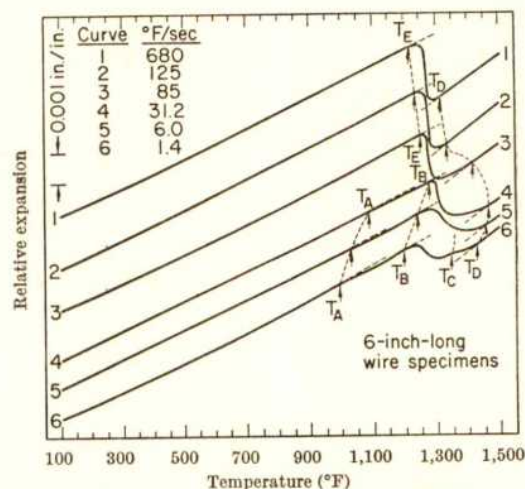


Fig. 1. The effect of heating rate on the dilatometric behaviour observed during the heating of a 300-grade 18 per cent nickel maraging steel. Before testing, specimens were annealed at 1,500° F in *vacuo* for 2 h. Wire specimens, resistance-heated, were used for heating rates of between 1.4° and 1,200° F/sec.

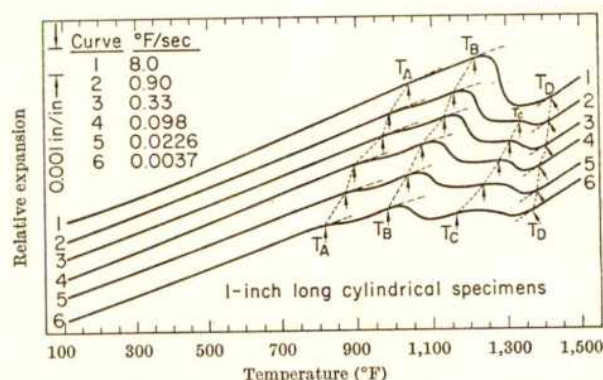


Fig. 2. The effect of heating rate on the dilatometric behaviour observed during the heating of a 300-grade 18 per cent nickel maraging steel. Before testing, specimens were annealed at 1,500° F in *vacuo* for 2 h. Cylindrical specimens, furnace-heated, were used for heating rates of between 0.0037° and 8° F/sec.

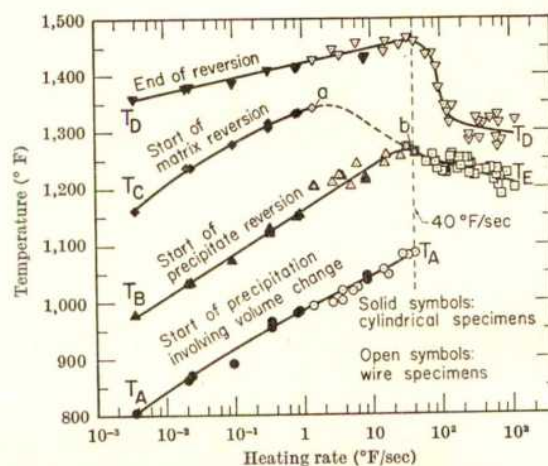


Fig. 3. Semilogarithmic plots of temperature against heating rate for both wire and cylindrical specimens. Temperatures correspond to the inflexion points on the heating curves of Figs. 1 and 2.

and nickel, which are elements that lower the  $M_s$  point<sup>7,10</sup>, much of this  $\gamma$  iron is retained on subsequent cooling.

During precipitation below  $T_B$  the martensite matrix was largely depleted in nickel; accordingly, the temperature at which  $\gamma$  iron starts to form is raised. The transformation of this nickel-poor M to  $\gamma$ , which is referred to as matrix reversion, is initiated, we believe, at the inflexion point,  $T_C$  (curve 6 of Fig. 1 and curves 2 to 6 of Fig. 2). During both stages of reversion, partitioning of alloying elements occurs between the M and  $\gamma$  phases. The transformation is completed at  $T_D$ ; large composition variations, however, are still present.

At rates above 40° F/sec both precipitation and precipitate reversion are absent. The transformation occurs completely by matrix reversion without any previous change in composition, such as occurs at low heating rates. To distinguish between the presence or absence of such a change in composition, matrix reversion at the high heating rates is designated by  $T_E$  instead of  $T_C$ . Between 40° and 100° F/sec, matrix reversion is accompanied by partitioning of alloying elements, which affects the transformation finish temperature,  $T_D$ . With less time for diffusion at increasingly higher rates, the degree of partitioning decreases rapidly, resulting in a sharp drop in  $T_D$  as a heating rate of 100° F/sec is approached. At still higher rates, diffusion is absent and only minor variations in  $T_E$  and  $T_D$  are observed.

Both  $T_C$  and  $T_E$  represent the initiation of matrix reversion and accordingly are joined in Fig. 3 by an arbitrary curve  $ab$ . Since  $T_C$  is absent on the dilatometric curves for heating rates between 2° and 40° F/sec, it may be that, in this range, matrix reversion is masked by the presence of concurrent precipitation and precipitate reversion. At  $a$  extensive precipitation and alloy depletion of the martensite occur before matrix reversion; at  $b$  this prior behaviour is absent. With an increase in heating rate between  $a$  and  $b$ , less time is available for precipitation, alloy depletion, and precipitate reversion before matrix reversion starts. Thus  $T_C$ , the  $\gamma$ -start temperature for matrix reversion, is lowered from  $a$  to  $b$ . As there is some uncertainty in assigning the data points at  $b$  specifically to  $T_B$ ,  $T_E$ , or  $T_C$ , a combination of symbols is used which represents all three curves.

It would generally be expected that diffusionless reactions should be independent of heating rate. Yet a decrease in reversion temperature is obtained with an increase in heating rate above 40°/sec. This may possibly be related to nucleation sites in the martensitic structure, which may be more readily retained at the elevated temperatures with faster heating rates. This behaviour may also be caused by the presence of two or more competing diffusionless transformations. It has been shown for the case of cooling that different  $M_s$  values are obtained for iron-nickel alloys, and depend on whether the diffusionless reaction is massive or shear<sup>11</sup>. We are now involved in studies directed toward clarifying the dependence of heating rate on the nature and temperature of the transformation. This work was performed under the auspices of the U.S. Atomic Energy Commission.

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## Crystal Structure of Solid Hydrogen Chloride and Deuterium Chloride

It has long been known from measurements of specific heat<sup>1</sup> that solid hydrogen chloride undergoes a first order phase transition at about 98.4° K. A similar phase transition was later found<sup>2</sup> in solid deuterium chloride at about 105.0° K. X-ray powder photographs<sup>3</sup> revealed that the crystal structure of hydrogen chloride was orthorhombic below the transition point and cubic above it. They also indicated that the chlorine atoms formed a face-centred lattice in both phases, but gave no clue regarding the location of the hydrogen atoms. Subsequent dielectric constant<sup>4</sup>, infra-red and Raman spectra<sup>5</sup> and nuclear magnetic resonance<sup>6</sup> investigations resulted in a variety of structural models for both phases of solid hydrogen chloride. Nevertheless, the indirect character of the structural information provided by these investigations did not allow an unambiguous choice between the various models.

Table 1

Substance	Temperature (°K $\pm$ 0.2 °K)	No. of observed powder lines	Lattice type	Lattice parameters (Å $\pm$ 0.001 Å)
Hydrogen chloride	92.4	51	face-centred orthorhombic	$a = 5.082$ $b = 5.410$ $c = 5.826$
Deuterium chloride	92.4	41	face-centred orthorhombic	$a = 5.068$ $b = 5.399$ $c = 5.828$
Hydrogen chloride	118.5	21	face-centred cubic	$a = 5.482$
Deuterium chloride	118.5	16	face-centred cubic	$a = 5.475$

To resolve this ambiguity, the structure of solid hydrogen chloride and deuterium chloride was investigated by X-ray and neutron powder diffraction methods. The X-ray powder patterns were recorded photographically using a Weissenberg camera, a temperature-controlled liquid nitrogen cryostat and copper radiation, monochromatized by a curved crystal of lithium fluoride. The results of this investigation are summarized in Table 1. They show (i) that the crystal structures of hydrogen chloride and deuterium chloride are isomorphous, although the unit cell of deuterium chloride is significantly smaller in both phases; (ii) that the reduction in the size of the orthorhombic unit cell of deuterium chloride is strongly anisotropic; it lies almost entirely in the (001) plane. This was interpreted as an indication that the bonds of hydrogen chloride and deuterium chloride are parallel to (001) plane.

The neutron powder patterns were recorded by an automatic neutron diffractometer using a temperature-controlled liquid nitrogen cryostat and crystal monochromatized thermal neutrons of wavelength 1.03 Å. The neutron powder pattern of orthorhombic deuterium chloride recorded at 77.4° K showed several new lines compared with the corresponding X-ray powder pattern. Some of the new lines were due to the cryostat, others to the specimen of deuterium chloride. All deuterium chloride lines could be unambiguously indexed on the basis of the orthorhombic lattice parameters given in Table 1. This confirmed that the orthorhombic unit cell derived from the X-ray powder pattern was the true unit cell of the structure, but it also revealed that the true unit cell was not face-centred as suggested by the X-ray data.

The systematic absences observed in the neutron powder pattern were compatible with two orthorhombic space groups:  $Bbmm$  and  $Bb2_1m$ . The centrosymmetric  $Bbmm$  space group had to be excluded, however, because none of the four-fold special positions allowed by this space group for the deuterium atoms could account for the intensity distribution of the observed neutron powder pattern.

The structure was finally solved on the basis of the non-centrosymmetric  $Bb2_1m$  space group. It was subsequently



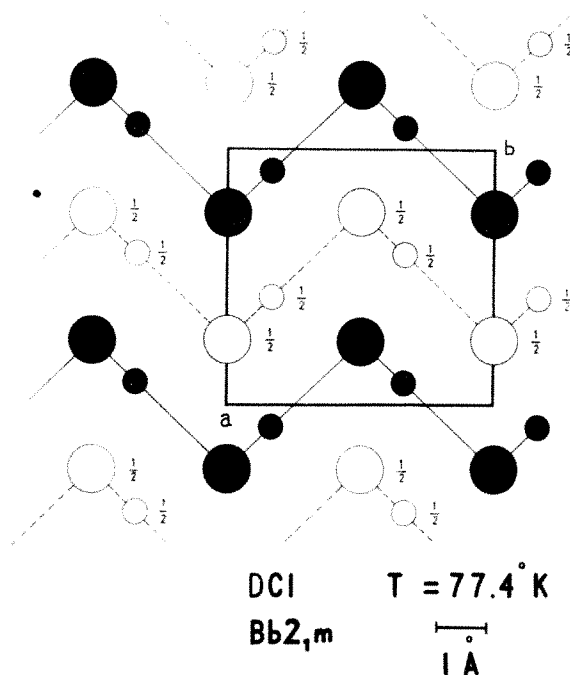


Fig. 1.

refined by the least-squares method using all the fifteen integrated intensities which could be derived with sufficient accuracy from the neutron powder pattern. Because of the rather small number of observed intensities, only isotropic thermal parameters were used in the refinement procedure. The results are summarized in Table 2 and a diagram of the structure is shown in Fig. 1. It can be seen that orthorhombic deuterium chloride consists of planar zigzag chains, all oriented in the same way and arranged in layers parallel to the (001) plane.

Table 2. STRUCTURAL DATA OF DEUTERIUM CHLORIDE AT 77.4° K

Lattice parameters:			
$a = 5.053 \pm 0.002$ Å	$b = 5.373 \pm 0.001$ Å	$c = 5.825 \pm 0.002$ Å	
Space group: $Bb2_m$ ; No. of molecules in the unit cell: 4			
Fractional positional parameters of the atoms:			
	$x$	$y$	$z$
Deuterium	$0.081 \pm 0.002$	$0.170 \pm 0.004$	0
Chlorine	0.25	0	0
Isotropic temperature factors of the atoms ( $B$ in Å <sup>2</sup> ):			
Deuterium	$2.7 \pm 0.5$	Chlorine	$1.71 \pm 0.01$
Bond lengths and bond angles in the plane of the chain:			
D-Cl	$1.25 \pm 0.02$ Å	Cl-Cl	$3.688 \pm 0.001$ Å
Cl-Cl-Cl	$93^\circ 31' \pm 2'$		
Final $R$ factor after six cycles of isotropic refinement: $R_1 = 2.9$ per cent.			

The neutron powder pattern of cubic deuterium chloride recorded at 111.5° K showed no new lines compared with the corresponding X-ray powder pattern. This confirmed that the face-centred cubic unit cell derived from the X-ray powder pattern was the true unit cell of the structure. It also indicated that in cubic deuterium chloride the deuterium atoms formed a similar face-centred lattice as the chlorine atoms. No ordered face-centred arrangement of the deuterium atoms could account for the observed intensity distribution of the neutron powder pattern. This discrepancy led to the conclusion that the face-centred cubic structure derived from the powder pattern was only the statistical average of a disordered structure.

Various disordered models were tested, but the small number of the integrated intensities which could be derived with sufficient accuracy from the neutron powder pattern did not allow an unambiguous choice between the models. We are attempting to increase the number of accurately measured integrated intensities by increasing both the angular range and the counting period as far as practicable.

No useful structural information could be extracted from the neutron powder patterns of the solid samples of hydrogen chloride because of the large incoherent scattering of the hydrogen atoms. Nevertheless, the close parallelism between the X-ray powder patterns as well as the specific heat and spectroscopic data of solid hydrogen chloride and deuterium chloride seem to indicate that their structure is isomorphous in both phases.

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### Effect of Pre-yield Relaxation on the Yielding of Iron

THE phenomenon of relaxation before yielding<sup>1-3</sup> has been investigated for polycrystalline iron. Specimens were loaded in tension by a small amount, and then the tensile machine was stopped to permit relaxation to occur. A sequence of such stops was continued until the occurrence of yielding as shown in Table 1. For specimens A-D at 22° C, before yielding, a halt in strain was followed immediately by a reduction of load. By contrast, stopping the machine at about the usual upper yield stress was followed by a period with almost no drop in load after which the specimen suddenly yielded (Fig. 1).

Some of the specimens were made from National Physical Laboratory iron 99.97 per cent pure by weight, with interstitial impurities of 0.0012 per cent carbon and 0.0016 per cent nitrogen. Others were made from this material zone-refined to reduce the interstitial impurities to 0.0004 per cent carbon and 0.0003 per cent nitrogen. Test-pieces with 4 BA threads at each end were machined from 3.2-mm swaged rod, recrystallized at 790° C, and either air-cooled or furnace-cooled (as noted in Table 1). They were then machined electrolytically<sup>4</sup> to uniform gauge sections of length 0.5 cm and diameter between 1.5 and 1.8 mm. In tensile tests these specimens gave the familiar stress-strain curve with a yield drop of up to 50 per cent.

The time allowed for relaxation was 3 min in the pre-yield region, and, after this, relaxation was very slow with a strain-rate of less than  $10^{-7}$  sec<sup>-1</sup>. When the specimen was replaced by massive steel connectors, the relaxation of the system was negligible, so that any load drop with a specimen resulted from deformation of the specimen. The elastic constant of the machine was determined using the massive connectors, a drop in load of 1 kg corresponding to a specimen extension of  $10^{-4}$  cm. The pre-yield stress drops therefore indicate strains of the order of  $10^{-4}$  to  $10^{-3}$ , which are much larger than those found in direct measurements of microstrain in polycrystalline iron<sup>5</sup>. It seems likely that at lower loads part of this strain arose from

Table 1. LOAD-RELAXATION PROGRAMME OF IRON SPECIMENS

Temperature °C	Iron, furnace-cooled, strain-rate $1.7 \times 10^{-4} \text{ sec}^{-1}$			Zone-refined iron, air-cooled, strain-rate $1.7 \times 10^{-4} \text{ sec}^{-1}$			Iron, air-cooled, strain-rate $1.7 \times 10^{-4} \text{ sec}^{-1}$			Iron, furnace-cooled, strain-rate $1.7 \times 10^{-4} \text{ sec}^{-1}$		
	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>
22	4.2	180	0.61	4.6	180	0.48	6.5	180	0.52	6.0	180	0.63
	7.8	180	0.51	6.8	180	0.46	9.8	180	0.55	12.1	180	0.69
	11.8	180	0.53	8.0	180	0.39	11.4	180	0.37	12.1	180	0.18
	12.9	180	0.20	9.1	180	0.36	13.0	180	0.33	15.1	2.3	0.06
	15.9	180	0.37	9.1	180	0.05	14.7	180	0.29			then
	17.7	180	0.28	10.3	180	0.41	16.3	6.0	0.07		180	6.9
	20.4	180	0.35	11.4	180	0.41			6.1			
	24.0	180	0.57	11.4	180	0.11		180				
	25.7	180	0.28	12.6	180	0.66*						
	27.9	2.7	0.03	13.8	180	0.32						
			then	13.8	180	0.09						
		180	15.6	14.9	180	0.36						
				16.0	180	0.36						
				16.0	90	0.02						
-80	Iron, furnace-cooled, strain-rate $1.7 \times 10^{-4} \text{ sec}^{-1}$											
	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>	Stress kg/mm <sup>2</sup>	Relaxation time sec	Stress drop kg/mm <sup>2</sup>
	15.0	180	3.1	5.8	180	1.1	15.0	180	0.12	14.9	180	3.9
	20.9	180	3.0	10.5	180	1.2	20.0	180	0.85	15.4	{ Strained for 74 sec	
	24.0	180	2.1	15.4	180	1.4	23.3	180	1.6	21.9	180	6.3
	27.0	180	2.1	19.9	180	1.3	25.8	180	3.8		{ Strained for 23 sec	
	29.9	36	1.5	23.4	180	1.6	27.9	180	5.8	23.4	180	6.9
		180	then	25.8	180	1.6	28.3	{ Strained for 47 sec		28.8	{ Strained for 180 sec	6.8
				28.0	180	1.3	29.4	180				
				29.3	180	0.82	29.2	180	8.8			
				30.4	180	0.64						
				31.8	180	0.76	28.1	{ Strained for 30 sec				
				32.8	180	0.76	28.1	180	7.8			
				33.9	126	0.70						
					then							
					180	13.5						

\* Specimen connectors knocked during relaxation, and increased load drop resulted.

post-yield relaxation of a small volume of material at stress concentrations in the specimen threads. It has been shown in associated work that the post-yield relaxation of iron is roughly constant up to 50 per cent extension, so that relaxation at the threads could continue to higher loads. At the 22°C yield point in these tests, however, there was initially no drop in load and, since there is no apparent reason for relaxation at specimen threads to decrease abruptly, this must mean that any such relaxation has become negligible, presumably because the threads bed-in.

The observed relaxation at stresses approaching the upper yield stress is therefore attributed to dislocation movement in the gauge length of the specimen. It has then to be explained why at the yield point there was a delay period with no measurable relaxation, followed by sudden yielding. The absence of relaxation indicates that the number of free dislocations, or of dislocation sources which could operate at the yield stress, was much reduced by the loading programme. This is supported by the observation that, on successive reloading to the same stress in the pre-yield region, the stress drop was much reduced (see Table 1 and ref. 2). The dislocations set moving during each stress increment will slow down during the subsequent relaxation, and there could be partial loss or relocking of dislocations; this could give a slower build-up of dislocation movement before the upper yield point than in a normal test. A large amount of dislocation movement could, however, still occur without being apparent, because the limit of detection of overall strain was  $2 \times 10^{-5}$ . The delay period could then be explained by an unlocking process; and since the delay is so long this indicates that to produce yielding a co-operative interaction of a large number of dislocations is required, which might be a piling of dislocations against grain boundaries, followed by a sudden release of fresh multiplying dislocations. Alternatively, the yield process might be dynamic, and require a critical amount of movement or momentum to be attained in part of the dislocation network to give

an unstable system and rapid multiplication, in which case obstruction to moving dislocations rather than unlocking could be the controlling factor. The experiments so far made do not distinguish between these explanations.

Delayed yielding is normally associated with fast strain-rates<sup>6-10</sup>, when for low stress delay times of several seconds are found at room temperature and up to 80 sec at low temperatures. Hutchison and Louat investigated for Armeo iron the effect of preloading at 65°, 100° and 150° C on the yield stress in a conventional tensile test at room temperature, and found that this was increased<sup>11</sup>; they did not report any change in the form of yield. The work most closely related to the present is that of Owen *et al.*<sup>12</sup> on pre-yield phenomena in mild steel. Some

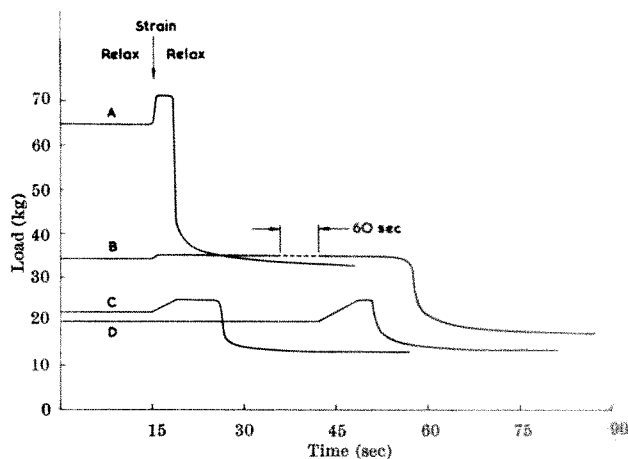


Fig. 1. Pre-yield relaxation followed by sudden yielding, in iron at 22°C. A, Pure iron, furnace-cooled, grain size 4.0 mm<sup>-0.5</sup>, area 2.55 mm<sup>2</sup>; B, zone-refined iron, air-cooled, grain size 3.9 mm<sup>-0.5</sup>, area 2.18 mm<sup>2</sup>; C, pure iron, air-cooled, grain size 4.0 mm<sup>-0.5</sup>, area 1.54 mm<sup>2</sup>; D, pure iron, furnace-cooled, grain size 4.0 mm<sup>-0.5</sup>, area 1.66 mm<sup>2</sup>. (Actual strain-rate  $1.7 \times 10^{-4} \text{ sec}^{-1}$  for curve A,  $1.7 \times 10^{-5} \text{ sec}^{-1}$  for B, C, D.)



specimens were extended at a low rate to a predetermined stress, held for several minutes, unloaded, and subsequently reloaded to a higher stress; a long period of microstrain (microcreep) preceding gross yield was obtained at liquid nitrogen temperature, but not at room temperature.

To check the effect of temperature with the present loading procedure, specimens were tested at  $-80^{\circ}\text{C}$  ( $E-H$  in Table 1). All gave a greater drop in load on relaxation in the pre-yield region than at  $22^{\circ}\text{C}$ . Specimens  $G$  and  $H$  yielded during a period of straining, at stresses of 28 and 15 kg/mm<sup>2</sup>, and their behaviour differed from that in a conventional test at  $-80^{\circ}\text{C}$  (see Table 1: the normal upper and lower yield stresses were 34 and 24 kg/mm<sup>2</sup>). Specimens  $E$  and  $F$  gave similar behaviour to that at  $22^{\circ}\text{C}$ , with sudden yielding during relaxation at about the upper yield stress, and delay times of 36 and 126 sec; however, there was a slow fall in load before the sudden yielding, and the fall in load on yielding was not so abrupt as at  $22^{\circ}\text{C}$ . These results accord with the effects of temperature already noted, showing greater dislocation movement at  $-80^{\circ}\text{C}$  than at  $22^{\circ}\text{C}$ .

The present work shows that the yield behaviour of iron when loading at conventional strain-rates depends on the loading programme. In interpreting these or other delay time experiments it is necessary to consider not only the unlocking of dislocations, but also the possibility that the mechanism controlling yield is the multiplication of free dislocations.

The work described above has been carried out at the National Physical Laboratory, with the participation of Miss J. M. Hills.

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## CHEMISTRY

### A New Type of *bis*-(Acetylacetonato)metal(II) Polymer: the Trimer of *bis*-(Acetylacetonato) Zinc

THE versatility of acetylacetonate (acac) as a ligand is well documented<sup>1</sup>. It forms metal complexes of the following structural types: (1) normal chelate compounds<sup>2,3</sup>, (2) polynuclear bridged species<sup>4-6</sup>, (3)  $\gamma$ -carbon bonded complexes<sup>7,8</sup>, and (4) condensation products formed from complexes of the third category<sup>9</sup>. We report here the structure of  $[\text{Zn}(\text{acac})_2]_3$ , which is an example of the second type with a new kind of stereochemistry in which zinc exhibits both five and six co-ordination.

Bullen, Mason and Pauling<sup>4</sup> inferred but could not prove a trimeric structure from preliminary cell measurements (now found to correspond to the non-standard monoclinic space group  $F2$ ). Recent measurements in this laboratory indicated possible space groups  $C2/m$ ,  $C2$  or  $Cm$ , with unit cell parameters of  $a=18.63$ ,  $b=8.437$ ,  $c=12.20$ , and  $\beta=112.68^{\circ}$ . The intensities of 1,928 reflexions were collected on a manual diffractometer; the Patterson synthesis unambiguously determined the space group as  $C2$ . The structure was solved by conventional Fourier and

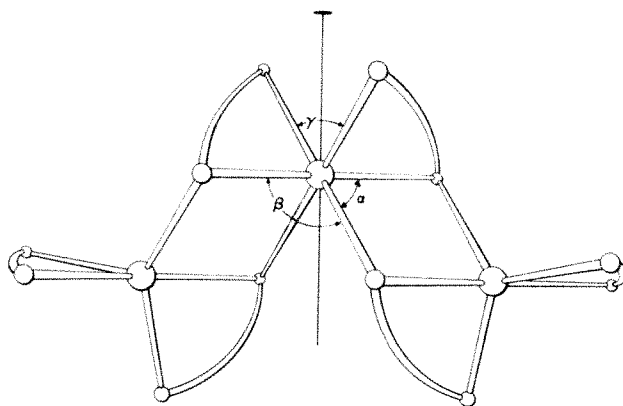


Fig. 1. Stereochemistry of  $\text{Zn}_3(\text{acac})_6$ . Carbon atoms of acetylacetonate ligands are omitted for clarity. The "octahedron" about the central zinc atom is appreciably distorted. The main deviations from  $90^{\circ}$  angles are in those marked as  $\alpha$ ,  $\beta$  and  $\gamma$  on the sketch;  $\alpha=75.1^{\circ}$ ;  $\beta=105.8^{\circ}$ ;  $\gamma=101.7^{\circ}$ . Other angles are within  $4^{\circ}$  of  $90^{\circ}$ . The arrangement of oxygen atoms about the terminal zinc atoms approximates fairly closely (average deviation  $5^{\circ}$ ) to that for a trigonal bipyramid.

least squares techniques and has refined isotropically to a present reliability index,  $(\Sigma||F_o| - |F_c||)/\Sigma|F_o|$ , of 0.08.

The main features of the molecular structure are shown in Fig. 1. The crystallographic and molecular  $C_2$  axes are coincident and contain the central octahedrally co-ordinated zinc atom. The two terminal zinc atoms have a slightly distorted trigonal bipyramidal co-ordination. Unlike  $\text{Ni}_3(\text{acac})_6$  (ref. 4) and  $\text{Co}_3(\text{acac})_6$  (ref. 5),  $\text{Zn}_3(\text{acac})_6$  contains no acetylacetonate ligands with both oxygens serving as bridges. The relative stability of pentaco-ordinate zinc in this system is evidenced by the preferential formation of products of the type  $\text{Zn}(\text{acac})_2L$  over  $\text{Zn}(\text{acac})_2L_2$  complexes<sup>2,3,10</sup>. The presence of the central octahedrally co-ordinated zinc atom indicates, however, that the difference in stability is not large because otherwise one might expect a structure involving only five-co-ordinate zinc, such as the dimer of trigonal bipyramids reported for *bis*(*N*-methylsalicylaldiminato)zinc(II) (ref. 11). Thus, the tendency of  $M(\text{acac})_2$  molecules to form polymers can be said to result from the preference of the metal atoms for co-ordination numbers higher than 4, but not necessarily for a co-ordination number of 6. These polynuclear structures have been different in each of the three compounds so far structurally characterized (for  $M=\text{Ni}$ ,  $\text{Co}$ ,  $\text{Zn}$ ) and the structures are evidently quite sensitive to the stereochemical idiosyncrasies of the individual metal ions.

A detailed report of the results of this investigation will be submitted when further refinement, including absorption corrections and a weighting scheme, is complete. The work was supported by the U.S. National Science Foundation. One of us (R. E.) is a Union Carbide predoctoral fellow.

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## Dipole Moment and Microwave Spectrum of Bromo-acetylene

THE electric dipole moments of the mono-halogen acetylenes are known to decrease to very small values as the electronegativity of the halogen decreases. The moment of bromo-acetylene, in particular, was reported to be indistinguishable from zero by Brockway and Coop<sup>1</sup>. Although no estimates of possible errors were stated for this molecule, the accuracy of the methods was in general such that the error was not expected to exceed 0.01 D. Tyler and Sheridan<sup>2</sup> prepared and characterized iodo-acetylene, and were unable to detect its microwave spectrum in sensitive instruments, although the  $B_0$  value assumed was subsequently proved to be correct by rotational Raman spectroscopy<sup>3</sup>. Hence it was concluded that the dipole moment of iodo-acetylene is not more than 0.05 D, perhaps much less. In view of the improbability that bromo- and iodo-acetylene should both possess such small dipole moments, we have examined the microwave rotation spectrum of bromo-acetylene. This spectrum proved easy to observe and indicated that the dipole moment of the molecule is more than 0.01 D.

The  $J = 2 \rightarrow 3$  and  $3 \rightarrow 4$  transitions were measured for <sup>79</sup>BrCCH and <sup>81</sup>BrCCH, and the  $J = 2 \rightarrow 3$  transitions for <sup>79</sup>BrCCD and <sup>81</sup>BrCCD. High electrostatic fields were required, as expected, for the production of resolvable Stark effects in the lines of the molecular ground states, but well resolved splittings were observed at fields of several thousand volts per cm. The nuclear quadrupole fine-structures, which showed the expected second-order perturbations, were analysed to give the following spectroscopic constants.

Table 1		
Molecule	$B_0$ (Mc/s $\pm$ 0.03 Mc/s)	eqQ of bromine (Mc/s $\pm$ 2 Mc/s)
<sup>79</sup> BrCCH	4,000.05	646
<sup>81</sup> BrCCH	3,978.44	541
<sup>79</sup> BrCCD	3,655.11	646
<sup>81</sup> BrCCD	3,634.98	541

Spectra caused by molecules excited in the bending-modes  $\omega_4$  and  $\omega_5$  were also easily observed, and distinguished by their much stronger Stark effects. The analysis of their overlapped fine-structures is in progress. Details of the Stark effects are being analysed to obtain accurate dipole moments in the ground- and vibrational-states. The preliminary indications are that the moment is of the order of 0.1 D.

The values of  $B_0$  yield a substitution bromine-hydrogen distance of 4.051 Å. From the inertial contributions of the carbon atoms, and the mass-centre condition, the bond lengths are derived with less accuracy as: bromine-carbon = 1.784 Å, carbon-carbon = 1.216 Å, and carbon-hydrogen = 1.051 Å. They agree well with the electron diffraction data<sup>1</sup> and expectations based on results for related substances<sup>2</sup>. The nuclear quadrupole coupling constants are very close to those found in methyl bromo-acetylene<sup>4</sup>.

The strength of the spectra indicates that work now in progress on species containing carbon-13 in natural concentration should lead to a more accurate complete set of bond lengths by the substitution method.

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## RADIOBIOLOGY

### Killing and Mutagenic Efficiencies of Heavy Ionizing Particles in *Arabidopsis thaliana*

THE killing and mutagenic effects of heavy ionizing particles, helium, carbon and argon, accelerated by an energy of 10.4 MeV/nucleon were investigated and compared with the effects produced by caesium-137 gamma-rays. The effects of heavy ions on several biological systems have been discussed by Brustad<sup>1</sup>, Tobias<sup>2</sup> and by Deering *et al.*<sup>3</sup>.

*Arabidopsis thaliana* (L.) Heynh. was used in this experiment. Dry  $F_2$  seeds obtained from  $F_1$  hybrids between a hairless mutant ( $gl^1$ ) and the wild hairy strain of race 'Landsberg' were subjected to gamma-rays and heavy ionizing particles.  $F_2$  seeds were used because it is difficult to obtain many  $F_1$  seeds<sup>4</sup>. Irradiation with heavy ionizing particles was carried out on a 'Hilac' machine<sup>5</sup>, and the dose rate for all irradiations was about 4-5 krad/min. The irradiations were carried out in air; before the beam reaches the seeds, it has first to penetrate various absorbers the thickness of which is equivalent to 12.7 mg/cm<sup>2</sup> of aluminium. Dry seeds were subjected to a surface dose of 1-5 krad of helium-4 and carbon-12 ions and 3 and 5 krad of argon-40 ions with initial particle energies of 41.6, 124.8 and 416 MeV respectively. To compare relative biological effectiveness (RBE) and establish a standard, 27 and 47 krad gamma-ray exposures were given to the same material at an intensity of 10 krad/h using a 6 kc. caesium-137 source. The seeds were sown about 30 h after the gamma-ray treatment.

Germination and survival rates in the control and in the two portions irradiated by gamma-rays were very high, amounting to about 90 per cent. Survival rates decreased markedly with increasing dosage in the portions irradiated by heavy ions, especially those treated with helium ions. Survival rate in the portions irradiated by a 5 krad dose of helium ions was reduced to about half of that in the control portion. The decrease of survival rates in portions irradiated by carbon ions was not so severe, and was about 66 per cent in the portion receiving the highest dosage. On the other hand, 3 and 5 krad treatment with argon ions showed a slight decrease of survival rate (Table 1).

Table 1. SEGREGATION RATIO AND MUTATION RATES IN IRRADIATED  $F_2$  POPULATIONS

Treatment (krad)	No. of seeds	Percentage of survivals	Hairy	Hairless	Expected No. of heterozygotes	No. of plants with hairless sectors (per cent)
Control	1,069	93.2	753	242	290	0
Caesium-137 $\gamma$ -ray	28 1,878	89.9	1,304	384	869.4	3 (0.34)
"	47 1,498	90.9	1,025	336	683.4	8 (1.16)
Helium ions	1 1,089	72.9	626	168	417.3	0
"	2 992	71.5	521	183	347.3	1 (0.29)
"	3 1,107	56.6	473	154	315.3	0
"	4 1,084	69.2	574	176	382.7	0
"	5 950	47.6	334	118	222.7	0
Carbon ions	1 1,066	74.7	620	176	418.3	4 (0.97)
"	2 696	79.9	424	132	282.7	5 (1.77)
"	3 1,067	77.5	641	186	427.3	8 (1.87)
"	4 1,156	80.4	676	217	450.7	9 (2.53)
Argon ions	3 1,101	87.2	724	236	482.6	1 (0.20)
"	5 1,100	87.5	776	187	517.3	2 (0.38)

About 67 per cent of the hairy plants can be assumed to be heterozygotes for the hairy hairless allele pair, so to find somatic mutation from dominant hairy to recessive hairless we had to look among those plants. The number per individual of mutated hairless leaves or leaf sectors showed a variation. When a leaf or a part of a leaf was hairless it was scored as one mutational event occurring in the dominant hairy allele, without reference to the number of mutated leaves or sectors per individual, in order to compare the relative mutation rates. The rates calculated by this method may not be equivalent to the real mutation frequencies, but they are useful for comparing

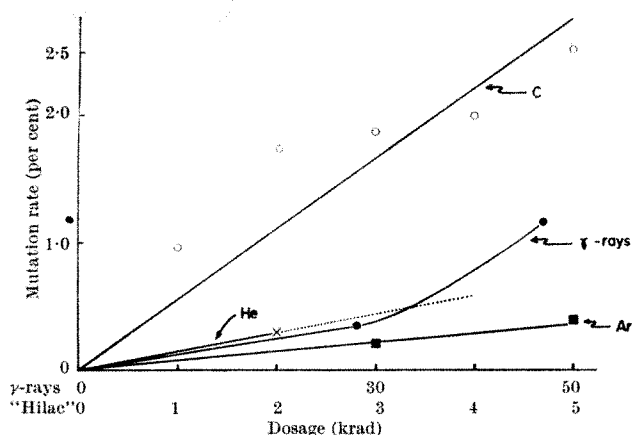


Fig. 1. Somatic mutation rates.

the mutation frequency induced by gamma-ray irradiation and heavy ionizing particles.

The frequency of plants with hairless sectors increased with increasing dosage in gamma-ray, carbon ion, and argon ion portions as shown in Table 1. Somatic mutation rates in the carbon ion portions were very high; those of argon ion portions were very much lower. On the other hand, only one mutated plant was observed in the portion irradiated by 2 krad helium ions, and the other 4 portions treated with helium ions showed no mutation. Irradiation experiments with helium ions were carried out again with a dose of 1 to 4 krad, but again no mutation was observed; a severe killing effect was found, however, which was in good agreement with the preceding experiments.

As shown in Fig. 1, curves of dose against mutation for the carbon and argon ion portions were fairly linear while an exponential curve was observed between mutation frequency and gamma-ray dosage. We could not draw a curve of dose against mutation frequency for the helium ion portion, because only one mutated plant occurred, although the experiment was repeated. A similar exponential curve for somatic mutation in gamma-ray treatments has previously been observed by Fujii<sup>4</sup>, and also an almost linear curve in neutron treatments. The exponential relation tends to predominate in radiations of low linear energy transfer (LET), such as gamma-rays and most X-rays, and at high doses or dose rates; the linear relation dominates in high LET tracks in general and at low doses or dose rates<sup>5,6</sup>. The shapes of dose-response curves are therefore different, and the RBE value of heavy ionizing particles cannot be expressed by a single parameter. The frequencies of somatic mutations of gamma-rays and heavy ionizing particles were compared at 0.5 per cent mutation rates from their dose against frequency curves. About 33 krad of gamma-rays was necessary to produce the 0.5 per cent mutation rate, and about 0.9 and 6.7 krad of carbon ion and argon ions could produce the same mutation frequency. From the results, RBE for somatic mutation at 0.5 per cent frequency was roughly estimated as 35 for carbon ions and 5 for argon ions. We were also unable to decide about the RBE of helium ions, but if the mutation rate shown by the 2 krad portion was sustained, their RBE for somatic mutation might be about 10.

The calculated RBE value for argon ions is rather tentative, since there remains the question of the penetration of the ion beams. The seeds were attached to filter paper and were irradiated with heavy particles. Because of their ellipsoidal shape, most of the seeds lie flat on the filter paper. We may therefore assume that the accelerated ion beams of heavy particles penetrate most seeds laterally and only a few longitudinally. The width of the seeds is about 0.28 mm, that is an average of 28 mg/cm<sup>2</sup>; and argon ions which have a residual range of 12.6 mg/cm<sup>2</sup> in

the tissue must be stopped within the seeds. These ions presumably cannot pass through the seeds, but they can penetrate into seed tissue, and might attack the embryo which is located almost in the centre of the seed. The residual range of helium ions and carbon ions is 133 and 47 mg/cm<sup>2</sup> respectively; these ions certainly can penetrate and pass through the seed tissue.

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### Increased Radiosensitivity in *Lens esculentum* Seeds irradiated in a Mononucleotide Solution

We know from previous work that it is possible to incorporate a mononucleotide into a polynucleotide by means of  $\gamma$ -radiation<sup>1-4</sup>. This communication describes the results of some experiments which we have carried out to explore the possibility that a similar model could be applied to biological systems and could take into account the development of mutations.

The method used was as follows: two groups of *Lens esculentum* seeds (I and IV) were incubated for 3 h in water and in a 0.02 molar solution of uridylic acid-2'-3' (UMP-2'-3'), respectively, and irradiated with a caesium-137 source at a rate of about 200 r./min and a total dose of  $6 \times 10^4$  r. Two control groups (III and IV) were handled in a similar fashion, but not irradiated. The four groups were planted on wet filter papers and the number of roots and cotyledons counted daily.

The data were plotted according to equation (1)

$$\log_e (1 - N/N_0) = A - Ct \quad (1)$$

where  $t$  is the time after irradiation,  $N_0$  is the total number of seeds in a group, and  $N/N_0$  is the fraction of seeds that have roots or cotyledons. In this equation the fraction of seeds without germination (or without cotyledons in the other case) is a logarithmic function of time after irradiation, at a given dose. We calculated the best line that fits our data; the regression equations for each group are shown in Table 1. As can be seen in Fig. 1 each group is characterized by a different slope. Lines (1) and (2) have statistically different slopes, but the main difference is their respective value for the constant  $A$ . On the other hand,  $A$  has approximately the same value in lines (3) and (4) (within the experimental error), but the lines have statistically different slopes. Furthermore, the slopes of these last lines are statistically different from the slopes of lines (1) and (2). Similar observations can be drawn from the lines shown in Fig. 2, which correspond to the development of cotyledons.

As we did not detect any damage in the seeds irradiated in water, and even observed a slight stimulation in their development, we assume that the relatively small dose of radiation used increased the total number of totipotential cells. It is remarkable that in the group of seeds irradiated with mononucleotide, the increasing effect on the initial

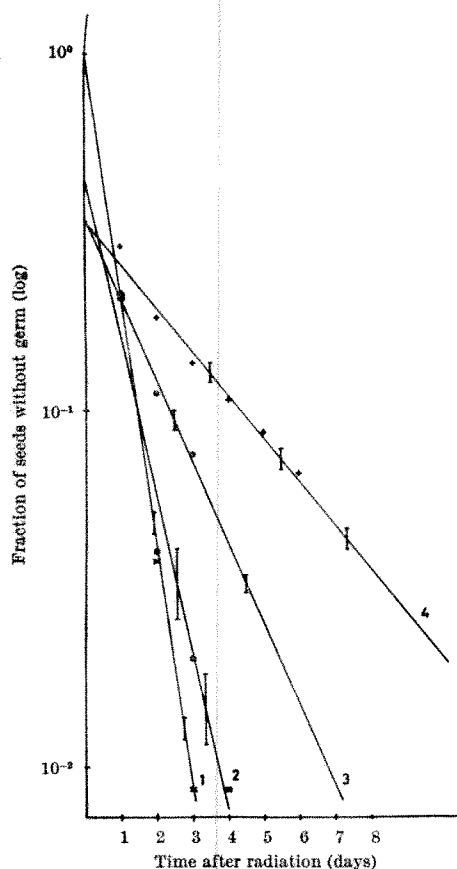


Fig. 1. Regression line for root development. (1) Seeds irradiated in water; (2) control seeds in water, without irradiation; (3) control seeds in 0.02 molar UMP-2'-3', without irradiation; (4) seeds irradiated in uridylic acid-2'-3' 0.02 molar. The standard deviations ( $S_{y/x}$ ) are drawn on each regression line. The regression lines 3 and 4 cannot be extrapolated.

process of cell multiplication and on the velocity of development, due to the irradiation, is absent. The mononucleotide on its own, however, has an inhibitory effect, which is increased in the presence of radiation and persists throughout the experiment.

In accordance with equation (1), which describes the damage to the seeds, a greater slope corresponds to a smaller damage. If we calculate the time taken to reach  $T_{37}$  (the point at which 37 per cent of the seeds are without roots or cotyledons), it is obvious that this gives directly an equivalent of the usual "sensitive volume" of the system and that is due to the sort of inverse plotting (as compared

Table 1. REGRESSION EQUATION

	Value of constant A	Value of slope B	Value of $S_{y/x}$
Roots			
I	0.00	-0.693	0.026
II	-0.3458	-0.4441	0.1
III	-0.529	-0.227	0.024
IV	-0.465	-0.1217	0.03
Cotyledons			
I	0.00	-0.529	0.04
II	-0.1288	-0.2741	0.039
III	-0.151	-0.1735	0.058
IV	-0.1084	-0.116	0.029

Groups I, irradiated seeds in water; II, control seeds in water without irradiation; III, control seeds in mononucleotide solution without irradiation; IV, irradiated seeds in mononucleotide solution.  $S_{y/x}$ , Means, standard deviation of the regression lines.

Table 2. VALUE OF THE EQUIVALENT OF THE SENSITIVE VOLUME

A, Root processes		B, Cotyledonary processes	
	Time of $T_{37}$		Time of $T_{37}$
I	0.63	I	0.82
II	0.98	II	1.58
III	1.9	III	2.5
IV	3.6	IV	3.7

Groups I, irradiated seeds in water; II, control seeds in water without irradiation; III, control seeds in mononucleotide solution; IV, irradiated seeds in mononucleotide solution.  $T_{37}$ , 37 per cent of seeds without roots or cotyledons.

with the usual one) which we adopted for graphs (1) and (2). Table 2 summarizes the sensitive volume of the two processes as the appearance of roots and cotyledons. Except for group IV the sensitive volume for the development of the cotyledons is larger than the volume obtained when the appearance of roots is measured. Moreover with seeds irradiated in water there is a decrease in this sensitive volume, which is greater for cotyledonary processes, in accordance with the slope of their respective regression lines. On the contrary, with irradiated seeds treated with nucleotide we find no difference between this sensitive volume of roots and the development of cotyledons; furthermore, these two sensitive volumes are the largest found, and about four times greater than those of the control seeds (group II) for root processes and twice for cotyledonary processes.

It is possible to imagine that seeds which are treated with mononucleotide and nucleotide plus radiation become damaged in such a way as to prevent normal growth and differentiation. The seeds treated with only nucleotide have a retarded development, and therefore we can interpret this persistent damage as arising from a high concentration of one mononucleotide inside the cell, in relation to the others; such a difference could promote a greater chance of error in the copying of genetic information for the processes of root growth and appearance of cotyledons. It follows from our data that the amount of damage is still greater in seeds irradiated in solutions of mononucleotide because here the sensitive volume of the two processes is equal, possibly as a result of a shared damage.

Bearing in mind our earlier findings and reports concerning the incorporation of mononucleotide into polynucleotide by  $\gamma$ -radiation, we can postulate that the greater cell damage in group IV is a consequence of the incorpora-

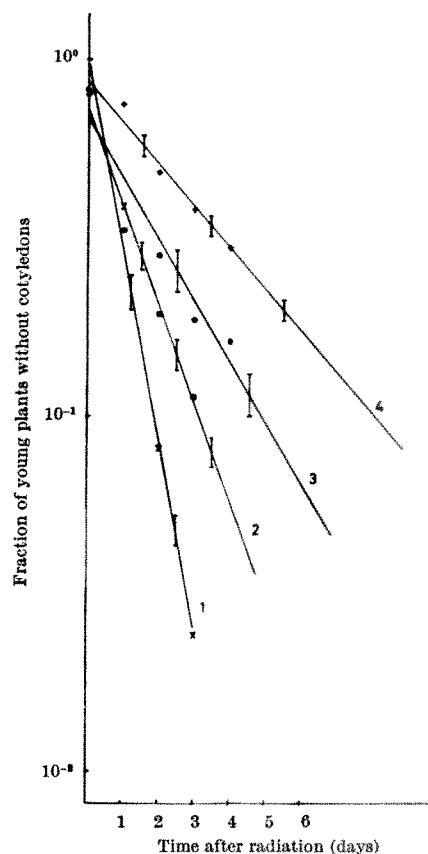


Fig. 2. Regression lines of cotyledon development. (1) Seeds irradiated in water; (2) control seeds in water, without irradiation; (3) control seeds in 0.02 molar UMP-2'-3', without irradiation; (4) irradiation seeds in 0.02 molar UMP-2'-3'. The standard deviations ( $S_{y/x}$ ) are drawn on each regression line. The experimental regression lines cannot be extrapolated.

tion of nucleotide into the cellular DNA or RNA. The damage remains throughout the experiment and for many cycles of cellular replication. It also inhibits the two processes studied, and is therefore more likely to involve damage of the DNA, especially as in seeds mRNA has a life of about 2 h (ref. 5).

With the view of gaining a better understanding of the mechanism of the cellular damage described here, we are continuing our experiments, and are especially looking out for the specificity of the nucleotide damage induced when seeds are irradiated.

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### Direct and Indirect Effects of Radiation: the Radiolysis of Sugar

OUR earlier communication<sup>1</sup> presented evidence that important biological consequences accrue from the presence of stable chemical products of radiolysis which are derived from sugar in the ambient culture media of plant cells or tissue explants. These effects became evident because they neutralized the effect of growth stimulatory substances which induce cell division and cause otherwise quiescent cells to grow rapidly. Thus the potency of the effect of radiated sugar could be assayed. We also showed that growth in the presence of the radiolysis products of sugar may result in abnormal mitotic and meiotic chromosomes in other types of material (root tips and pollen mother cells).

In selected earlier works, we<sup>1</sup> found some claims that products of irradiation of the external medium could exert effects on cells and organisms with which they come into contact; these effects are termed indirect. The work of Phillips<sup>2,3</sup> also showed that much is known about the radiolysis of sugars.

There is other evidence for indirect biological effects of radiation. Chopra has detected such effects by increased mutations of bacteria<sup>4</sup>; Berry *et al.* have recognized cytotoxic effects on human and animal cells<sup>5</sup>, and Rinehart and Ratty saw a significant increase in sex-linked recessive lethals in *Drosophila*<sup>6</sup>, but of particular importance is the work of Shaw<sup>7</sup> on human cells in culture. The adverse comments of Goldblith<sup>8</sup> do not require further notice<sup>9</sup>.

Later work has been carried out in an attempt to isolate and identify the biologically active constituents in the many radiolysis products from sugar which has been exposed to radiation from a cobalt-60 source. Previously some furan derivatives of known structure, which were isolated after fructose breakdown under acidic conditions by Dr. C. T. Moye in Australia, were supplied to us for test in our assay conditions. Some of these exhibited ultra-violet absorbance, but others did not. Our tests so far show that these compounds were neither identical with

the most potent fractions from irradiated sugar nor were they similarly active in our assay system.

Some 280 g of sucrose (as a 2 per cent aqueous solution) were irradiated in batches (2 megarads delivered during a 2-h period). From this material, 263 g of unchanged, biologically neutral, cane sugar were recovered, as well as 476 mg of a white crystalline compound which suppressed growth. This proved to be a salt of formic acid—possibly derived from formaldehyde. The formic acid (as its sodium salt) was critically identified by chemical analysis and by infra-red absorption spectra and nuclear magnetic resonance; the identification in the Cornell laboratories was made with the help of Dr. J. Meinwald of the Department of Chemistry and was carried out independently by Dr. Sugii, then in Japan. Neither the data which prove this identification nor the assay data which record the biological activity will be reported here. It can, however, be stated that on both points the evidence was unequivocal. How far the formic acid contributes to both the cytotoxic and to the cytological or genetic effects is, however, not yet known. At the same time, approximately 1 g of crude, yellow syrup of high ultra-violet absorbance (at 265 mμ), analogous to that previously described<sup>1</sup>, was isolated. This fraction definitely inhibited growth in the carrot assay system. With the help of Dr. E. M. Shantz of this laboratory, this material has been still further purified and fractionated on activated charcoal and silica gel columns. We have obtained various subfractions which show both ultra-violet absorbance and varying effects in the culture system. Among these subfractions are some which are inhibitory in the carrot explant assay system, but the identification of any one fraction, or the evaluation of their interactions, is still incomplete. Similar tests made on irradiated glucose and fructose have produced comparable biological results.

Although the evidence is incomplete, there can be no doubt that when sucrose, as pure as this reagent can be, is in de-ionized distilled water a certain percentage of its total carbon is converted to other compounds under the influence of γ-radiation from cobalt-60. The number of compounds so formed is widely known to be large. Among the acidic compounds which did not absorb ultra-violet light is formic acid, but the most elusive biologically active compounds are among those which have characteristic ultra-violet absorption spectra in acidic and alkaline solution, and which are separable by column chromatography into several components.

The inhibitory effects of these radiolysis products on growth have been preserved during storage for long periods at low temperatures, at least when the products have been autoclaved in the appropriate media before use. When the active substances are fully identified, however, it may be possible for them to be assayed in such complex media as radiated foods, and their longevity and stability when subjected to varying treatments in various media may be determined, and they will be tested against a variety of different assay systems.

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## BIOPHYSICS

Guard-rings, Surface Films and Artefacts  
in the Viscometry of Human  
Blood

THE realization that blood is a non-Newtonian and shear-thinning or thixotropic fluid is quite recent, and in great measure is due to the pioneering efforts of Wells and Merrill<sup>1,2</sup>, who first used a 'Brookfield' cone-plate viscometer and later a more complex coaxial-cylinder viscometer. Shortly afterwards, however, they observed<sup>3</sup> some artefacts which influenced their viscosity readings and which they attributed to the formation of a rigid surface film, which was believed to transmit torque from the rotating to the suspended member of the viscometer. Believing that their observations negated the existence or the amount of shear-thinning in blood at low rates of shear (in the vicinity of  $1 \text{ sec}^{-1}$ ), they rejected some of their earlier data.

In my studies of blood viscosity and thixotropy, I have used a cone-in-cone viscometer since the beginning of 1961, and freshly shed blood without anticoagulants. The data observed were reported in a series of publications<sup>4,5</sup> and led to some suggestions on the role of high blood viscosity, especially at low shear rates (0.01 to 1 reciprocal second), in circulatory diseases and in the pathogenesis of thrombosis.

Following the suggestion by Wells<sup>6</sup> that the results obtained by means of my cone-in-cone viscometer should also be affected by the formation of a "rigid surface film", I decided to investigate this phenomenon more closely. In this I used guard-rings which according to Wells and Merrill should prevent the transmission of a torque from the rotating to the suspended member, and thus avoid the effects of the rigid surface film.

Five guard-rings were made from 'Teflon'; three had cylindrical and two conical geometry. The gaps between the edge of the inner cone and the inner surface of the guard-rings varied from  $16 \times 10^{-3}$  to  $120 \times 10^{-3}$  in. (from 0.4 to 3 mm). Tests were carried out on water, low-viscosity paraffin oil, freshly shed and fresh anticoagulated blood, and one-week-old plasma and blood.

The tests carried out on distilled water showed that stationary guard-rings decrease deflexions of the inner cone to a considerable extent. The diameter of the ring is of no great importance, although some small variations in deflexions (up to 10 per cent at high speeds) were observed. The addition of a detergent markedly reduces the deflexions obtained in the absence of guard-rings, and reduces the discrepancy observed between deflexions in the presence and in the absence of stationary guard-rings. The discrepancy is proportional to the angular velocity of the external cone.

The addition of detergent to water, when any of the guard-rings is used, makes very little if any difference to the deflexions of the internal cone.

The rings were tested not only in a stationary position (which is proper for the guard-ring), but they also were allowed to rotate with the external cone in order to estimate the additional drag caused by their presence. Drag was small or negligible in the presence of a drop of detergent; without detergent, the drag was highest for the smallest ring, and absent for the largest ring. The effect of detergent suggests that some phenomenon related to surface tension is involved.

When low viscosity paraffin oil (3 to 5 centipoises) of Newtonian rheology was used, the deflexions were always proportional to the angular velocity of the external cone. The "braking" or "accelerating" action of the ring was caused by the ring being stationary or rotating with the external cone, respectively. The mean value of the deflexions of the inner cone, obtained in both ways, was nearly identical (within 1 to 2 per cent) to the deflexion observed in the absence of rings. It suggests that no

surface film of any consequence is present on the surface of paraffin oil.

The situation becomes much more complex when blood is used. In the first series of experiments, freshly shed blood was collected into a plastic syringe containing heparin as anticoagulant. The large ring, which rides on the edge of the external cone, did not affect the deflexions (or blood viscosity reading) at any rate of shear. The small ring, on the other hand, acted as a single unit with the inner cone; that is, when stationary, it locked the inner cone in a stationary position; when rotating, it locked the inner cone and caused it to rotate at the angular velocity of the rotating cone. The intermediate rings behaved in an intermediate manner; that is, when stationary, they caused a "braking" effect and decreased the deflexion of the inner cone; when rotating, they induced a higher deflexion of the inner cone.

Further tests on older blood and on plasma showed that, in general, the action of the guard-rings depends on the gap existing between the inner surface of the ring and the edge of the inner cone. The rings had no effect in one-hour old plasma or one-hour-old anticoagulated blood if this gap was larger than 0.056 in. (1.42 mm). A strong braking and/or locking effect was found in anticoagulated blood more than a few minutes old, when the gap was less than 0.033 in. (0.84 mm). Locking-braking actions could be observed with intermediate rings, the effect depending on the age of the blood and increasing with the age of the blood. Consequently, even a ring of large diameter could lock the inner cone, if the blood was old enough.

These experiments indicate the following:

(1) The nature of the film formed at the blood-air interface is completely different from that observed on the distilled water air interface. (2) The effect of this film on the blood viscosity test depends on the geometry of the guard-ring used. Although a small ring may act as a complete brake and may lock the inner cone, a large ring may have no effect whatsoever. (3) The action of a guard-ring depends on the age of blood used, and the locking and braking effects increase with the age of the blood. (4) In the systems in which a ring acts as a partial brake or a partial accelerator, the effect is roughly proportional to the angular velocity of the external cone. (5) In freshly shed (not anticoagulated) blood, the presence of large and intermediate size rings is of no importance.

It does seem, then, that there is no rigid surface film formed on freshly shed blood in the cone-in-cone viscometer. If a film of the type described by Wells and Merrill were present, the application of any guard-ring would be noticeable, and the geometry of the ring would not be important.

It must be concluded that while a surface film is formed on anticoagulated blood, it is a peculiar property of this film that its action decreases with the increasing gap between the inner and the outer member of the viscometer. Another peculiar property of this film is that it increases in strength when blood ages.

It is possible that the low magnitude of the surface effects in the cone-in-cone viscometer, and their complete absence in the freshly shed blood, is a lucky consequence of the fact that the gap between the inner and outer cone edges is about 1.8 to 2 mm. It is quite likely that in instruments with very narrow gaps, such surface films might be very noticeable. The critical "locking" range (up to 0.8–1 mm) may, however, be quite different in the narrow-gap instruments. The ratio of the frictional area of the suspended member to the frictional effects of the guard-ring might be relevant, and will be quite different in viscometers of different geometries.

While these experiments with guard-rings of different geometry confirm the validity of viscosity data obtained on freshly shed blood by means of the cone-in-cone viscometer, they raise many interesting questions. The molecular structure of this surface film on blood or the nature of the "surface film" in distilled water are matters

of speculation. One may wonder whether the rejection of their earlier data by Wells and Merrill was not too hasty; perhaps a guard-ring of another geometry might have behaved differently.

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## PHYSIOLOGY

### Movement of Solutes across Luminal Cell Membranes in Kidney Tubules of the Rabbit

SLICES of kidney cortex have been used to advantage in the investigation of the mechanism of renal transport systems<sup>1</sup>, although the technique has definite limitations. Thus it could be calculated that the contribution of the membrane which separates the cell from the tubular lumen (luminal membrane) to the turnover of paramino hippurate in slices is negligible<sup>2</sup>. Similarly, the luminal membrane appears to play no part in the turnover of potassium in slices<sup>3</sup>; only the peritubular membrane seems to be involved here. Among possible reasons for this is the length of the path of diffusion through the convoluted tubule and/or the collapse of the tubular lumen as a result of continued reabsorption of salt and water<sup>4</sup>. The influence of the length of the tubule is seen, for example, in a preparation of teased flounder kidney. In this case, efflux of dyes from the lumen is dependent on energy<sup>5</sup>, which suggests that the path of efflux lies across the cell and that there is little loss of dye directly from the lumen to the medium.

Some of the inherent limitations of the technique which employs slices can be overcome by the use of fragments of tubules<sup>6</sup>. In such a preparation the destruction of tubular architecture causes the exposure of luminal cell membranes to the incubation medium. This is illustrated in Fig. 1 which shows a comparison of solute fluxes in fragments and slices. It further indicates that in physiological conditions the net passage of sodium out of the cell during the process of reabsorption of sodium occurs across the peritubular membrane. In contrast, paramino hippurate is transported through the cell across the luminal membrane in the opposite direction. It is only in fragments, therefore, that the efflux of paramino hippurate from cells can be measured in the normal physiological sense. The unmasking of the luminal membrane on fragmentation of the tubule is thus expected to cause a greater acceleration of efflux of paramino hippurate and other secreted substances than of sodium.

To test this hypothesis tissues were preloaded with either sodium-22 or paramino hippurate or tetraethylammonium labelled with carbon-14 by incubation with these substances at 28° C for 30–40 min. For the accumulation of sodium-22, Ringer's solution free of potassium was used. Solute efflux was followed in tissues suspended in Ringer solution (free of paramino hippurate or tetraethylammonium) after washing in an isotonic solution of choline chloride (for efflux of sodium-22) or saline (carbon-14). Washing of fragments involved centrifuga-

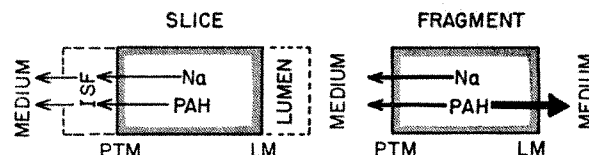


Fig. 1. Schematic presentation of efflux of sodium and paramino hippurate (PAH) from slices and fragments. PTM, Peritubular cell membrane; LM, luminal cell membrane; ISF, interstitial fluid. Thickness of arrows indicates relative magnitude of net fluxes. Note that in slices the presence of the lumen effectively prevents the return of solute from cell to medium across the luminal membrane. For further details, see text.

tion and resuspension in solution kept ice-cold by the addition of crushed frozen pieces of the same solution; this slurry was again spun down, and the packed washed cells were resuspended in efflux medium. Rapid separation of fragments from efflux medium was achieved by filtration on a Buchner funnel fitted with Whatman No. 541 filter paper. The fall in the concentration of tracer in the tissue was determined at time intervals selected so that the final percentage activities remaining in slices and fragments were similar, and the initial fast loss from the interstitial fluid spaces in slices was avoided, so that efflux during the period of observation approximately obeyed first order kinetics. The following times, measured from the moment of the suspension of tissue in efflux medium, circumscribe the intervals chosen: for sodium, 1–2 min and 0–10 sec for slices and fragments respectively; for carbon-14, similarly, 5–25 min and 0–20 sec.

An increase in the rate of efflux of sodium of approximately ten-fold was observed in fragments compared with slices (see Table 1). A similar result was obtained in experiments in which the use of an isotonic solution of choline chloride provided for sodium the same high efflux gradient as for paramino hippurate. Among the possible reasons for the relatively high rate of efflux of sodium from fragments may be the shortening of the path of diffusion through the interstitial fluid compartment of slices and the setting free of the intercellular membranes<sup>7</sup>. The same factors should also modify rates of efflux for other solutes. A much larger acceleration of efflux was seen, however, especially in the case of paramino hippurate. This supports the view that efflux of this substance does occur largely across the luminal membrane. Presumably, the same applies to tetraethyl ammonium.

Table 1. EFFLUX RATES FROM SLICES AND FRAGMENTS OF RABBIT KIDNEY CORTX

Solute	Slices			Fragments		
	Mean	Range	No. of experiments	Mean	Range	No. of experiments
Sodium	0.36	0.09–0.73	16	3.4	3.1–3.8	6
PAH	0.016	0.002–0.020	11	0.94	0.74–1.30	7
TEA	0.016	0.003–0.027	5	0.41	0.25–0.71	7

Each experimental value represents the mean of five or more independent measurements.

If efflux of paramino hippurate from fragments does, as suggested, occur across the luminal membrane, then a high sensitivity to 'Benemid' of this efflux can be predicted on the basis of a recent analysis of the kinetics of the secretion of paramino hippurate *in vivo*<sup>8</sup>. This work led to the conclusion that 'Benemid' inhibits the transfer of this substance across both the peritubular and luminal membranes. In agreement with this we now find that 10<sup>-3</sup> molar 'Benemid' depresses efflux of paramino hippurate from fragments by 70 per cent. The fact that 'Benemid' so strongly inhibits this efflux argues against the likelihood that a large fraction of the efflux occurs across the intercellular membranes. It seems improbable that these membranes should possess specific carrier systems sensitive to 'Benemid'. The effect of 'Benemid' thus further supports the view that efflux of paramino hippurate from fragments is primarily a function of the luminal membrane.

Implicit in this view is the conclusion that because of the leak through the luminal membrane, fragments will not be able to accumulate paramino hippurate to the same high steady-state level as can slices. On the basis of experiments involving 2 h periods of incubation it has been claimed that accumulation of paramino hippurate by slices and fragments is equally high<sup>6</sup>. In our conditions the fragments, prepared by digestion with collagenase, invariably failed to yield the same high ratios of concentration of paramino hippurate in tissue as compared with medium. Thus, in five experiments, the following tissue: medium ratios were obtained after a 3 h period of incubation at 28° C in bicarbonate Ringer solutions, (0.08 mmolar paramino hippurate): for slices, 16 (a range of 12–24); for fragments, 7 (a range of 4–9). Relatively low final values of tissue: medium in a preparation of tubule have been reported<sup>9</sup>. All our observations therefore support the proposal that comparison of solute fluxes in slices and in suitably prepared renal tubular fragments can provide a measure of the properties of the luminal membrane.

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### Comparison of DNA revealed by Feulgen and by Ultra-violet Light in Rabbit Spermatozoa after Storage in the Male Efferent Ducts

PUBLISHED data concerning the correlation of the content of DNA of spermatozoa and their capacity to fertilize are contradictory. We have therefore investigated thirteen sexually mature rabbits with normal spermatogenesis. Ejaculates were collected from all the rabbits, and the content of DNA in single spermatozoa (twenty on a slide) was measured in visible light at 560 mμ, after they had been stained with Feulgen by a standard technique, and in ultra-violet light at 260 mμ while still unstained. The measurements were made with an automatic scanning microspectrophotometer<sup>1</sup>. Testicular degeneration was induced in ten rabbits by means of a hot water spray on the scrotum (10' at 46° C). The three remaining rabbits served as controls. Both the controls and the treated animals were killed at different intervals after treatment and spermatozoa were collected from the caput epididymis, the cauda epididymis and the ampullae. In these spermatozoa, too, the contents of DNA were measured in twenty cells after staining with Feulgen, and in the ultra-violet spectrum.

The ejaculated spermatozoa collected before treatment showed a very variable content of DNA revealed by Feulgen in contrast to the constant values for DNA given in ultra-violet light. The mean value given by Feulgen was  $4.11 \pm 1.07$  with a coefficient of variation of 0.25, and the mean DNA value in ultra-violet light was  $5.18 \pm 0.08$ , and the coefficient of variation was 0.015 (Table 1). The same variability was found in different ejaculates from the same rabbits after staining with Feulgen (a mean value of

$4.52 \pm 0.95$ , and a coefficient of variation of 0.21), but the DNA in rabbits shown in ultra-violet light was constant (a mean value of  $5.21 \pm 0.11$  and a coefficient of variation of 0.02). Spermatozoa with normal or low DNA values shown by Feulgen, however, gave similar curves for hydrolysis.

Application of heat provoked severe testicular lesions but did not influence either the content of DNA shown by Feulgen or the content of DNA in ultra-violet light. In the spermatozoa, the mean value of DNA before and after treatment was not significantly different. After staining with Feulgen, however, there was great variability within and between rabbits; the mean value of DNA shown by Feulgen before treatment was  $4.06 \pm 1.06$  and the coefficient of variability was 0.26; after treatment the mean value was  $4.31 \pm 1.51$  and the coefficient was 0.35 (Table 1).

Table 1. MEAN VALUES OF DNA SHOWN BY FEULGEN AND ULTRA-VIOLET LIGHT AND STANDARD DEVIATION IN THE FIRST EJACULATES OF THIRTEEN RABBITS AND IN SPERMATOCYTES RECOVERED FROM DIFFERENT PARTS OF THE MALE DUCTS

Rabbit	Origin of spermatozoa	Mean value	Standard deviation	Mean value	Standard deviation
(1)	E	5.25	0.32		
(2)	E	4.68	0.38		
(3)	E	4.23	0.16		
(Control)	CE	4.73	0.20		
	C	4.85	0.85		
	A	2.86	1.12		
(4)	E	2.25	0.40		
	CE	2.71	1.26		
	C	2.10	0.68		
	A	1.72	0.66		
(5)	E	3.31	0.33		
	CE	4.79	1.40		
	C	1.60	0.52		
	A	0.67	0.32		
(6)	E	2.92	0.30	5.20	0.20
(Control)	CE	3.81	0.74	5.16	0.34
	C	3.56	0.36	5.25	0.28
	A	3.07	0.45	5.12	0.26
(7)	E	4.55	0.30	5.16	0.51
	CE	5.31	0.24	5.07	0.20
	C	4.98	0.34	5.11	0.24
	A	3.53	0.74	4.97	0.24
(8)	E	2.70	0.38	5.21	0.43
	CE	5.28	0.39	5.21	0.22
	C	5.09	0.37	5.17	0.21
	A	4.06	0.37	5.25	0.30
(9)	E	4.60	0.22	5.12	0.51
	CE	5.11	0.41	5.23	0.27
	C	4.72	0.27	5.18	0.22
	A	4.91	0.40	5.21	0.35
(10)	E	4.27	0.19	5.04	0.30
(Control)	CE	6.31	0.40	5.27	0.28
	C	5.09	0.55	5.16	0.27
	A	3.02	1.41	5.17	0.28
(11)	E	4.44	0.74	5.29	0.34
	CE	5.53	0.74	5.12	0.32
	C	5.22	0.28	5.10	0.36
	A	4.19	0.75	5.09	0.16
(12)	E	5.23	0.19	5.20	0.22
	CE			5.13	0.15
	C	6.59	0.36	5.13	0.32
	A	4.88	0.15	5.22	0.32
(13)	E	5.08	0.20	5.23	0.32
	CE	6.29	0.32	5.20	0.32
	C	6.55	0.42	5.12	0.25
	A	4.52	0.39	5.07	0.26

E, Ejaculate; CE, caput epididymis; C, cauda epididymis; A, ampullae.

The high coefficients of variation were mainly the result of a gradually decreasing content of DNA shown by Feulgen in the spermatozoa from the caput to the ampullae. The least significant difference<sup>2</sup> was 0.72; the DNA values given by Feulgen in the ampullae (3.41) were significantly lower than in the caput (5.00) and in the cauda (4.67). The differences between caput and corpus were not significant. The ejaculates yielded DNA values with Feulgen which were intermediate between those of the epididymis and those of the ampullae. For the DNA seen in ultra-violet light very constant results (mean value of  $5.18 \pm 0.05$  and a coefficient of variability of 0.01) were obtained at death and no correlation could be detected between this content of DNA and the site of collection (Table 1).

These experiments reveal an effect of ageing in the male efferent ducts. A similar trend has been demonstrated in

spermatozoa of bulls after ageing *in vitro*<sup>3,4</sup>. The basic mechanism for this decrease in DNA which stains with Feulgen when storage is carried out *in vivo* is not yet clear, but the constant values for DNA in ultra-violet light in all spermatozoa measured suggest that the DNA molecules show a decreased quantitative response to the Feulgen reagent during storage. Hydrolysis curves of the same shape for spermatozoa with normal or low DNA values would upset the hypothesis that DNA would become more sensitive to hydrolysis in normal hydrochloric acid at 60° C after storage *in vivo*, but this requires further confirmation. On the other hand the very stable DNA values in ultra-violet light, even in these spermatozoa with extremely low DNA values shown by Feulgen, suggest that the nucleotides are intact and that no hydrolysis has commenced during the storage processes in the male ducts.

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*Note added in proof.* Since this paper was submitted, Gledhill *et al.* have found testicular spermatozoa in the bull to have a higher Feulgen DNA content than epididymal spermatozoa (*Exp. Cell. Res.*, **41**, 652; 1966).

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### Effects of Oestradiol on Calf Endometrial Tissue *in vitro*

CURRENT concepts of the mode of action of steroid hormones involve activation or inactivation of enzymes; alteration of the permeability of cellular or nuclear membranes; and activation or inactivation of the genome by gene derepression or repression<sup>1-3</sup>. More recent work with oestrogens appears to support the latter hypothesis. Among the reported biochemical effects, detectable only a few hours after hormone administration *in vivo*, are increased RNA (especially nuclear RNA) and protein biosynthesis, and increased activities of various enzymes, for example, RNA polymerase, enzymes which activate amino-acids and alkaline phosphatase<sup>4-6</sup>. These reactions occur relatively early, but they may be considered to be secondary to an undetected primary event.

A major problem of the use of rat uteri, the most common test object for biochemical investigations, has been the difficulty of clear-cut isolation of the endometrium in useful quantities. It was considered that unequivocal biochemical results could be obtained if specific target organs were available in large quantities. Several histochemical investigations have referred to the non-uniformity of the uterus, which would be expected to give rise to misleading biochemical data derived from the use of whole tissue extracts. With calf endometrium *in vitro* we have overcome these problems.

An essential prerequisite for investigation of isolated target tissues incubated with hormones is that the test

object should show a similarity of morphological and biochemical changes which are known to occur *in vivo*. In the past 15 years very few *in vitro* effects of oestrogens have been observed<sup>2</sup>, and the reports generally refer to whole rat uteri incubated for only a few hours. Our findings, however, support the idea that explants of endometrial tissue of the calf, maintained in tissue culture for up to 2 weeks, remain responsive to oestradiol when it is added to the incubation medium, and that they exhibit the same morphological and biochemical effects as are caused by the injected hormone.

The explants (2 mm × 2 mm; 0.02–0.15 mm thick), preserving the surface epithelium and the bulk of the lamina propria, were dissected from the middle portion of the opened uterine horns of freshly killed virgin heifers which were about 8 weeks old. Two tissue fragments were placed in Rose chambers under dialysis membranes and incubated at 37° C with 1.7 ml. of Eagle's basal medium containing 10 per cent foetal bovine serum free of oestradiol, 100 u/ml. of penicillin and 50 µg/ml. of streptomycin. The medium was replaced every second day except in chambers where low pH values suggested the need for more frequent feeding. After 6 days, the medium was exchanged for a medium containing  $1.2 \times 10^{-6}$  molar oestradiol-17 $\beta$ . No bacterial contamination was observed during incubation. When technical difficulties were encountered, they were thought to be caused in part by the hormonal status of the animals (for example, age, feeding, pretreatment with diethylstilboestrol) and partly by the incubation conditions (for example, the use of bovine serum with an unknown hormone content, or an unsuitable concentration of antibiotics). The explants were examined by time-lapse cinematography using Zeiss phase contrast optics (one or eight frames/min). The activity of alkaline phosphatase was demonstrated using Burstone's method for frozen tissue<sup>7</sup>.

Time-lapse cinematography of sections of the stratum compactum of intercaruncular areas revealed that the

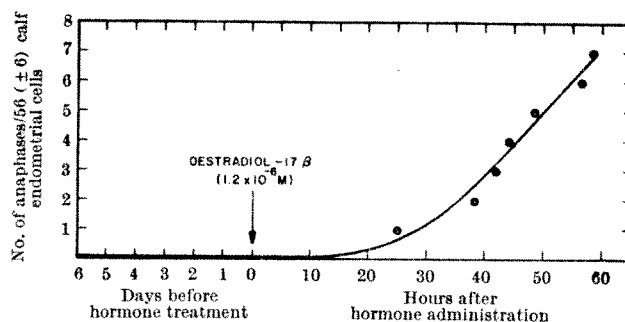


Fig. 1. Frequency of mitoses before and after administration of oestradiol to explanted calf endometrial tissue, examined by time-lapse cinematography.

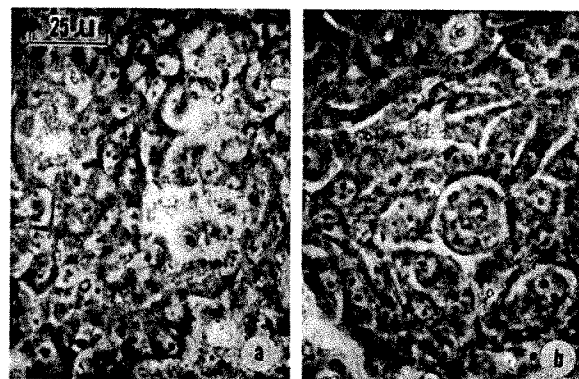


Fig. 2. (a) Calf endometrial tissue after 7 days *in vitro*. (b) Cells from the same culture chamber as (a) after 19 h of treatment with  $1.2 \times 10^{-6}$  molar oestradiol.



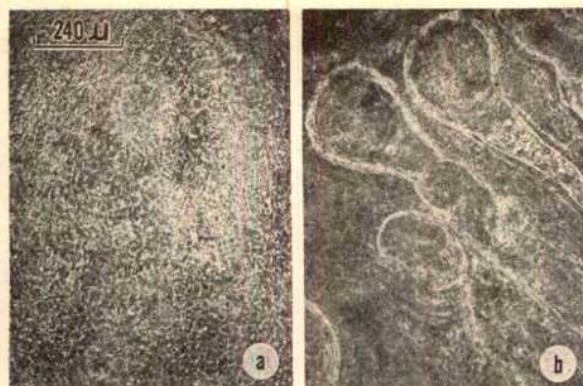


Fig. 3. (a) Untreated calf endometrial tissue after 8 days in culture. (b) Calf endometrial tissue after 46 h of treatment with  $1.2 \times 10^{-6}$  molar oestradiol.

first statistically significant burst of mitoses occurred about 40 h after the administration of hormone (Fig. 1). Regardless of the morphological difference, this is rather later than the 24 h reported for the appearance of numerous mitoses in the luminal and glandular epithelium of the rat uterus<sup>8</sup>. Occasional mitoses were noted after 20 h, however. Enlargement of the nuclei (two to threefold), nucleoli, and of the cells, which was probably caused by increased nuclear activities and imbibition of water respectively, was detected after about 19 h (Fig. 2), which agrees with the findings of Asdell *et al.*<sup>9</sup> after injection of oestrogen into ovariectomized heifers. Virtually no mitoses were observed during the 6 days before treatment with the hormone.

In a series of experiments with sections showing few indistinct glands of small diameter ( $20\mu$ ), the height of the glandular epithelial cells increased two to threefold after treatment with  $10^{-6}$  molar oestradiol for 24 h. These cells became columnar and contained ovoid, basal, vesicular nuclei and filamentous mitochondria. The numbers of glands increased significantly over a period of 46 h (Fig. 3). When the explants were incubated in the presence of solutions of oestradiol of decreasing molarities ( $1.2 \times 10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$  and  $10^{-10}$  molar),  $10^{-9}$  molar oestradiol appeared to be limiting for a definitive response; equivocal results were obtained with  $10^{-10}$  molar oestradiol.

Alkaline phosphatase activities were followed using explants incubated for 2 h, 4 h, 6 h and 18 h in Eagle's basal medium containing  $1.2 \times 10^{-6}$  molar oestradiol without the bovine serum. Some weak staining of the peripheral cells was noted, in particular where the lining epithelial cells were preserved, but increasing enzyme activities in the glandular epithelial cells compared with the controls were disclosed after 4 h of treatment and were at a maximum after 6 h and 18 h. No distinction could be made between the stainability of the basal, mid and neck gland portions.

The application of tissue culture techniques provides a refinement of biochemical investigations of uterine alkaline phosphatase of mice and rats<sup>8</sup>. These *in vitro* findings agree with *in vivo* reports by Sykes *et al.*<sup>10</sup>, but are somewhat inconsistent with Kenny's results<sup>11</sup>. This could be explained by the presence of different concentrations of progesterone.

Attempts were made to follow the effects of oestradiol on single cell cultures consisting predominantly of surface epithelial cells. These cells, obtained by the trypsin technique<sup>12</sup>, were viable and exhibited outgrowth for 8 days. No significant stimulatory or inhibitory effects caused by hormone concentrations ranging from  $1.2 \times 10^{-6}$  to  $5.8 \times 10^{-8}$  molar could be detected.

As far as we know, there have been no investigations of bovine endometrium in tissue culture which show a specific and pronounced morphological and biochemical sensitivity to administered oestradiol (ref. 13 and personal

communication from Biggers). As long as concentrations of natural hormone in the tissue and medium are carefully controlled, our results seem to recommend tissue cultures of hormone target organs as the experimental system; thus possible effects of the blood and nerve supply are eliminated.

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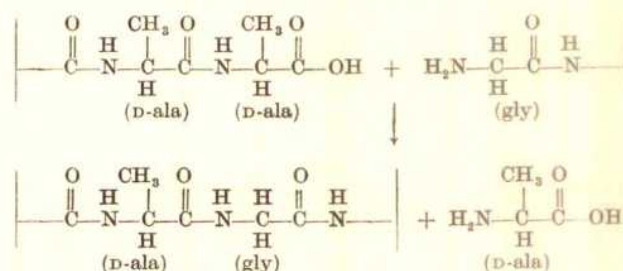
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## MICROBIOLOGY

### Mode of Action of Penicillin

A BIOCHEMICAL explanation for the antimicrobial activity of penicillin has been proposed<sup>1</sup>. In this hypothesis the structure of penicillin was related to that of intermediates known to be involved in the synthesis of cell wall material<sup>2</sup>. It was suggested that penicillin interferes with a transpeptidation reaction responsible for the cross-linking of mucopeptide chains in the cell wall polymer. The reaction is represented as follows:



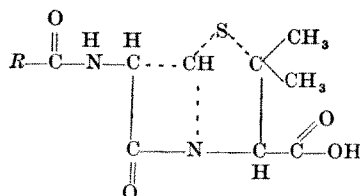
The ability of penicillin to interfere with this reaction was attributed to its putative structural resemblance to the D-alanyl-D-alanine portion of the peptide chain.

We have used a similar approach to investigate the mode of action of penicillin, and are encouraged to report our findings in order to stimulate further experimentation and to prevent needless duplication.

Our approach was strictly enzymological, and deliberately ignored what was known of the biochemical steps in cell wall synthesis. We started with the assumption that penicillin was a potent competitive inhibitor of an enzyme that participated in some phase of cell wall synthesis. On the basis of experience with other enzymes<sup>3</sup>, the high activity of penicillin seemed to be caused by its rigidly constrained structure, part of which was in the same conformation as that of a vital



substrate when bound to the enzyme. In other words, we assumed that there were two parts to the penicillin molecule, one portion that mimicked the substrate and another that was responsible for maintaining the first in the proper conformation. The fact that the activity of penicillin is lost on hydrolysis of the amide bond of the  $\beta$ -lactam ring, as well as some other considerations, led us to the conclusion that the two parts of the molecule were as follows:



where - - - represents constraining bonds and — represents substrate bonds. The substrate analogue of penicillin *G* would therefore be phenacetylglucyl D-valine; that of penicillin *V* would be phenoxyacetylglucyl D-valine. These compounds as well as a number of closely related ones were synthesized and are listed in Table 1 with their melting points and specific rotations. All gave satisfactory elementary analyses.

Table 1. ACYLATED DIPEPTIDES\*

Compound	Melting point	Specific rotation $[\alpha]_D^{25}$
Phenylacetylglucyl D-valine	167	+5.5, $c=1$ , methanol
" " L-valine	167	-5.5, $c=1$ , methanol
" " DL-valine	143	—
" " DL-alanine	202 (dec)	—
" " D-alanine	193	+18.7, $c=0.5$ , 1 per cent sodium bicarbonate
" " L-alanine	190	-17.8, $c=1$ , " " "
" " D-serine	165	-10.0, $c=1$ , " " "
" " D-leucine	133	+17.0, $c=1$ , " " "
Phenoxyacetylglucyl D-valine	160	+7.5, $c=1$ , " " "
" " DL-alanine	124	— " " "

\* All the derivatives were prepared by acylation in the presence of excess aqueous bicarbonate of the free dipeptides which were synthesized by known procedures or purchased from Mann Research Laboratories, Inc., New York.

According to the hypothesis, the acylated glucyl D-valine or the related derivatives should show biological activity. For example, one might expect that they would have antimicrobial activity, but, because they are not structurally constrained, relatively high concentrations may be needed. On the other hand, they could antagonize the action of penicillin if they could be utilized by the target enzyme to make cell wall material. More recent findings<sup>3</sup> make the latter possibility unlikely, but when our work was carried out it could not be eliminated *a priori*.

Both of these possibilities were tested for all the compounds listed in Table 1. Three test organisms were used: penicillin-sensitive strains of *B. subtilis* (0.3 $\gamma$  penicillin/ml.) and *Staphylococcus aureus* (0.2 $\gamma$  penicillin/ml.) and a penicillin-resistant *Staphylococcus aureus* (200 $\gamma$  penicillin/ml.). Several experimental techniques were used: observation of growth in liquid media after a fixed period (22 h), nephelometric monitoring of liquid cultures throughout a 10 h period, and the use of disks impregnated with peptide. In the last case, the organism was seeded heavily on agar plates containing various concentrations of penicillin (including plates lacking the antibiotic) before the application of the disks. Antibacterial activity would have been indicated by the presence of a clear area around the disks if penicillin were absent from the medium. Neutralization of the action of penicillin would have caused the formation of a zone of growth around the disk implanted on a medium containing penicillin. With respect to experiments in liquid media, 20 per cent sucrose was present in order to prevent lysis of the organism<sup>4</sup> so that the action of penicillin could be a reversible one.

Concentrations as high as 2,000  $\gamma$ /ml. had no effect on growth of the test organisms nor was any interference

with the action of penicillin noted, even when borderline concentrations of the antibiotic were used. In accord with the hypothesis of Tipper and Strominger<sup>1</sup>, phenylacetyl D-alanyl-D-alanine was also synthesized (melting point 210° C;  $[\alpha]_D^{25} + 75.8$ ,  $c = 0.5$  in 1 per cent sodium bicarbonate) from D-alanyl-D-alanine<sup>5</sup>. It, too, possessed no biological activity in our test system at concentrations as high as  $4 \times 10^{-2}$  molar.

In view of our negative results, it was necessary to establish that our system and techniques were capable of revealing a competitive antagonism when it was known to exist. We therefore attempted to show reversal of the antibacterial action of D-cycloserine by D-alanine, as was done with mycobacteria<sup>6</sup>, *S. faecalis*<sup>7</sup> and with *Bedsoniae*<sup>8</sup>. A truly competitive reversal could be demonstrated over a range of concentrations of D-cycloserine at a ratio of D-alanine to the antibiotic of approximately 1:1 using our strain of *B. subtilis* as the test organism.

Despite the successful results with the D-cycloserine-D-alanine system, it is possible that whole cell preparations are unsatisfactory for the investigation of the activity of the acylated peptides. Perhaps sufficiently high concentrations of peptide cannot penetrate the intact cell. It must be noted that the uptake of penicillin is an irreversible process<sup>9</sup> and therefore not dependent on a high concentration gradient. Presumably, the binding of penicillin is followed by a chemical reaction leading to a covalent bond with the enzyme. Previous results<sup>10</sup> suggest that methicillin reacts with penicillinase in a similar manner. It would appear, then, that a final decision as to the validity of earlier suggestions<sup>1</sup> and of those outlined in this communication must await experiments with the proper cell-free systems.

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### Salmonellin—a New Colicin-like Antibiotic

SALMONELLIN is a new colicin-like antibiotic characterized by its wide range of activity on the salmonellae and its failure to affect strains of *Escherichia coli*. Other *Salmonella* colicins have a much more restricted activity<sup>1,2</sup>. It is produced by a strain of *Salmonella eastbourne*, SC 11, from my collection of local isolates. During an investigation of about 2,000 strains of *Salmonella* for colicin-like activity, the new antibiotic was found only in this one strain. Using Fredericq's double layer technique<sup>3</sup> with the indicator organism, *Salmonella gallinarum*, which is useful in the detection of certain colicin-like reactions and bacteriophage, *Salmonella* SC 11 produced a very large, clear, ill-defined zone of inhibition. On nine strains of *E. coli*, including K 12 58/161 and phi (said to be sensitive to all colicins), SC 11 had no action.

On other sensitive salmonellae, SC 11 produced a characteristic large, double zone consisting of an inner clear area with a few colonies and an outer area with many colonies or reduced growth, often surrounded by a halo (Fig. 1). Strains sensitive to salmonellin (called sal-s), including a number of colicin producers, were found in Kauffmann-White groups, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, V, and W for which the numbers of sensitive

Table 1. SENSITIVITY OF SALMONELLAE TO SALMONELLIN  
Kauffmann-White group

	No. of strains	
Sensitive		Resistant
A	—	4
B	56(22)	9(1)
C <sub>1</sub>	51(1)	4
C <sub>2</sub>	65(20)	2(1)
C <sub>3</sub>	2	2
D <sub>1</sub>	30(2)	14
E <sub>1</sub>	36(6)	5(1)
E <sub>2</sub>	8(3)	1(1)
E <sub>3</sub>	4	1
E <sub>4</sub>	2(1)	—
F	2	—
G <sub>1</sub>	1	—
G <sub>2</sub>	5(4)	—
H	3(1)	1
I	5	2
J	2	1
K	1	—
L	1	—
M	1	—
N	1	—
O	24(13)	15(9)
P	4	—
Q	1	—
R	2	—
S	5	—
T	1	1
V	1	—
W	1	—
Y	—	1
Z	—	1
Total	315(72)	64(13)

Figures in brackets are the numbers of colicin producers among the test strains.

and resistant strains are given in Table 1. Sal-s strains were also found in the Arizona group listed as *Salmonella* sub-genus III<sup>4</sup>.

Table 1 shows that some strains of *Salmonella*, including some colicin producers, were naturally resistant to salmonellin (designated sal-r) and sal-r strains of many sal-s salmonellae were obtained by picking colonies from their inhibition zones. It seemed possible that some of the naturally sal-r strains owed their resistance to production of salmonellin. They were, therefore, tested by the double layer technique, with fifteen different sal-s salmonellae as indicators. A few strains showed atypical zones on one or two indicators, but none resembled salmonellin either in range of activity or in appearance of the zone. SC 11 remained the only natural salmonellin producer (sal<sup>+</sup>) strain so far found.

The ability of SC 11 to transmit the sal factor to other sal<sup>-</sup> strains was indicated by the isolation of sal<sup>+</sup> forms of streptomycin resistant recipients of the *Salmonella*, Arizona and *Escherichia* groups from mixed broth cultures with SC 11. These sal<sup>+</sup> strains were distinguished from the donor, SC 11, not only by streptomycin resistance, but also by having the serological and biochemical characters of the recipient. They inhibited the characteristic wide range of salmonellae and showed the typical

inhibition zone of salmonellin. Two sal<sup>+</sup> strains, obtained from *E. coli*, still retained their full sensitivity to my salmonellae which produce colicin.

Salmonellin has not yet been detected in broth cultures, but it has been obtained in cell-free extracts of agar cultures. These extracts, though weak, retained activity after heating for at least one hour at 60° C or at least 20 min at 100° C. In agar cultures, *Salmonella* readily diffused through dialysis tubing and the large size of the inhibition zone suggested rapid diffusion in agar.

With certain strains of *Salmonella*, SC 11 showed bacteriophage action and was lysogenic for at least one type of bacteriophage, and, as plaque types suggest, probably more. The relation, if any, between salmonellin and bacteriophage activity is being investigated.

Among salmonellae, the salmonellin system provides two new markers, the sal factor (sal<sup>+</sup> or sal<sup>-</sup>) and the sensitivity factor (sal-s or sal-r). It also offers a number of other interesting possibilities for investigation of the relationship between Gram negative bacilli. Thus, transmission of the sal factor among various groups of Gram negative rods may uncover relationships not previously recognized. Furthermore, genetic exchange may be achieved, along with the sal factor, between genera that do not readily cross.

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### Neuraminidase Activity of a Bovine Strain of Parainfluenza 3 Virus

In a discussion of the taxonomic implications of the term "myxovirus", it has been suggested that it is the property of myxophily and not morphology which establishes a true member of the myxovirus group of viruses. It was further suggested that some bovine strains of parainfluenza 3 (PI 3) virus lack the enzyme neuraminidase and that, because their relation with mucoprotein is limited to attachment (that is, they do not elute), they should not be considered as myxoviruses but rather as pseudomyxoviruses<sup>1</sup>. It has also been suggested elsewhere that bovine strains of PI 3 virus do not possess neuraminidase<sup>2</sup>. We have been unable to trace any published evidence to support these suggestions. During the course of an investigation into the role of PI 3 virus in the aetiology of bovine respiratory disease, one of us (P. S. D.) considered that both adsorption to and elution from guinea-pig erythrocytes occurred in the haemagglutination reaction with bovine strains of the virus. This is supported by other work<sup>3</sup>. The present report describes briefly some experiments made to test the neuraminidase activity of a bovine strain of PI 3 virus.

The T1 strain of bovine virus which we used was isolated from the tonsils of two calves<sup>4</sup>. The virus was grown in primary monolayers of calf kidney cells maintained in Earle's saline containing 0.5 per cent lactalbumin hydrolysate and 0.01 per cent yeast extract. The medium did not contain serum.

The cultures were collected 4 days after inoculation with the virus and, after freezing and thawing rapidly three times, they were clarified by light centrifugation. The virus suspension was then concentrated approximately thirty-fold by centrifugation at 105,000g for 60 min. The resulting concentrated viral suspension, used for the estimation of enzyme activity, had a haemagglutinating titre<sup>5</sup> of between 1 in 1,024 and 1 in 2,048/0.2 ml. Control preparations consisted of fluid collected from uninfected tissue cultures treated in a similar manner to the virus

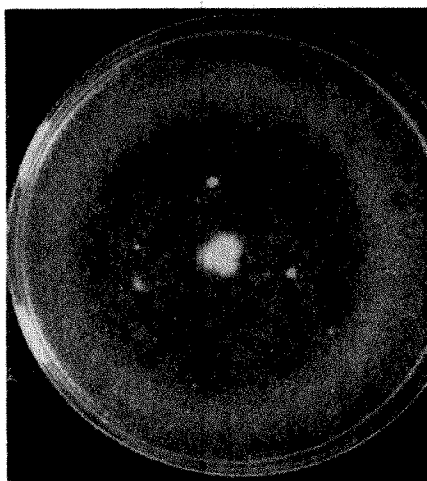


Fig. 1. Appearance of a salmonellin zone of inhibition. The double layer technique was carried out on an agar plate.

Table 1. ACTIVITY OF NEURAMINIDASE IN TEST PREPARATIONS

Preparation	$\mu\text{g}$ of N-acetyl neuraminic acid liberated from bovine glycoproteins by 0.2 ml. of preparation in 2 h at 37° C	
	pH 5.8	pH 7.4
Bovine strain PI 3 virus	1.16	0.52
Control tissue culture fluid	0.07	0.02
Neuraminidase	24.5	17.3

suspension, and a commercially available preparation of neuraminidase (extract of *Vibrio cholerae*).

Serum from calves deprived of colostrum (globulin-free) and normal adult bovine serum were used as the source of glycoprotein substrate. The activity of neuraminidase was assayed by the incubation at 37° C for 2 h of 0.2 ml. of the test preparation, 0.2 ml. bovine serum (containing 180  $\mu\text{g}$  of total sialic acid<sup>6,7</sup>) and 0.1 ml. of a 0.2 molar phosphate buffer (final pH 5.8 or 7.4). After subjection to 100° C for 1 min to inactivate the enzyme, free sialic acid was estimated in the reaction mixture by a method<sup>8</sup> which was specific for N-acetylneuraminic acid. The results obtained with adult bovine serum as the substrate are shown in Table 1. Similar results were obtained when colostrum-deprived calf serum was used.

The suspension of PI 3 virus showed significant enzyme activity at pH 5.8 and 7.4. At pH 5.8, the optimal hydrogen ion concentration for viral neuraminidases<sup>9</sup>, the enzyme activity of the virus suspension was more than twice that observed at pH 7.4. The control preparation of neuraminidase, which has an optimal activity at pH 5.6 (ref. 10), also showed somewhat greater activity at pH 5.8 than at pH 7.4. The control, concentrated fluid collected from tissue culture, showed only very slight neuraminidase activity at either pH (Table 1).

These results show that T1 bovine strain of PI 3 virus possesses neuraminidase activity. If the criteria proposed<sup>1</sup> are accepted, then at least this bovine strain of virus should correctly be considered as a myxovirus.

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### Failure of Function of the "Early Protein" induced by an Influenza Virus in Cells infected by Newcastle Disease Virus

AFTER infection of chick embryo cells with fowl plague virus (FPV), an influenza A virus, or with Newcastle disease virus (NDV), a para-influenza virus, "early proteins" are synthesized before viral RNA-synthesis starts<sup>1-4</sup>. These "early proteins" might function as an RNA-dependent RNA polymerase as has been demonstrated for picornaviruses<sup>5</sup>. In contrast to the picornaviruses, the "early proteins" of the myxoviruses are stable<sup>1-4</sup>. The purpose of this investigation was to determine whether or not the "early protein" induced by FPV is able to function for the multiplication of NDV.

The cell system and virus strains used and the procedure of infection have been published previously<sup>1</sup>. *p*-Fluorophenylalanine (FPA) has been used as an inhibitor for virus multiplication and the demonstration of the "early protein"<sup>1,2</sup>. If FPA is added to the tissue cultures immediately after infection, no viral RNA is synthesized.

The normal complement of viral RNA is produced, however, if the inhibitor is added 2 h post infection to FPV infected cells or 3 h post infection to NDV infected cells, respectively<sup>1,2,4</sup>.

In the present experiments chick embryo cells were infected with FPV and superinfected with NDV 2 h later. After the adsorption of NDV, 300  $\mu\text{g}/\text{ml}$ . FPA and 5  $\mu\text{g}/\text{ml}$ . actinomycin were added. Actinomycin prevents the synthesis of cellular and FPV-RNA, but not the synthesis of NDV-RNA<sup>6,7</sup>. Six hours after addition of the inhibitors 0.5  $\mu\text{C}$ . <sup>14</sup>C-uridine per culture were added and the radioactivity in the RNA was determined 2 h thereafter as described before<sup>8</sup>. The following controls were used: (1) FPA was omitted; (2) FPA was added 4 h after superinfection with NDV; (3) NDV and FPA were omitted; (4) tissue cultures alone were incubated with the isotope.

Table 1. SYNTHESIS OF NDV-RNA IN CELLS PRETREATED WITH FPV

FPV	NDV	Actino- mycin	Addition of FPA, time after superinfection	C.p.m. in RNA
-	-	-	-	17,000
+	-	-	-	199
+	+	+	0 h	110
+	+	+	4 h	1,470
+	+	+	-	1,041

Essentially the same results were obtained in four independent experiments. The results of one representative experiment are given in Table 1. It can be seen that the "early protein" of FPV is not able to stimulate the synthesis of RNA after superinfection with NDV if FPA and actinomycin are added immediately after superinfection. As already mentioned, actinomycin prevents the synthesis of cellular and FPV-RNA and, under the conditions used (addition of FPA immediately after superinfection), FPA interferes with the production of the "early protein" of NDV. Thus the "early protein" of FPV synthesized before FPA is added is not able to act as a substitute for the "early protein" of NDV. The control experiments show that the superinfected cells are able to synthesize NDV-RNA in the presence of actinomycin if FPA is either omitted or added after the "early protein" of NDV is already synthesized.

Two different possibilities might be considered to explain the fact that the "early protein" induced by FPV does not function in the NDV-system. It is known that the RNA of FPV is synthesized within the cell nucleus<sup>9</sup>, while NDV-RNA very probably replicates in the cytoplasm<sup>10</sup>. Thus the "early protein" of FPV might not be able to leave the nucleus. As shown with two different RNA-containing phages the RNA-dependent RNA polymerase (RNA replicase) is very specific in the sense that it uses only that RNA as template which has induced its synthesis<sup>11</sup>. A similar situation might apply to different myxoviruses.

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### *In vitro* Transformation of Cells of Hamster Brain by Adenovirus Type 12

THERE has been some investigation of the *in vitro* transformation of cultured mammalian cells by adenovirus type 12<sup>1-4</sup>. There is, however, only one report about the reproducibility of the phenomenon and the malignancy of the transformed cells. Transformed cells have never been examined for the presence of tumour antigen. A pathological investigation showed that the target cells of adenotumours were undifferentiated Schwann cells or mantle cells of the peripheral nervous system, which correspond to glial cells in the central nervous system<sup>5</sup>. We have observed earlier and more consistent transformation than has been reported previously, by the use of dispersed cells of the brain tissues of newborn hamsters. Preparation of the cells, the incidence of transformation and the biological characteristics of the transformed cells are described briefly in this communication.

Dissected brain tissues of several newborn hamsters were cut into pieces with scissors and incubated with serum containing modified Eagle's medium, at 37° C and left for 24 h. These incubated brain fragments were collected and digested with 0.01 per cent pronase P (preparation of protease from *Streptomyces griseus*), in Hanks physiological solution at 37° C for 30 min. The brain cells thus dispersed were cultured in square bottles for a week to obtain confluent growth of cells using the medium described above. The cells were dispersed in pronase and used as seed cultures for the transformation test: 1 ml. containing  $2 \times 10^5$  dispersed cells, suspended in the culture medium, was dispensed into each test-tube and incubated at 37° C for 2 days. The medium was then decanted and the virus stock fluid was immediately added to the culture. The virus stock fluid was prepared, by freezing and thawing, from a 7 day culture of adenovirus type 12 (Huie strain) and transferred serially to KB cell culture. The titre of the virus stock was  $10^{2.5}$  T.C.I.D.<sub>50</sub>/ml. when KB cells were used as the indicator cells. The cultures inoculated with virus were incubated for 1.5–48 h with the virus in the quantities shown in Table 1. After the adsorption of virus the cultures were fed again with the culture medium and incubated for about 2 months during which they were observed microscopically each day for the appearance of the transformed foci. Fig. 1 shows the appearance of the transformed foci. No foci were found in the control cultures into which virus was not inoculated. The first detection of transformed foci was 15–33 days after infection, which is earlier than that previously reported. The foci grew rapidly and reached a size of about 2 mm in diameter in about 15 days. This incidence of the transformation was not obtained if BHK21 hamster kidney cell line and the other embryonal hamster cell line (HE-32) were used.

The morphology of the transformed cells is quite distinct in that they are small epithelioid cells with large nuclei and a small basophilic cytoplasmic area. They had a tendency to aggregate and pile up. The more specific character of the transformed culture was that it grew

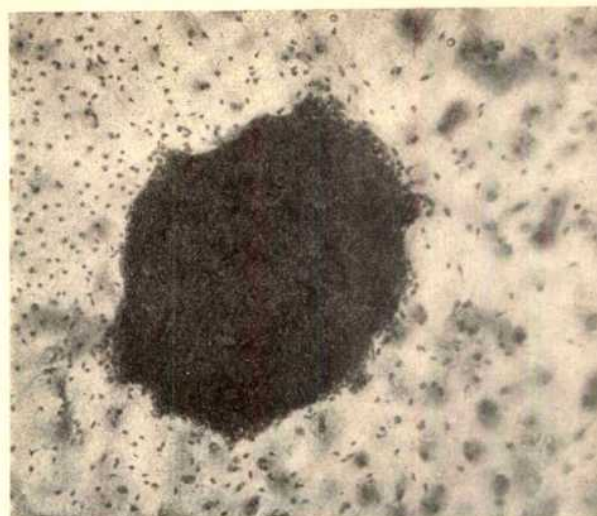


Fig. 1. Appearance of the transformed colony in culture of cells of newborn Syrian hamster, 34 days after inoculation with virus. Stained with Giemsa. ( $\times 210$ .) Note multilayered focus.

better in an atmosphere of 5 per cent carbon dioxide. The cells often failed to grow in the usual conditions of bottle culture, even with a large inoculum (20,000 cells/ml.) unrelated to the medium pH, but grew when 5 per cent carbon dioxide was supplied, even with a relatively small inoculum (500 cells/ml.). These characteristics were similar to those of the cells which were isolated from the tumour tissue induced *in vivo* by the virus and cultured serially *in vitro* and were not observed with KB and HeLa lines. Two cell lines obtained from two foci were similar in their tumorigenicity. Table 2 shows the result of the malignancy test expressed as the numbers of implanted cells sufficient to kill 50 per cent of the hamsters by development of tumours. Attempts to detect adenovirus in *in vitro* cultures of transformed cells were made in the following ways. (1) Transformed cells were cultured for several days in medium containing 2 per cent bovine serum and frozen and thawed three times. The supernatant was inoculated with the indicator culture of KB cells. (2) Transformed cells were mixed with KB and PS (pig kidney) cells and subcultured to observe the cytopathogenic effect induced by the virus. (3) The lysed cell supernatant was inoculated with the KB and PS cells. (4) Transformed cells were treated with 0.1–0.5  $\mu$ g/ml. of mitomycin C to induce adenovirus. (5) Electron microscopy of the transformed cells was carried out to detect any virus particles which were present. None of these methods has revealed virus in the transformed cells. The presence of tumour antigen<sup>6</sup>, however, was detected in the transformed cells as well as in the cultured tumour cells, with the use of the serum of a tumour-bearing hamster (Table 3).

Table 1. INCIDENCE OF TRANSFORMATION BY ADENOVIRUS TYPE 12 INOCULATION IN SYRIAN HAMSTER BRAIN CELLS *in vitro*

Experimental designation	Titre of inoculated (T.C.I.D. <sub>50</sub> /ml.)	Time for virus adsorption (h)	Medium used	Frequency of transformation (foci/test-tubes or bottles)
HB-1	Control (without virus)	—	MEM + 15 per cent bovine serum	0/3
	$10^{2.5}$	48	MEM + 15 per cent bovine serum	1/2
HB3-5	Control (without virus)	—	MEM + 10 per cent bovine serum	0/3
	$10^{2.5}$	24	MEM + 10 per cent bovine serum	5/3
HB-12	Control (without virus)	—	MEM + 5 per cent calf serum	0/6
	$10^{2.5}$	1.5	MEM + 5 per cent calf serum	0/3
	"	6	MEM + 5 per cent calf serum	2/3
	Control*	—	MEM + 5 per cent calf serum	0/2
	(without virus)	—	MEM + 5 per cent calf serum	2/2
	$10^{2.5}$	24	MEM + 5 per cent calf serum	0/2

Medium was Eagle's MEM (plus 0.03 per cent Bactopeptone, 110 mg of pyruvate and 10 mg of serine/l.) supplemented with serum. Cells were seeded with  $20 \times 10^4$  cells/test-tube and  $100 \times 10^4$  cells/bottle.

\* This experiment was performed with a culture bottle containing 5 ml. of medium.

Table 2. SUBCUTANEOUS TRANSPLANTABILITY TO NEWBORN SYRIAN HAMSTERS

Passage	Cell dosage	Animals inoculated	Animals with tumour	Cell No. of <i>E.I.D.</i> <sub>50</sub>
Control (normal brain cells)	1 × 10 <sup>6</sup>	6	0	
Second	1 × 10 <sup>6</sup>	7	7	
Highth	1 × 10 <sup>6</sup>	—	—*	
	1 × 10 <sup>5</sup>	6	5	< 10 <sup>3.7</sup>
	1 × 10 <sup>3</sup>	6	0	
Twenty-first	1 × 10 <sup>6</sup>	13	13	
	1 × 10 <sup>5</sup>	12	11	
	1 × 10 <sup>4</sup>	9	8	10 <sup>3.4</sup>
	5 × 10 <sup>3</sup>	10	4	
	1 × 10 <sup>3</sup>	5	0	

Results noted within 7 days of transformation of HT-2 cells *in vitro* by adenovirus type 12.

\* Animals lost by accident.

Table 3. TITRE OF COMPLEMENT FIXATION ANTIGEN OF THE *in vitro* TRANSFORMED AND CULTURED TUMOUR CELL EXTRACTS FOR THE SERUM OF A TUMOUR BEARING HAMSTER

Source of soluble antigens	1:1	1:2	1:8	1:16
HT-2 40T	4	4	4	0
AC*	0	0	0	0*
H-4 80T	4	4	4	0
AC	0	0	0	0
T-6	4	4	4	4
AC	0*	0	0	0

HT-2, *in vitro* transformed cells; H-4, tumour cells cultured serially *in vitro*; T-6, a hamster tumour caused by the inoculation of the virus. The titre of a serum of a tumour bearing hamster was 1:64 for T-6 tumour extract.

Anticomplementary effect.

These data strongly suggest that the observed event is malignant transformation *in vitro* by the adenovirus, such as polyoma virus and SV40 types, although it is still of lower incidence than transformation by the latter viruses.

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## Role of Sialidase in the Initial Phase of Infection with Influenza Virus

THERE have been numerous investigations of the role of sialidase (neuraminidase), but definitive evidence of its functional significance is lacking: a result of technical problems and inadequate experimental designs<sup>1</sup>. It has been proposed that the enzyme is involved: (a) in the initial phase of the infectious process; (b) during intracellular multiplication; and (c) in the release of virion from host cells. Thus a specific sialidase inhibitor could conceivably be used to investigate directly the function of the enzyme in the infectious cycle of virus multiplication<sup>1</sup>. At present, appropriate inhibitors are not available<sup>2</sup>, but it seemed possible to evaluate (a) by an experimental approach which involved specific anti-sialidase antiserum, and by determination of its effect on the infectivity of A2/Japan 305/57 influenza virus in chick embryonated eggs.

For this purpose "purified" enzyme was prepared from Japan 305 virus and used for the immunization of rabbits for the production of anti-sialidase antiserum by methods previously reported<sup>3,4</sup>. Virus preparations were prepared,

assayed for haemagglutinins and egg infectivity (*E.I.D.*<sub>50</sub>) as described previously<sup>5,6</sup>. Enzyme analyses and enzyme inhibition assays were carried out in the same way as before<sup>3,4</sup>. The degree of inhibition of haemagglutination and of enzyme activity by rabbit anti-sialidase antisera is shown in Table 1. In addition, the antibody activities of two antisera, designated as AS544 and AS28, are also included. These were prepared with crude enzyme and virus preparations which had been treated with ether. It can be seen that antiserum AS544 and purified  $\gamma$ -globulin antiserum AS28 were reactive to both the enzyme and to the haemagglutinin. On the other hand, purified  $\gamma$ -globulin antisera AS19, AS23, AS26 and unfractionated antiserum ASR were reactive to the enzyme and were essentially unreactive to the haemagglutinin (< 1-10). This indicates that these four antisera are highly specific for the enzyme. It was found that antiserum prepared with enzymes which had been purified by gel filtration with 'Sephadex G-75' alone frequently inhibited haemagglutination<sup>3</sup>. Further purification of the enzyme obtained from 'Sephadex' columns by gel electrophoresis<sup>4</sup> allowed injection of rabbits with enzyme prepared in this way to give antisera which did not inhibit haemagglutination (see AS19, AS23 and AS26; Table 1).

Table 2 shows the effect of sialidase antisera on the *E.I.D.*<sub>50</sub> of Japan 305 virus. The non-specific antisera, AS544 and AS28, significantly neutralized the virus particles; whereas AS19, AS23, AS26 and ASR did not significantly neutralize the *E.I.D.*<sub>50</sub> of Japan 305 virus. Additional experiments were carried out to show that the lack of neutralization is not attributable to possible dissociation of virus and antibody complexes<sup>6</sup>. AS23 antiserum was added to serial ten-fold dilutions of virus and the mixtures of virus and serum were incubated at 37° C for 2 h and then at 0° C-5° C for 20 h. AS23 did not significantly neutralize the *E.I.D.*<sub>50</sub> of Japan 305 virus (*t* = 1.55) under these conditions of virus neutralization by antiserum; nor did AS23 diluted serially four-fold from 1-4 to 1-64 and added to a constant amount of virus (100 *E.I.D.*<sub>50</sub> dose) neutralize the virus. It is not possible to detect any enzyme activity at these dilutions of the virus.

Table 1. INHIBITION OF HAEMAGGLUTINATION AND SIALIDASE BY RABBIT ANTI-SIALIDASE ANTISERA

Rabbit serum No.	Titre causing inhibition of haemagglutinin	Enzyme inhibition Serum dilution*	Per cent
AS544	1-320	10 <sup>-1</sup>	90
AS28	1-320	10 <sup>-2</sup>	71
AS19	< 1-10	10 <sup>-2</sup>	91
AS23	< 1-10	10 <sup>-2</sup>	84
AS26	< 1-10	10 <sup>-1</sup>	87
ASR	< 1-10	10 <sup>-2</sup>	69

Assays of inhibition of haemagglutination and enzyme inhibition analyses were carried out as described previously<sup>3,4</sup>. AS544 and ASR were whole serum; the others were salt fractionated immune  $\gamma$ -globulin and further purified by chromatography on diethylaminoethyl cellulose column<sup>4</sup>. AS19, AS23, and AS26 were from rabbits immunized with enzyme initially purified on 'Sephadex G-75' column and then by polyacrylamide gel electrophoresis<sup>4</sup>.

\* The dilutions represent first serial ten-fold dilution which inhibited the enzyme reaction by less than 100 per cent.

Table 2. EFFECT OF ANTI-SIALIDASE ANTISERA ON THE EGG INFECTIVITY OF INFLUENZA A2/JAPAN 305/57

Experiment	Virus and antiserum*	<i>E.I.D.</i> <sub>50</sub> †	<i>t</i> ‡
(1)	Virus alone	7.50 ± 0.23	
	Virus and AS544	1.63 ± 0.32	14.00 + +
(2)	Virus alone	8.17 ± 0.23	
	Virus and AS28	2.50 ± 0.23	11.80 + +
(3)	Virus alone	8.57 ± 0.23	
	Virus and AS19	8.28 ± 0.23	0.81 n.s.
(4)	Virus alone	8.69 ± 0.41	
	Virus and AS23	7.83 ± 0.39	1.50 n.s.
(5)	Virus alone	7.50 ± 0.23	
	Virus and AS26	7.50 ± 0.45	0.00 n.s.
(6)	Virus alone	7.68 ± 0.40	
	Virus and ASR	7.50 ± 0.33	0.35 n.s.

One part of the antiserum was added to one part of serial ten-fold dilutions of Japan 305 virus. Virus-serum mixtures were allowed to react at room temperature for 30 min and then embryonated eggs of chick were challenged with 0.1 ml. of a combination of virus and serum.

\* Sera were assayed at 10<sup>-1</sup> dilutions.

† *E.I.D.*<sub>50</sub> expressed as log<sub>10</sub> ± standard error/ml.

‡ Student's *t* values calculated as follows:  $\frac{E.I.D._{50} - E.I.D._{50}}{\sqrt{((S.E._1)^2 + (S.E._2)^2)}}$  at infinite degrees of freedom; n.s., not significant; + +, highly significant.



These data indicate that the viral enzyme of influenza A, Japan 305, is not required for the initial phase of the infectious cycle of influenza virus in embryonated eggs. Virus multiplication occurred in the presence of adequate concentrations of specific anti-sialidase antibodies, which *in vitro* inhibited completely the enzyme present in virus particles diluted 1,000 or more times<sup>3</sup>. It has been reported that virus and antibody concentrations are directly related to enzyme activity and inhibition by antiserum<sup>2</sup>. The lack of effect of the antiserum to sialidase on the infectivity does not preclude the possible role of the viral enzyme during the intracellular phase of virus multiplication<sup>10,11</sup> or in the release of newly replicated virus from host cells<sup>12</sup>.

We reported previously that "purified" sialidase could be characterized by gel electrophoresis and immunoelectrophoresis, respectively<sup>4</sup>. The present data on enzyme inhibition, inhibition of haemagglutination, and *E.I.D.*<sub>50</sub> assays with mixtures of antibodies and specific antibodies show that these methods will be useful for further characterization of, in particular, the purity of influenza virus surface antigens. This is mainly because of the extreme sensitivity of the biological methods, inhibition of haemagglutination and assays of *E.I.D.*<sub>50</sub>.

These results further show that haemagglutinin and sialidase are distinct surface antigenic components of Japan 305 virus, which is in agreement with reported findings<sup>5,13</sup>. In addition, the lack of neutralization of the *E.I.D.*<sub>50</sub> of Japan 305 virus by sialidase antiserum supports the concept that (a) the haemagglutinin is responsible for the initial attachment of virus particles to host cells<sup>14</sup>; (b) sialidase is possibly involved during a phase of the infectious process of influenza virus in eggs after the initial phase<sup>10-12</sup>. Further work in this connexion is in progress.

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## GENETICS

### Cytogenetic Studies of Two Species of Porpoise

THIS investigation was undertaken to compare the chromosomes of two species of porpoise, the Atlantic bottlenose (*Tursiops truncatus*) and the Pacific white-striped porpoise (*Lagenorhynchus obliquidens*), both small odontacete cetaceans of the family Delphinidae. The chromosome number and karyotypes were established for a male and female

*T. truncatus* and for two males and one female *L. obliquidens*.

The animals used in this study are members of the cetacean research colony, maintained at the Marine Biology Facility, U.S. Naval Missile Center, in California. The two species differ markedly in external appearance. *T. truncatus* dwells mostly in coastal or inshore waters, often swimming into bays and rivers<sup>1</sup>. They are common in the Atlantic Ocean along the East and Gulf coasts of the United States, as well as in the Mediterranean Sea and several other areas of the world. *L. obliquidens* ranges from Mexico to Alaska in the deeper offshore waters of the eastern Pacific ocean.

Blood samples were obtained by venipuncture of small vessels which run laterally down the centre of each side of the fluke. About 10 ml. of heparinized blood were withdrawn and refrigerated or kept at room temperature for periods not exceeding 2 h before a culture was set up. The microtechnique for culturing leucocytes from whole blood described by Arakaki and Sparkes<sup>2</sup> was modified slightly for use with porpoise blood. The type of medium used, duration of incubation of the cultures and the amount of phytohaemagglutinin (PHA) were varied to determine the optimal conditions for preparation of metaphase chromosomes. The samples from *L. obliquidens* were the easiest to obtain and to culture; almost every modification produced satisfactory preparations, while those from *T. truncatus* were of limited success. Table 1 summarizes these results. In general the chromosome preparations were inferior to those obtained from human blood, but were satisfactory for counting chromosomes and for establishing karyotypes for these mammals.

Table 1. SUMMARY OF RESULTS WITH VARIED TISSUE CULTURE CONDITIONS

Culture media	Culture results	
	<i>L. obliquidens</i>	<i>T. truncatus</i>
(1) Eagle's MEM (2 ml.) with: 20 per cent foetal calf serum, 0.2 ml. of whole blood and 0.01 ml. of PHA-P*	Good	Poor
(2) TC 199 (5 ml.) with: 20 per cent foetal calf serum, 0.7 ml. of leucocyte rich plasma and 0.2 ml. of PHA-M*	Good	Fair
(3) Eagle's MEM Spinner (5 ml.) with: 20 per cent foetal calf serum, 0.7 ml. of leucocyte rich plasma and 0.2 ml. of PHA-M*	Good	Good metaphases but few in number
(4) NCTC 109 (5 ml.) with: 20 per cent foetal calf serum, 0.7 ml. of leucocyte rich plasma and 0.2 ml. of PHA-M*	Very good	Poor

Incubation time: 3-4 days—acceptable results.

More than 4 days—clots formed and few metaphases seen.

Optimum conditions: *L. obliquidens*: NCTC 109 incubated for 3 days.

*T. truncatus*: Eagle's MEM Spinner incubated for 3 days.

\* 'Difco', as reconstituted.

Metaphases were accumulated by exposure to colchicine for 4 h. After incubation the cells were subjected to hypotonic treatment with 0.95 per cent sodium citrate, washed in methyl alcohol and glacial acetic acid fixative (3:1) mounted on slides by the air-dry method and stained with aceto-orcein. To establish a karyotype which could be regarded as representative, the chromosomes of several cells from each porpoise were counted, photographed and karyotyped. These results are summarized in Table 2 and the karyotypes are illustrated in Figs. 1 and 2.

The modal chromosome number is constant at forty-four for both species, male and female, and the chromosomes can be readily paired. There seemed to be sufficient diversity in size and morphology of the chromosomes to warrant an attempt to establish provisional karyotypes for these animals. Comparison of karyotypes between

Table 2. SUMMARY OF METAPHASE ANALYSES

Animal studied	No. of cells in which chromosomes were counted	No. of chromosomes/cell	No. of karyotypes
<i>Tursiops truncatus</i> , male	5	44	4
<i>Tursiops truncatus</i> , female	5	44	5
<i>Lagenorhynchus obliquidens</i> , male (A)	5	44	4
<i>Lagenorhynchus obliquidens</i> , male (B)	4	44	3
<i>Lagenorhynchus obliquidens</i> , female	8	44	4

individuals of the same and different species supported the establishment of a similar karyotype for both species.

The chromosomes are sub-classified into groups given designations of A, B, C, D, and the sex chromosomes (X, Y). Within the chromosome groups most of the individual pairs of chromosomes can be recognized and identified. Group A contains the first five pairs of large sub-metacentrics: the centromere of A-1 is more medial than A-2 although both pairs are about the same size; the centromere of A-3 is less medial than A-4 and is slightly larger; A-5 is the smallest of this group. The long arms of one A-2 chromosome seemed to be consistently longer than those of its paired chromosome. Group B comprises the smaller sub-metacentrics arranged in order of decreasing size. The centromere position is also characteristic in that it is: sub-medial in B-6; sub-terminal in B-7; sub-terminal in B-8; sub-medial in B-9; sub-terminal in B-10; sub-medial in B-11; and sub-medial in B-12.

Group C consists of four metacentric or very nearly metacentric pairs of chromosomes. C-13 is distinctly larger than the other three. C-14 to C-16 become progressively smaller; C-16 is slightly smaller than the last sub-metacentric, B-12. Group D is composed of acrocentric pairs; D-17 is considerably larger than any of the other pairs in group D. D-21 is the smallest of the autosomes. Because of the size of this pair in our preparations it was not possible to determine accurately whether

it was best grouped with the acrocentrics or sub-metacentrics, but as the smallest it was given the last of the autosomal designations.

The sex chromosomes are of the X,Y type, characteristic of mammals. The males have one X chromosome and one Y, the females two X chromosomes. The Y chromosome is the smallest of all the chromosomes. In two preparations it appeared to be sub-metacentric but generally the centromere was indistinct. The X chromosome is metacentric and intermediate in size between C-14 and C-15 and very nearly the size of B-11; the difference between the two is in the position of the centromere. In most of the cells examined, the X chromosome had arms which appeared more compact and rounded than those of any of the other chromosomes.

Using testicular squash preparations, Makino found a chromosome complement of forty-four in *Phocoenoides dallii*<sup>3</sup>. An accurate comparison between his spermatogonial chromosomes and the somatic cell metaphases of the present investigation is not possible, but he also found the Y chromosome to be the smallest and the X chromosome of medium size comparable with pair 10 or 11. Walen and Madin<sup>4</sup> investigated the chromosomes (prepared by tissue culture of kidney cells) of *Tursiops truncatus* (female) and pilot whale (*Globicephala scammonii*, male and female), and demonstrated a diploid (2n) chromosome number of forty-four for both species with the karyotype of *T. truncatus* in close agreement with our observations.

A diploid (2n) chromosome number of forty-four has been found for two species of porpoise, *Tursiops truncatus* and *Lagenorhynchus obliquidens*. Except for differences in sex chromosomes between male and female, there appear to be no obvious gross differences in chromosomal constitution between sexes in the same species nor between the two species (Figs. 1 and 2). Provisional karyotypes for male and female members of both species have been established.

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## ANATOMY

### Histochemical Demonstration of Melanocytes by the Use of 5,6-Diacetoxyindole as Substrate for Tyrosinase

The stages in the formation of melanin from tyrosine proposed by Raper<sup>1</sup> are generally accepted as the metabolic pathway of melanogenesis in mammalian pigment cells. Most of the stages have been examined in some detail by experimental systems *in vitro*, but the dehydrogenation of 5,6-dihydroxyindole by normal human melanocyte tyrosinase had not been established. It was considered that this could be determined by a comparison of the

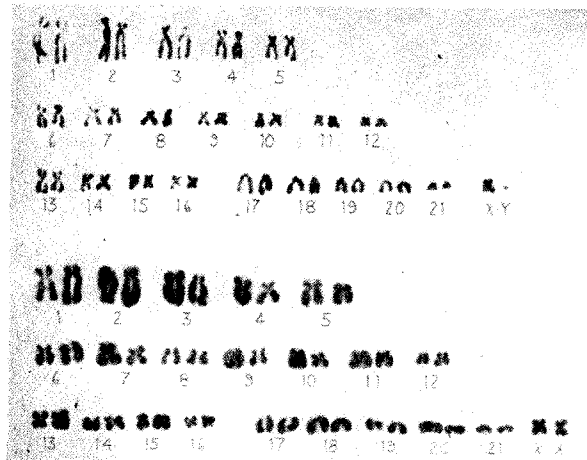


Fig. 1. Representative karyotypes of male (top) and female (bottom) of porpoise species *T. truncatus*. The pairs of chromosomes have been numbered and grouped according to scheme outlined in text. The male and female seem to differ only in their sex chromosome make-up. The very small Y chromosome is paired with the X in the male.

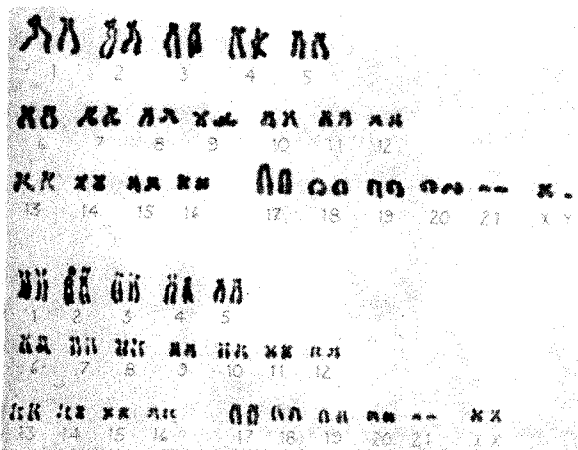


Fig. 2. Representative karyotypes of male (top) and female (bottom) of porpoise species *L. obliquidens*. Comparison with the karyotypes of species *T. truncatus* in Fig. 1 shows no obvious gross differences.



distribution of reaction product in sections of normal skin incubated in appropriate conditions in solutions of dihydroxyphenylalanine (DOPA) and dihydroxyindole. It was, however, impossible to use the indole as a histochemical substrate because of the very rapid autoxidation which it undergoes. A substituted derivative, 5,6-diacetoxyindole, was supplied by Dr. R. J. S. Beer from material prepared synthetically<sup>2</sup>. This substance was suitable for use as a histochemical substrate after recrystallization from light petroleum.

The incubation medium was made from 0.1 per cent diacetoxyindole dissolved in ethylene glycol monoethyl ether (final concentration 3 per cent), made up with distilled water and with pH adjusted to 7.2 with solid sodium bicarbonate. Cryostat sections of normal human abdominal skin fixed with formol and calcium<sup>3</sup> were incubated in this medium for 3 h at 37° C. Control sections were incubated in a medium containing 0.1 per cent DOPA made up in the same way. These incubations were repeated subsequently with the inclusion of sections of skin from an albino subject and pigmented and depigmented skin from a patient with vitiligo. After incubation the sections were washed in distilled water and mounted in water-mounting medium for inspection.

Examination of the material showed that a black reaction product was present in cells situated in relation to the basal layer of the epidermis in the normal skin and in the material from a pigmented region of the skin of the patient with vitiligo. These results were comparable to those seen in the sections incubated in DOPA, and the DOPA-like distribution of the staining is shown in Fig. 1. No reaction product was observed in sections of albino or depigmented vitiligo skin.

These results suggest that the indole is enzymatically oxidized by tyrosinase. This conclusion is, however, questionable, for if there were an indole 5,6-acetyl esterase present in certain cells (for example melanocytes) it would, by hydrolysis of the substrate 5,6-diacetoxyindole, liberate 5,6-dihydroxyindole which could undergo spontaneous oxidation to the corresponding quinone, followed by polymerization to form melanin. For this reason sections of the normal skin giving the positive results described above were incubated in diacetoxyindole medium to which an azo dye had been added. This esterase incubation medium was prepared by the addition of 1 mg of hexazotized pararosaniline<sup>4</sup> to 10 ml. of indole solution made up as described. Control sections were incubated in medium which did not contain diacetoxyindole.

The result after 3 h of incubation at 37° C is shown in Fig. 2. Indole acetyl esterase activity was widespread in the cells of the epidermis and the distribution of reaction



Fig. 2. Normal human skin incubated in indole esterase medium. Reaction product is widely distributed in the epidermis in contrast to Fig. 1.

product was in marked contrast to the melanocyte staining in sections incubated with 5,6-diacetoxyindole alone.

It is, therefore, reasonable to deduce from these results that the enzymatic nature of the oxidation leading to the formation of indole 5,6-quinone is substantially confirmed, and it may be concluded in the absence of positive reactions in sections of albino and vitiligo skin that the oxidations of DOPA and dihydroxyindole are both brought about by tyrosinase.

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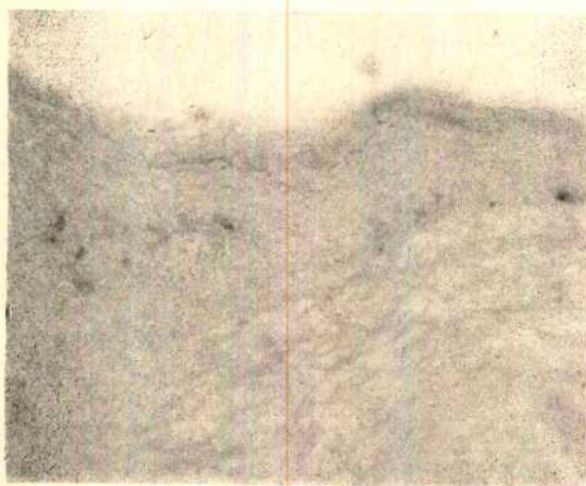


Fig. 1. Normal human skin incubated in 5,6-diacetoxyindole medium. Black reaction product is present in melanocytes at the base of the epidermis.

## BIOLOGY

### Leopard Frogs raised in Partially Controlled Conditions

THE difficulty of raising leopard frogs, *Rana pipiens*, to maturity in controlled conditions makes them impractical for many experiments<sup>1</sup>. Tadpoles have previously died soon after metamorphosis<sup>2</sup>, but we have now raised frogs to sexual maturity with a low mortality rate. Thus sibling adults can be used for experimental purposes, and using our methods (described here) it should be possible to carry out genetic studies over several generations and to develop partially inbred lines of these laboratory animals.

In April 1962, fertilized eggs from a pond in Springville, New York, were divided into several groups, each of which was put into an uncovered plastic pan, measuring 32 × 22 × 7 cm. The pans were half filled with pond water or well water. The animals grew and developed equally well in both types of water.



Fig. 1. Two generations of laboratory frogs. The first generation animals are 3 years old. The smaller animals, progeny of siblings of the older frogs, are 6 months old.

Dead eggs and embryos were removed as soon as they were identified. By stage 22 (prominent gills) the larvae were separated into groups of twenty to twenty-five, and were transferred to clean pans every second or third day. After each transfer, one or two pellets of Purina guinea-pig chow were added to each container. The tadpoles consumed the disintegrated pellets eagerly, and as they grew they were further separated until eventually five to ten were kept in each box.

The animals were transferred to polyethylene dishes measuring  $38 \times 33 \times 15$  cm when their front legs emerged. The pans were propped at an angle of  $10^\circ$ , and approximately 500 ml. of well water formed a pool at the lower end of each pan. The upper end of the pan provided dry space for feeding. The pans were covered with a metal screen lid.

The nutritional requirements of newly metamorphosed leopard frogs are unknown. In a preliminary experiment, the animals ate one or two small meal worms (*Tenebrio molitor*) at each thrice-weekly feeding. In a few months, however, the frogs died, often severely bloated, and this was not prevented by injections of B-complex vitamins. For this reason, the newly metamorphosed frogs were transferred to outdoor pens measuring  $4.5 \times 4 \times 2$  ft., with 0.25 in. mesh hardware cloth for the floor and sides, and chicken wire for the roof. The floor of each pen was covered with leaf mould to a depth of approximately 1 in. A shallow plastic pan with a sloping gravel floor and containing water was placed in the pen. Laboratory meal worms and an assortment of naturally occurring insects (mainly crickets and flies) were put into the pen daily. The animals were free to capture whatever other insects were available. In September the frogs were replaced in the dishes in the laboratory and maintained exclusively on a diet of meal worms. In succeeding summers, the meal worm diet was supplemented with flies and crickets. During the summer of 1963 the frogs were returned to the outdoor pens, but during the summers of 1964 and 1965 they were kept in the laboratory.

In November 1963, 19 months after the collection of the eggs, an attempt was made to obtain a second generation. Motile spermatozoa were observed in a suspension of minced testis from a representative male. Accordingly, 1  $\mu$  of 'Turttox' pituitary extract was injected into a female sibling to determine whether eggs could be obtained, but they could not be stripped from this female. In April 1964, a second female 24 months old was treated with the pituitaries of several frogs. This animal showed the increase in pigmentation and shedding of skin expected after such treatment, and eggs were obtained after 4 days, but they were misshapen and did not divide after exposure to motile spermatozoa from a sibling.

In December 1964, a third female, 33 months old, which had been treated with 1  $\mu$  of 'Turttox' pituitary suspension provided a small yield of apparently normal eggs. These eggs were fertilized with spermatozoa from a sibling male, and thirty apparently normal tadpoles developed,

eighteen of which metamorphosed into normal frogs (Fig. 1). To our knowledge, this is the only second generation of *Rana pipiens* produced in captivity.

We investigated the deficiency of the meal worm diet. Newly metamorphosed frogs in 1963 were divided into four groups. The first was fed meal worms alone; the second was given the varied diet of meal worms, flies, crickets and other wild insects; the third was fed meal worms which, on two occasions, were dipped in a suspension of adult frog faeces; and the fourth group was fed meal worms supplemented on three occasions by cod liver oil. The animals of the second group, which were given meal worms and insects during early life, were the only animals that showed satisfactory weight

gains and survival (Figs. 2 and 3).

The proteins of a sample of meal worms, a wild frog, and ten frogs deficient in nutrients were isolated by precipitation with trichloroacetic acid and washed with water, acetone and chloroform. The material was hydrolysed and the amino-acid content was determined. The glycine, lysine and cysteine content of the protein from meal worm was less than that of protein from frog, but the former contained all the measured amino-acids in moderate abundance (Table 1). There was no pronounced difference in amino-acid composition between the wild frog and the frogs on the diet of meal worms alone.

Our experiments do not identify the deficiency in the meal worm diet. The deficiency does not appear to be caused by a lack of amino-acids or fat soluble vitamins. If intestinal micro-organisms are required to produce some other growth factor, the organisms are not maintained in the frog gut but must be supplied frequently. Frogs fed on meal worms and two inocula of normal faeces failed to demonstrate nutritional competence.

Meal worms could possess some substance which is toxic to young frogs but tolerated by mature frogs. In our laboratory we have had no excess mortality in adult frogs maintained during the winter on meal worms alone. Furthermore, we have observed no ill effects in adult

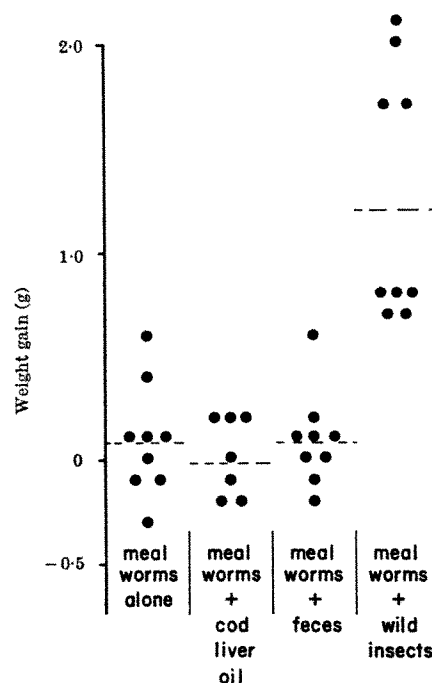


Fig. 2. Weight gains of frogs fed on various diets. Each dot indicates gain or loss in weight of the surviving individual frogs between November 23 and December 14, 1963 (when supplements were no longer administered).



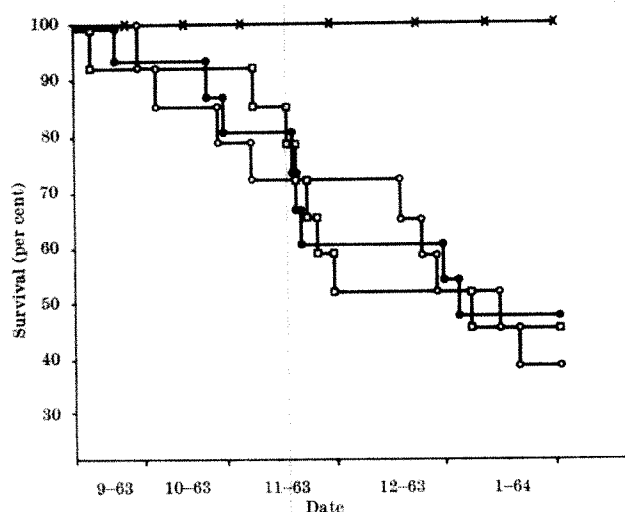


Fig. 3. Survival of frogs fed on various diets. The diets and numbers of animals in each group were as follows: —○—, meal worms alone (13); —□—, meal worms and cod liver oil (14); —△—, meal worms and adult faeces (15); —×—, meal worms and wild insects (9). Wild fertilized eggs were brought into the laboratory on April 27, 1963, metamorphosis into frogs was complete between July 2 and July 15. The experiment was terminated at the end of January 1964. All the frogs fed on wild insects survived until April 1964, when they were released.

wild frogs which were brought into the laboratory and fed on a diet consisting of meal worms alone. The deficiency of a meal worm diet appeared only in newly metamorphosed animals.

Table 1. AMINO-ACID CONTENT OF FROGS AND MEAL WORMS

Amino-acid	Abundance in protein sample (mole per cent)	Wild frog*	Deficient frog	Meal worm
Glutamic acid	13.1	12.1	11.3	11.3
Glycine	12.2	16.7	9.0	9.0
Aspartic acid	9.9	9.0	9.5	9.5
Alanine	8.4	8.4	9.3	9.3
Lysine	7.9	7.2	5.8	5.8
Leucine	7.6	6.9	8.0	8.0
Serine	5.8	5.6	5.6	5.6
Valine	5.4	5.1	8.9	8.9
Proline	5.4	6.2	5.3	5.3
Arginine	4.7	4.8	4.0	4.0
Threonine	4.7	4.4	4.6	4.6
Isoleucine	4.4	3.9	4.7	4.7
Phenylalanine	3.3	2.9	3.6	3.6
Tyrosine	2.4	2.2	6.0	6.0
Methionine	1.9	1.8	1.3	1.3
Histidine	1.9	1.5	2.4	2.4
Cysteine	1.0	1.1	0.7	0.7

\* A small amount of collagen-like protein was lost from this sample.

It seems likely that frogs can be more easily fed and maintained if the tadpoles are maintained at reduced temperatures. When our tadpoles were kept at approximately 24° C the time span from egg to metamorphosis of the first frog was 60 days. These tadpoles grew to about 5 cm before metamorphosis. In a later experiment, in which the water temperature was 17° C, the first frog developed in 110 days; these tadpoles grew to about 7 cm in length. The animals appeared to eat just as vigorously at the lower temperature as at the higher, and the frogs were larger at the time of metamorphosis.

It is now possible to raise frogs to maturity in a laboratory environment in spite of the difficulty of obtaining a completely controlled diet. Such frogs should provide better material for study than the captured wild animals now in use.

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## Transplantability and Life Span of Mammary Gland during Serial Transplantation in Mice

SMALL segments of mammary tissues obtained from a 358 day old virgin female *C57BL* mouse and from a 734 day old virgin female *CBA* mouse, when transplanted into genetically compatible mice, regenerated glands which appeared normal and which responded to either exogenous or endogenous hormonal stimulation in a manner similar to that of the mammary glands of the host, and secreted milk when the hosts nursed young<sup>1,2</sup>. The quiescent status of the mammary glands of old mice was attributed to their environment, particularly the hormonal status of the animal rather than to the ageing of mammary tissues themselves.

The present experiment was carried out to determine whether mammary tissues could survive for prolonged periods *in vivo* when environments are renewed repeatedly.

From the third mammary gland on the right side of a 135 day old female mouse of the *CBA* strain, segments of mammary duct, 0.6 mm in length, and a little surrounding adipose tissue, were transplanted into the fat pads from which the fourth mammary gland had been removed in young adult female host mice<sup>3</sup>. These were the  $T_1$  generation for serial transplantation. The hosts were hybrid mice (female *BCB* × male *CBA*, designated as  $BA_1$ ; and female *CBA* × male *BCB*, designated as  $BA_2$ ). Mammary gland tissues derived from the original donor were transplanted serially in  $BA_1$  hosts for six transplant generations ( $BA_1$  line) and in  $BA_2$  hosts for seven generations ( $BA_2$  line). Serial transplantations were made approximately every 3 months. Some host mice of  $BA_1$  and  $BA_2$  lines were not mated until after segments of these transplanted mammary glands had been transplanted to mice of the succeeding generations (virgin line). At the second transplantation-generation ( $T_2$ ) of both  $BA_1$  and  $BA_2$  lines, the hosts were mated soon after they received mammary grafts. After their first litters were weaned at 21 days *post partum*, they were not mated until they donated mammary tissues to mice of the succeeding generation. In this subline (parous line), the same procedures were repeated at each subsequent generation of serial transplantation. All the hosts carrying mammary transplants were allowed to rear litters as long as they were fertile and were kept until mammary tumours developed or their death seemed imminent.

The incidence of successful transplants is summarized in Table 1. The data concerning the incidence of tumours will be reported elsewhere. The transplantability of mammary tissues was comparable in all lines. The transplantability was not uniform in different generations. In  $BA_1$  line, the relative number of successful transplants declined to 4/38 in  $T_3$  of virgin line and to 1/34 in  $T_4$  of parous line. In  $BA_2$  line, a similar decline took place in  $T_5$  of virgin

Table 1. INCIDENCE OF SUCCESSFUL MAMMARY TRANSPLANTS AT DIFFERENT GENERATIONS OF SERIAL TRANSPLANTATION

Transplantation generation	Virgin line				Parous line			
	No. of successful grafts	No. of transplants	(Per cent)	No. of host mice	No. of successful grafts	No. of transplants	(Per cent)	No. of host mice
$BA_1$ host line:								
$T_1$	9	14	64.3	7				
$T_2$	33	66	50.0	33				
$T_3$	4	38	10.5	19	11	26	42.3	13
$T_4$	11	20	55.0	10	1	34	2.9	17
$T_5$	12	18	66.7	9	6	16	37.5	8
$T_6$	26	36	72.2	18	23	36	63.9	18
Total $T_2-T_6$	53	112	47.3	56	41	112	36.6	56
$T_1-T_6$	95	192	49.5	96				
$BA_2$ host line:								
$T_1$	8	14	57.1	7				
$T_2$	18	34	52.9	17				
$T_3$	19	34	55.9	17	26	36	72.2	18
$T_4$	18	32	56.3	16	3	36	8.3	18
$T_5$	3	40	7.5	20	19	40	47.5	20
$T_6$	20	42	47.6	21	25	40	62.5	20
$T_7$	11	38	28.4	19	2	14	14.3	7
Total $T_2-T_7$	71	186	38.2	93	75	166	45.2	88
$T_1-T_7$	97	234	41.5	117				



line (3/40) and in  $T_4$  of parous line (3/36). During later generations the mammary grafts grew in relatively more hosts. The longest life span of mammary tissues of the original donor *CBA* mouse throughout seven generations of serial transplantation was 1,379 days (3 years and 9.5 months). This transplanted mammary segment had developed into a branched duct system that occupied the whole area of the fat pad without mammary gland of the  $BA_2$  host mouse in  $T_7$  virgin line when this host was killed at 812 days old. A few hyperplastic alveolar nodules had developed in this mammary gland. The transplanted mammary tissue was comparable with the mammary glands of the host.

Our experiment showed that selected and surviving mammary gland tissues can regenerate and grow the normal structures of mammary glands when placed in a younger animal. Whether the reduced transplantability of mammary tissues at three or four generations of serial transplantation was due to the lack of transplantability of the adjacent adipose tissue and connective tissues or of the mammary epithelium is not known. It has been claimed that white adipose tissue is not readily transplanted<sup>1,5,6</sup>. Mammary gland that had been maintained for 1,379 days and mammary gland in the seventh generation responded to endogenous hormonal stimulation in a manner similar to that of the mammary glands of the hosts. This could suggest the capability of mammary parenchymal tissue to survive *in vivo* indefinitely if the environmental conditions are favourable.

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### Transplantability of Mammary Gland in Brown Fat Pads of Mice

THE importance of white adipose tissue as a stroma for the growth of transplanted mammary glands has been reported<sup>1-4</sup>. It has also been reported that interscapular brown fat tissues, which had been transplanted into mammary white fat pads in mice, sometimes survived and retained the characteristics of brown fat on transplantation and the mammary glands of the host could grow into them<sup>5</sup>.

Brown fat is morphologically, biochemically, physiologically and endocrinologically different from white fat<sup>6,7</sup>. Its physiological function is still obscure, but it has been reported to be closely related to endocrine function, particularly with ACTH, adrenocortical hormones, insulin, thyroid hormone and sex hormones.

Peripheral denervation of interscapular brown fat is followed by an increase in its content of water, glycogen

and lipids, and also by histological changes so that it comes to resemble white fat<sup>8-9</sup>. The concentration of 3-methylcholanthrene was found to be higher in interscapular brown fat pads than in mammary white fat pads when carcinogen, administered intraperitoneally, was recovered from these tissues spectrophotofluorometrically<sup>9</sup>. There are, however, apparently no reports of the regeneration and growth of mammary glands which have been isografted into intact or denervated interscapular brown fat pads in mice. Our experiment was undertaken to investigate interrelationships between mammary growth and stromal conditions.

Sixty-two segments of mammary duct, each 0.6 mm long, were obtained from the thoracic (third) pair of mammary glands of two female *CBA* mice by a "quantitative transplantation" method described previously<sup>3,4</sup>. With the aid of a dissecting microscope, single mammary transplants were inserted, using watchmakers' forceps, into the centre of each of the interscapular brown fat pads of the host mice. From a 4 month old donor *CBA* mouse, thirty-two segments of duct were transplanted into sixteen female *CBA* hosts 3 to 3.5 months old (Series I). The other thirty mammary segments obtained from a 3 month old female *CBA* mouse were transferred to fifteen female (female *CBA* × male *C57BL*) $F_1$  hybrid mice 1.5 months old (Series II). Immediately before mammary transplantation, the interscapular brown fat pads on one side of the host mice were peripherally denervated by a technique used by Sidman and Fawcett<sup>8</sup>; on the left side in Series I and on the right side in Series II. A sham operation was performed on the interscapular brown fat pads on the other side of the animals.

The host mice received injections of 1 mg of progesterone and one-third  $\mu$ g of oestradiol benzoate dissolved in 0.02 ml. of sesame oil daily for 3 weeks from the day after mammary transplantation. The site of injection was the dorsal subcutaneous area adjacent to the root of the tail to avoid local reaction to the transplantation sites caused by the injections. The host mice were killed on the day following the last injections. All interscapular brown fat pads were removed, fixed in 10 per cent buffered formol, sectioned serially after embedding in paraffin and stained with haematoxylin and eosin.

Mammary grafts survived transplantation equally well in both the denervated and sham-operated interscapular brown fat pads (Table 1). The denervated brown fat pads were larger and paler than the sham-operated pads on the opposite sides of the same host mice. The denervated brown fat was histologically different from the sham-operated fat. The fat droplets increased in size and decreased in number in the cytoplasm of denervated brown fat cells. Brown fat cells with multilocular features with the granular cytoplasm (Fig. 1) were often converted later to monolocular fat cells resembling in appearance those of white fat (Fig. 2). Sometimes the area adjacent to mammary grafts in sham-operated brown fat pads contained enlarged monolocular fat droplets (Fig. 3). It is not known whether or not these are surviving white fat tissues that had been transplanted together with segments of mammary duct. In either environment created by the different conditions of brown fat pads, transplanted mammary glands actively regenerated glandular structures, and responded to exogenous hormonal stimulation in a similar way (Figs. 1-3).

Table 1. RATES OF RECOVERY OF REGENERATING MAMMARY GRAFTS FROM EITHER SHAM-OPERATED OR PERIPHERALLY DENERVATED INTERSCAPULAR BROWN FAT PADS

Experimental group	Site of transplantation	
	Sham-operated brown fat pads	Denervated brown fat pads
Series I: female <i>CBA</i> donor to female host <i>CBA</i> mice	10/16* (62.5 per cent)	14/16 (87.5 per cent)
Series II: female <i>CBA</i> donor to female (female <i>CBA</i> × male <i>C57BL</i> ) $F_1$ hybrid host mice	13/15 (86.7 per cent)	12/15 (80.0 per cent)

\* Number of successful mammary grafts/number of mammary transplants. Differences in rates of recovery are not statistically significant.

Mammary glands of the axillary (second) or thoracic (third) pairs in mice usually do not grow into and occupy the whole bodies of brown fat pads that are apparently continuous with white fat surrounding those mammary glands. It has been reported that intervening structures, such as the fascial connective sheath between the fatty tissue and the mammary transplants, interfered with growth of transplanted mammary glands<sup>2,5</sup>. There seems to be, however, no fascial intervention between white fat of the second or third pairs of mammary glands and adjacent interscapular brown fat. It is, therefore, hard to explain this phenomenon which occurs in a physiological condition, because in both the previous<sup>6</sup> and the present experiments it was demonstrated that mammary gland tissues of the third pair and other pairs can survive on transplantation, regenerate and grow in brown fat tissues. Interrelationships between mammary growth and stromal conditions, particularly of adipose tissues, should be further explored.

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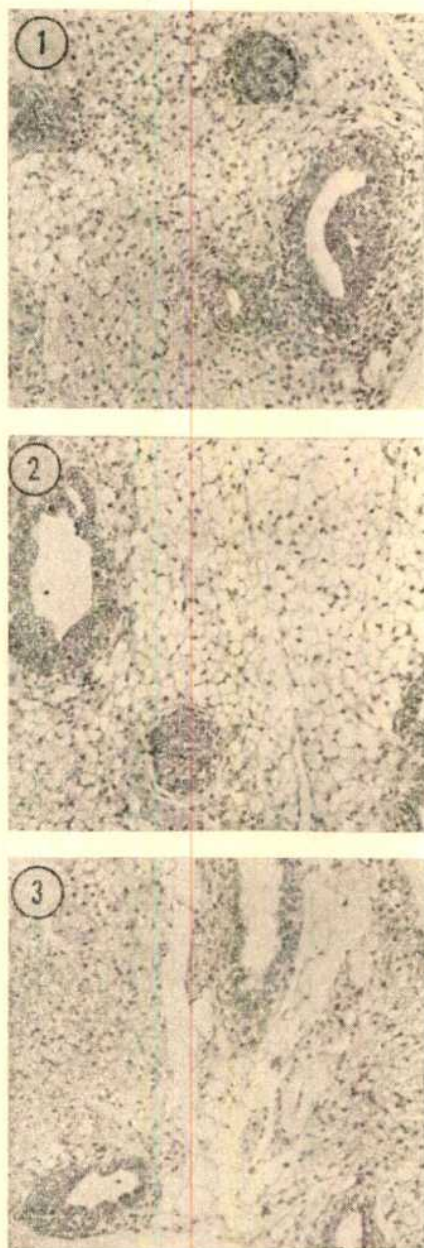
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### Influence of Multiple Anterior Pituitary Allografts on the Oestrous Cycle of the Syrian Hamster

TRANSPLANT studies of anterior pituitary tissue indicate that the hypothalamus exerts an inhibitory influence on the release of lactogenic hormone and that this hormone is luteotropic in the rat<sup>1</sup>. Comparable studies were undertaken as a preliminary investigation into the role of the pituitary in luteal maintenance in the hamster. Earlier studies indicate that autotransplants of anterior pituitary<sup>2,3</sup> (1-4 days *post coitum*) to the cheek pouch or the kidney capsule of the pregnant hamster do not supply enough lactogenic hormone to cause the corpora lutea to secrete progesterone in amounts sufficient for nidation. It was noted<sup>2</sup> that progesterone alone, in contrast with the rat<sup>4</sup> which requires oestrogen for nidation, can maintain blastocysts through nidation in the ovariectomized hamster. Similarly, our results indicate that more than one allograft of anterior pituitary to the cheek pouch is necessary to influence the oestrous cycle of the hamster.

The cheek pouch is used as the site of transplantation because of the simplicity of the technique and the peculiar properties of the pouch, which make it suitable<sup>5</sup>. Donors (adult male or female hamsters, *Mesocricetus auratus*) were decapitated under light ether anaesthesia. The anterior pituitary tissue was obtained by raising the cranium and removing the brain; the posterior pituitary was always discarded. Hosts were anaesthetized with sodium pentobarbital, a cheek pouch was everted and pinned on to a pine wood operating board and cleansed with an antiseptic solution. Both lobes of the anterior pituitary from a donor were inserted directly through a small incision in the cheek pouch epithelium, and the incision was closed with 5-0 catgut. The hosts received either male or female anterior pituitary tissue (one, four or six transplants/animal), and each transplant was inserted through a separate incision. Oestrous behaviour was checked daily with vasectomized or intact males from 3-5 h after the onset of darkness. Lighting was controlled to give a 12 h day. Both intact and hysterectomized females were used as hosts; sub-total hysterectomy (from the tubo-uterine junction to the cervico-vaginal junction) had been performed several weeks before transplantation.

Table 1 indicates the influence of a varying number of anterior pituitary allografts and the sex of the donors on the oestrous cycle of the hosts. The 4 day cyclic activity of hysterectomized hosts was completely unaffected by one anterior pituitary graft. Only one animal that received four or six grafts had normal cyclic activity; she was an intact host that had received six female grafts. Six allografts to the cheek pouch are probably necessary to influence the oestrous cycle of intact or hysterectom-



Figs. 1-3.



Table 1. INFLUENCE OF ANTERIOR PITUITARY ALLOGRAFTS ON THE OESTROUS CYCLE OF THE SYRIAN HAMSTER

Host	No. of hosts	No. of anterior pituitary and donor sex	Length of observation (days)	No. (%) incidence of cycles > 4 days	Dioestrous interval average (range) (days)
Intact	4	6 ♂	43-113	19 (50%)	8.7 (7-16)
"	3	6 ♀	43-114	8 (17%)	8.8 (7-9)
Hysterectomized	4	1 ♂	40-50	0 (0%)	0 (0)
"	4	4 ♂	43-45	5 (15%)	9.4 (8-13)
"	1	6 ♀	120	2 (8%)	11.0 (8, 14)
"	3	6 ♂	57-85	10 (56%)	17.7 (11-36)

ized females in a more or less consistent manner, that is, to prolong the dioestrous interval (8.7 and 17.7 days, respectively) to be comparable with that of pseudopregnancy in intact (10 days) or hysterectomized (18.5 days) females<sup>7</sup>. The onset of the first prolonged dioestrous interval occurred within 15 days (average) after transplantation. Although most of the donor groups were male, it is probable that allografts of male anterior pituitary are at least as effective as female anterior pituitary in causing prolonged dioestrous intervals.

We suggest that the occurrence of long cycles is due to the influence of lactogenic hormone from the transplants on the function of the corpora lutea and that lactogenic hormone is luteotropic in the hamster. Other work<sup>6</sup> suggested that exogenous prolactin (bovine) is also luteotropic in the hamster. The difference in the length of the long cycle between intact and hysterectomized females (with six grafts) suggests that the uterus has a controlling influence over the maintenance of the corpora lutea in the hamster. We have shown that uterine grafts may have a luteolytic activity since they tend to shorten the length of pseudopregnancy in the hysterectomized hamster<sup>7</sup>. Results from a recent investigation with hypophysectomized sows<sup>8</sup> suggest that the uterus has a direct luteolytic influence on the corpora lutea. The luteolytic influence of the uterus in the rat may, however, be mediated by the anterior pituitary, for autotransplants of this tissue can sustain luteal function for several months in the otherwise intact animal<sup>9</sup>.

When all transplants were removed from a host, oestrus occurred 1-3 days later; in each instance heat came sooner than would be expected if a single and brief release of lactogenic hormone in the intact animal could maintain corpora lutea of the hamster throughout a pseudopregnancy. Continued prolactin injection is necessary to maintain corpora lutea in the intact rat<sup>10</sup>. It is probable, therefore, that endogenous lactogenic hormone is released more than once or continuously to stimulate function of the corpora lutea during pseudopregnancy in the hamster.

Oestrus is recurrent in intact hosts, and so the luteotropic influence of the transplants is probably discontinuous. Similar observations have been made in rats<sup>1</sup> and mice<sup>11,12</sup>. It is possible that such grafts secrete lactogenic hormone discontinuously. This hypothesis is not attractive, for there is no known negative feedback mechanism that controls release of the hormone, and excess gonadotropins (human chorionic gonadotropin and pregnant mare serum)<sup>13</sup>, oestrogen and progesterone<sup>14</sup> all increase the content of lactogenic hormone in the serum of the rat over controls.

An alternative explanation is that corpora lutea become refractory to lactogenic hormone because of the luteolytic influence of the uterus. It is also possible that the uterus suppresses the release of lactogenic hormone from the intact pituitary and that there is a negative feedback relationship between the uterus and the hormone. Hysterectomy does not prolong luteal life indefinitely in the pseudopregnant hamster or the hamster and rat<sup>1</sup> with anterior pituitary transplants; there must also be a non-uterine source of luteolytic activity. Luteinizing hormone, for example, has been shown to have a luteolytic effect on "ageing" corpora lutea in the rat<sup>15</sup> and the rabbit<sup>16</sup>. We suggest that there may be a cyclic

activity of brain tissue (perhaps reticular formation)<sup>17</sup> which affects hypothalamic nuclei that produce factors which release follicle stimulating hormone and/or luteinizing hormone and the factor which inhibits lactogenic hormone. Cyclic changes have been shown in the endometrium and vagina of the hypophysectomized-ovariectomized-adrenalectomized rat<sup>18</sup>. There is no known explanation for these cyclic changes, but their existence allows for the possibility that brain tissue may have cyclic activity which affects phenomena of the oestrous cycle and that it can be exhibited in the absence of the pituitary, the ovaries, and the adrenal glands.

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### Phase Dependent Changes of Circadian Frequency after Light Steps

DIURNAL rhythm has been shown in a great variety of species to be self sustaining, that is, under constant conditions of light and temperature the periodicity continues. The period length of the free running rhythm ( $\tau$ ) generally deviates from the exact 24 h cycle of the solar day; it is then called a "circadian" rhythm. The "circadian rule"<sup>1</sup> states that the period length of circadian rhythms in constant light is a function of the light intensity. The effect of light on the free running rhythm has been regarded as a parametric or proportional influence, that is, a continuous action of light on the biological oscillation<sup>1,2</sup>. This interpretation implies that the following period would not be affected by the position of the cycle at which the light intensity is altered.

Preliminary experiments with the beetle *Tenebrio molitor* showed that the organism responds to shifts from lower to higher light intensities according to the circadian rule<sup>3</sup>. But it was unexpectedly found that the time of the shift (relative to the circadian rhythm of the animal) had a definite influence on the magnitude of the period change ( $\Delta\tau$ ). Increases in light intensity from 0.01 to 2 lux and from 2 to 100 lux were used. The data were pooled, because the results were similar in both groups of experiments. Because different lengths of period had to be dealt with, the phase position in the cycle of the light steps is given in degrees (0°, onset of activity; 360°, full period).

Fig. 1 shows a periodic change of  $\Delta\tau$  as a function of the time of the shift. The magnitude of  $\Delta\tau$  is maximal when the increase in light intensity occurs 2-3 h after the

onset of activity; it is minimal about 12 h later. It is not clear from these results whether the curve reaches the zero line, that is, whether there is any phase in the circadian period where increasing light intensity causes no change in the period length. Although at the lowest part of the curve (at about 220°) the data show a large variance, there is a statistically significant difference between the mean of the high data in the range 322°–360° and 0°–75° and the mean of the low data in the range 75°–322° ( $P < 0.001$ ).

These results were, however, obtained from frequency measurements over an average of only 7.7 periods. This makes it difficult to determine whether the measured values are steady state frequencies or transients. There are two points in favour of the former possibility: (1) no real transients have ever been found in *Tenebrio*, as can be deduced, for example, from Fig. 5 of ref. 3 (this was confirmed by Prof. Aschoff's further examination of the original data). (2) A "transient slope" response curve dependent on phase is unlikely, especially as there is no correlation between phase shifts and the phase position of the cue (which might cause a phase dependent transient slope under certain conditions).

Changes in period length generated in a similar way have been described only by Harker<sup>4</sup> and Pittendrigh<sup>5</sup>. Pittendrigh describes phenomena which he calls "after-effects" and distinguishes between four kinds of immediate pretreatment which result in period changes: (1) the frequency of preceding entrainment; (2) the preceding constant light conditions; (3) preceding transients caused by light pulses (of 12 h duration); (4) preceding transients caused by a short period of entrainment. We have not found any dependence of these after-effects on phase.

Harker<sup>4</sup> investigated the dependence on phase of after-effects caused by short periods of entrainment. Cockroaches (*Blaberus*) were kept in constant darkness for 15 days. They were then subjected to a light-dark cycle of 12:12 h for 5 days, and then returned to complete darkness. The curve (Fig. 12, ref. 4) has a maximum positive value of  $\Delta\tau$  when the first onset of light was applied 4–7 h before the onset of activity; its maximum of negative changes occurs 4 h after the onset of activity. The changes, however, are small and do not exceed 15 min. Nearly half the data in Harker's figure have been derived from Roberts's experiments with *Leucophaea*<sup>6</sup>, and Roberts himself hesitates to interpret his data as conforming to the results obtained with *Blaberus*.

Period-response curves, such as that published (but not interpreted) by Harker and the curve described here, suggest a very different effect of light on the circadian mechanism from the parametric influence mentioned in the introduction. The degree to which the system is changed depends on the phase position of the cue. This kind of influence on the oscillating system can be called

differential, in contrast with the parametric influence already mentioned. Harker's curve is, to my knowledge, the first example of a phase-dependent differential effect of environmental conditions on the free running period.

Phase-dependent shift responses ( $\Delta\phi$ ) have been interpreted as a mechanism of entrainment, but phase-dependent period responses ( $\Delta\tau$ ) cannot be so easily explained in terms of their biological utility. Harker's results do not show conclusively which part of the light cycle (for example, dusk or dawn or the middle of one semi-period) applied the effective stimulus in her experiments. Harker takes the onset of light as the effective stimulus, but it could just as well be the termination; in this case the curve would have to be shifted by 180°. The curve might also be produced by a combination of light-on and light-off stimuli. This would explain the weak effect on  $\Delta\tau$  compared with the step-up effects on *Tenebrio*. It would furthermore explain the most striking difference between the response curves: whereas the "two direction" stimulus of Harker's and Roberts's light cycle elicits a "two directional" response curve in the cockroach (that is, produces positive and negative  $\Delta\tau$  values), the "one direction" stimulus of a light step results in a "one directional"  $\Delta\tau$  response curve in *Tenebrio*.

Not only is there an apparent similarity between step-ups in light intensity and light-on stimuli of a light-dark (LD) Zeitgeber, but there is also evidence that the step-up may function as a light-on stimulus in the experiments with *Tenebrio*. The normal phase relation of the activity rhythm of *Tenebrio* to a 12:12 h light-dark cycle is characterized by the onset of activity about 2 h before darkness begins<sup>3</sup>. In other words, the light-on stimulus of the light cycle occurs 10 h before or 14 h after the onset of activity. Fig. 1 shows that at this time (about 220°) the response to a step-up stimulus is least. If the  $\Delta\tau$ -response curve of *Tenebrio* is similarly shaped in LD, the light-on stimulus would coincide with the part of the curve in which practically no period changes are generated. Such a phase relationship between Zeitgeber and response curve would be expected because it promises stability. If the hypothesis is correct, it would suggest that the differentially caused changes in period length are somehow involved in the procedure of entrainment.

In conclusion, I would like to propose the term " $\Delta\tau$ -response curve" or "period-response curve" for results such as those shown here. The usual response curves should then be called " $\Delta\tau$ -response curves" or "shift-response curves" in order better to distinguish these two types of "phase-response curves".

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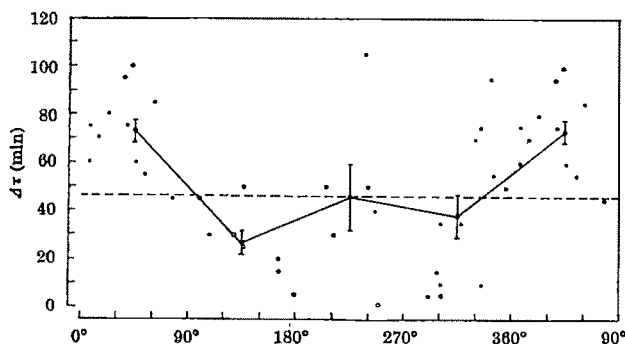


Fig. 1. Changes of circadian frequency ( $\Delta\tau$  in minutes of ordinate) plotted as a function of the phase position of light steps from a lower to a higher intensity ( $\phi$  in degrees after onset of activity; onset = 0°/360°). Black circles indicate light steps from 0.01 to 2 lux, open circles 2 to 100 lux. High means with standard deviation including data between 322° and 75°; low mean including data between 75° and 322°. Statistical significance between high and low means:  $P < 0.001$ .

### Aseptic Rearing of *Aedes aegypti* Linn.

INVESTIGATIONS involving the use of *Aedes aegypti* mosquitoes reared under aseptic conditions have been chiefly concerned with the nutrition of the larva. Whereas published techniques<sup>1-5</sup> were satisfactory for small numbers of insects, they were found unsuitable when large numbers of pupae were required for tissue culture purposes. The method for producing bacteriologically sterile pupae described here has been in use for 2 years, and has been found convenient and simple.

*Aedes aegypti* eggs laid on filter paper were obtained from a laboratory colony and stored over saturated potassium chloride at room temperature until required. Batches of eggs on small (1 × 1 cm) pieces of paper were sterilized in a 2-in. glass Petri dish by immersion in 5 ml. of 10 per cent benzalkonium chloride for 40 min, followed by 80 per cent ethanol for 15 min and two rinses in sterile distilled water. The eggs and paper were transferred with aseptic precautions to a 40 ml. tube with 25 ml. sterile water; a loose-fitting cap was fitted and the eggs induced to hatch by reducing the pressure in a vacuum desiccator. Eggs less than 1 month old tended to hatch during sterilization, and eggs 1–2 months old were usually used.

Eight ounce culture bottles were prepared by adding about 1 g white bread to 80 ml. tap water or distilled water; the bottles were closed with a screw cap, autoclaved for 10 min at 10 lb. pressure and stored at room temperature. Before use, 20 ml. of a 5 per cent solution of 'Marmite', which had been passed through a Seitz filter and stored in 20 ml. portions in universal containers at room temperature, were added with penicillin (final concentration 100 u/ml.) and streptomycin (final 100 µg/ml.). Between 400 and 500 freshly hatched larvae were transferred to a bottle, the screw cap was replaced with a loose fitting 'Oxoid' metal cap and the whole incubated at 28° C. Loose caps were used, because anoxia occurred if more than 50 third or fourth instar larvae were confined in a bottle with a screw cap. Batches of larvae were split into further bottles as they grew; where possible all handling was under a sterile hood. Culture of fragments of larvae or medium inoculated on to blood-agar or nutrient-agar plates incubated aerobically remained sterile; occasional mould contamination (easily detected in the early stages by the "lumpy" appearance of the bread) was encountered. The use of mycostatin was found to be unsatisfactory; 100 u/ml. suppressed mould growth but slowed down larval development considerably, and even at 25 u/ml. pupae were fewer and smaller than when mycostatin was omitted. In practice, it was found more convenient to discard the occasional contaminated bottle.

Under these conditions, pupae formed in 10–12 days were comparable in size with those from a normal laboratory colony. For use, they were removed from the culture bottles with a sterile pipette and washed twice in sterile water.

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### Synthesis of a New Amphidiploid Species of *Brassica*: *B. amarifolia* Narain and Prakash

THERE have been reports of the raising of artificial amphidiploid varieties of *Brassica juncea* Coss synthesized with members of different interfertile species of *Brassica* with twenty chromosomes as one of the parents and species of *B. nigra* Koch with sixteen chromosomes as the other parent<sup>1,2</sup>. Any attempt to cross *B. tournefortii* Gouan (also  $2n = 20$ ) with *B. nigra* has been unsuccessful<sup>3,4</sup>, and *B. tournefortii* does not give fertile hybrids with any of the *Brassica* species with twenty chromosomes<sup>4,5</sup>. Olsson has therefore assigned it a status as a separate species quite distinct from the other species of the genus with twenty chromosomes, which have been lumped under different subspecies of *B. campestris*<sup>5</sup>. We have also noted that there is very little homology between the A genomes

of *Brassica* and of *B. tournefortii* in their  $F_1$  hybrids, because twenty to sixteen univalents and nought to two bivalents were found in first metaphase plates and their genomes can be distinguished. An amphidiploid arising from the cross between *B. tournefortii* and *B. nigra* is therefore likely to be a new species combining good agronomic characteristics, dwarf size, profuse tillering habit, narrow flowering range and aphid resistance<sup>6</sup> of the wild donor parent, *B. tournefortii*.

With the foregoing objective in view, 4,000 flowers of *B. tournefortii* were crossed with *B. nigra* in 1962–63 and 1963–64. The seeds set fairly well and generally gave rise to matrophorphic plants. Only one seed was a genuine hybrid, of which the chromosomes doubled spontaneously early in its ontogeny. The new amphidiploid was fully fertile (86 per cent pod fertility), stable and true breeding for two generations. Meiosis was quite regular with eighteen bivalents in the first metaphase plate (Fig. 1). Its crossability with *B. juncea* was also very low; it gave no true hybrid with the latter. Details of the morphological characters of the amphidiploid are as follows.

The hybrid is a dwarf, erect (3–4 ft. high), branching annual, with a profuse tillering habit (Fig. 2). The stem is bloomed with a purplish tinge at the joints, and there

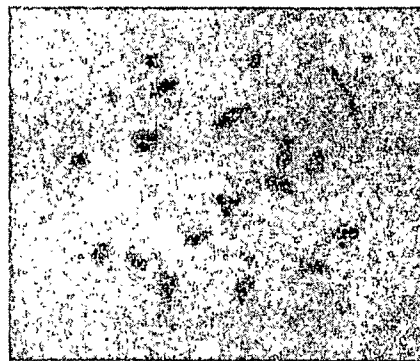


Fig. 1.

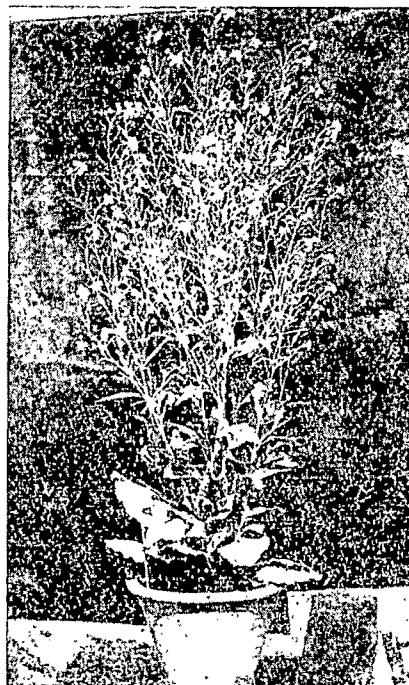


Fig. 2.



are many branches, which arise from the main stem at an angle of 50°. Leaves taste very bitter; the radical leaves form a rosette, and they are slightly petiolated, stiff and crisp, lyrate-pinnatisect, slightly lobed, with a serrate-dentate margin, sometimes sinuate, and hispid with white hairs. The lamina is uneven and dark green, and the terminal lobe is very large, forming about three quarters of the area of the leaf. The other lobes are smaller, and there are three or four of them. The main lobe is sparsely dotted with irregular protuberances on the upper surface. The cauline leaves are lyrate with a serrate-dentate margin, and are less hairy, while the upper leaves are lanceolate, entire and smooth. The inflorescence is a corymbose raceme. The flowers are bright yellow with petals separated by spaces. The anthers have introrse dehiscence. Pods are smooth and form an angle of 40°–50° with the main axis, are 5 cm long, with a beak of 7.5 mm, and are torulose. There are many seeds, seventeen to twenty-eight seeds/pod; they are brown with reticulation on the surface.

The amphidiploid looks suitable for direct cultivation. It has a narrow flowering range, gives a high yield, is resistant to aphids in field conditions and bears 30 per cent oil in the seed. It has been named *Brassica amari-folia* because the leaves taste very bitter.

We thank M. S. Swaminathan, head of the Division of Botany, Indian Agricultural Research Institute, New Delhi, for advice.

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## PATHOLOGY

### Atypical Reaction to Inhaled Silica

THE classical reaction of the lung to the deposition of air-borne silica is the formation of discrete nodules, which in man exhibit a characteristic disposition of dust and connective tissue fibres<sup>1,2</sup>. Although the experimental lesion does not reproduce all the human features<sup>3</sup>, I have confirmed that it remains focal and discrete<sup>4,5</sup>. In recent experiments on the disposal of inhaled particles within the lung parenchyma, specific pathogen-free (SPF) rats have been used in an attempt to eliminate the complication of pulmonary infection which not infrequently affects standard laboratory rats. The response of SPF rats differed greatly from that of the standard ones used in the earlier studies.

Some SPF rats inhaled Minusil quartz (particle size < 5 $\mu$ ) and others Dörentrup quartz (particle size < 3 $\mu$ ) for 600 and 1,200 h over periods of 6 and 12 weeks respectively. In the case of Minusil the mean atmospheric concentration of dust was approximately 40 mg/m<sup>3</sup> and with Dörentrup 30 mg/m<sup>3</sup>. Rats have so far survived for up to a year after exposure. Each pair of lungs contained a mean of 20 mg Minusil or 11 mg Dörentrup quartz (courtesy of Dr. A. Critchlow).

Grossly the lungs in both groups were large and collapsed only partially on removal. They showed extensive and irregular yellowish-white consolidation but no typical silicotic nodules. Histologically, fibrosis was limited to a few small areas of irregular outline and tended to be intra-alveolar; it was usually associated with a peripheral accumulation of foamy histiocytes, which often contained periodic acid-Schiff positive (diastase resistant)

granules and sometimes sudanophil fat. Fibrosis apparently started as an outgrowth from the walls of alveoli in which foamy macrophages had collected. Most of the remaining alveolar spaces were filled with eosinophilic granular material that also gave a positive periodic acid-Schiff reaction (diastase resistant). In this granular exudate birefringent particles of silica were identified in incinerated preparations. Rat lung thus consolidated bore a distinct resemblance to pulmonary alveolar proteinosis in man<sup>6</sup>, a similarity which was increased by the presence in the rat exudate of acicular birefringent crystals, occurring singly or in bundles. As survival increased after dust exposure, the granular material tended to diminish in amount, scattered macrophages and polymorphs appeared, and it may have been removed via the bronchial tree. The alveolar walls for the most part persisted apparently unchanged, but occasionally a further unusual feature was seen in the form of localized areas of epithelialization of alveolar walls resembling adenomatosis, the lining cells being cuboidal, columnar or ciliated. There was no evidence of pulmonary infection.

The nature of this exceptional response to quartz has yet to be elucidated, but the alveolar exudate seems to be derived from disintegrated cells, possibly macrophages, as in alveolar proteinosis<sup>6</sup>. The failure to develop typical fibrotic nodules in the presence of silica is striking, as is the feeble aggregation of the dust into foci and its prolonged retention in large areas of the parenchyma. The fibrogenicity of the two samples of quartz was established by intraperitoneal injection into standard and SPF rats. The widespread deposition and retention of a relatively large amount of quartz in a period of 6 to 12 weeks might lead to necrosis of macrophages as quickly as they are produced and in some way interfere with fibrogenesis. It is not yet possible to say whether the atypical reaction to quartz is related to the use of SPF rats.

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### Effect of Chlordiazepoxide on Eosinopenia of Stress in Rabbits

CHLORDIAZEPOXIDE, a new psychotherapeutic drug, chemically unrelated to either phenothiazine derivatives or indole-alkaloid derivatives, has been reported to show a number of pharmacological activities<sup>1</sup>. A possible hypothalamic activity of chlordiazepoxide has been reported<sup>2</sup>. We have investigated the effect of this drug on a physiological activity controlled by the hypothalamus, that is, eosinopenia after emotional stress. The emotional stress was applied to rabbits weighing 1.5–2 kg by the method of O'Connor and Verney as adopted by Colfer *et al.*<sup>3</sup>. Blood samples were taken from the ear veins of rabbits once before and again 3.5 h after the emotional stress (electric shock) was administered. The strength of the stimulus given by an electronic stimulator was such that it produced signs of fright in the animal. A freshly prepared solution of chlordiazepoxide (50 mg/kg) was given intraperitoneally 0.5 h before the electric shock and by this time the animal was showing the tranquillizing effect of the drug. Electrical stimulus was not repeated for 2 days. After pretreatment with chlordiazepoxide the rabbits still showed signs of fright after a similar electric shock. The experiments were carried out on three rabbits, each animal acting as its own control. Average means of blood counts are as follows. Before administration of

drug and stimulus, total white blood cell count was 4,860/cm, and the total number of eosinophils was 486 (10 per cent)/cm. Total white blood cell count 3.5 h after electric shock was 6,187/cm (a rise of 28.5 per cent), and the total number of eosinophils was 309.35 (5 per cent)/cm (a significant fall). Total white blood cell count 3.5 h after administration of chlordiazepoxide and electric shock was 5,175/cm (a rise of 6.4 per cent), and the total number of eosinophils was 466/cm (8.25 per cent, no significant change).

When the experiments were repeated after 2 days, the blood counts before and after electric shock were found to be similar to those obtained in the control experiments. The eosinophils again registered a marked and significant fall; the eosinophil count before stimulation was 603 (9 per cent), whereas it was 268 (4 per cent) only 3.5 h after stimulation. The experiments were repeated three times on each rabbit.

The foregoing results show quite clearly that administration of chlordiazepoxide (50 mg/kg) given intraperitoneally 0.5 h before the electric shock effectively prevents the usual fall in eosinophil count that follows emotional stress in rabbits. In animals under stress with intact pituitary-adrenal mechanism adrenocorticotrophin (ACTH) is responsible for the production of eosinopenia by causing a liberation of 11-corticosteroids. The release of ACTH in such conditions of stress is again in the control of the hypothalamus. These experimental results, therefore, point towards a possibility of chlordiazepoxide acting on the hypothalamus and thereby preventing the liberation of ACTH induced by acute stress. There is, however, the possibility of the drug acting peripherally as well. The effects of chlordiazepoxide on eosinopenia after the administration of adrenocorticotrophin and cortisone in the same rabbits were studied. ACTH (2  $\mu$ ) was given intraperitoneally and cortisone (2 mg/kg) intramuscularly instead of electric shock as in the previous experiments. Chlordiazepoxide (50 mg/kg) was given intraperitoneally 0.5 h before either of the foregoing drugs. Results with ACTH were as follows. Before administration of ACTH and/or chlordiazepoxide the total white blood cell count was 4,937/cm, and the total number of eosinophils was 543 (11 per cent)/cm. Total white blood cell count 3.5 h after administration of 2  $\mu$  of ACTH was 8,000/cm, and the total number of eosinophils was 320 (4 per cent)/cm. Total white blood cell count 3.5 h after chlordiazepoxide (50 mg/kg given intraperitoneally) and 2  $\mu$  of ACTH given intramuscularly was 4,600/cm, while the total number of eosinophils was 506 (11 per cent)/cm. These results show that while ACTH itself caused a marked eosinopenia, ACTH given after premedication with chlordiazepoxide failed totally to produce eosinopenia.

With cortisone, results were as follows. The total white blood cell count before cortisone and/or chlordiazepoxide was 4,950/cm and the total number of eosinophils was 445 (9 per cent)/cm. The total white blood cell count 3.5 h after cortisone (2 mg/kg given intramuscularly) was 7,750/cm, and the total number of eosinophils was 155 (2 per cent)/cm. The total white blood cell count 3.5 h after chlordiazepoxide and cortisone was 7,100/cm, and there were no eosinophils. Cortisone (2 mg/kg) caused a marked eosinopenia in the rabbit. Whereas in the control count eosinophils formed 9 per cent of the total white blood cell count 3.5 h after administration of cortisone, eosinophils accounted for 2 per cent only. The most interesting finding is that chlordiazepoxide could not prevent the eosinopenia caused by cortisone. Moreover, while ACTH alone caused a large rise in the total white blood cell count (8,000 as against 4,937 in controls), the total white blood cell count was only 4,600 when ACTH was administered 0.5 h after chlordiazepoxide.

Premedication with chlordiazepoxide prevented eosinopenia of emotional stress (fright caused by electric shock); chlordiazepoxide similarly prevented eosinopenia after

the administration of ACTH itself, and chlordiazepoxide completely failed to prevent eosinopenia following the administration of cortisone. The foregoing results, therefore, suggest that in preventing eosinopenia of emotional stress in rabbits, chlordiazepoxide acted in some way against ACTH itself, but the mechanisms are not clear from the present experiments.

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### Induction of Dental Caries in Gnotobiotic Rats by Streptococci of Human Origin

ORLAND *et al.*<sup>1</sup> have shown that an enterococcus isolated from conventional rats with dental caries could induce caries when it was used to infect germfree rats receiving a diet rich in sugar. Fitzgerald *et al.*<sup>2</sup> later obtained similar results with a previously undescribed anaerobic streptococcus (Strain FA-1), and Fitzgerald and Keyes<sup>3</sup> described the induction of caries in a conventional strain of albino hamsters with still another strain of anaerobic streptococcus (Strain HS-1). Zinner *et al.*<sup>4</sup> isolated streptococci, designated AHT, from human caries. These streptococci could induce caries in hamsters. These human streptococcal strains were identical morphologically, antigenically and in biochemical fermentation patterns to the hamster (HS-1) types. The present communication reports the isolation from human caries lesions of streptococci which cause caries in gnotobiotic rats.

The streptococcus was isolated from human carious debris by the fluorescent antibody method<sup>5</sup> using rabbit antisera prepared against the rat cariogenic streptococcal strain FA-1 (ref. 2). This human streptococcus (Strain BHT) was identical to the rat strain morphologically, antigenically and in its response to the various biochemical tests used for its classification. Like the rat strain, it conforms to none of the currently named species of streptococci.

The results reported are based on two separate experiments using young germfree rats of the National Institutes of Health Sprague-Dawley line. In the first experiment thirteen animals 38 days old were infected orally with cotton swabs. The swabs were soaked in a culture of strain BHT. They were maintained in sterile isolators for 90 days with coarse particle cariogenic diet 585-V (ref. 6), which contains 25 per cent sucrose, available *ad libitum*. The diet was sterilized by 4 Mrads radiation in a Van der Graff accelerator.

In the second experiment nine germfree rats were similarly infected with a substrain of BHT isolated from the animals of the first experiment. They were 35 days old at the time of infection and were maintained on a fine particle cariogenic diet 2000 V (ref. 7) which contains 56 per cent sucrose. This diet was available *ad libitum* and was also sterilized by irradiation.

Ten of the thirteen animals in the first experiment showed decay of one or more molar teeth. Twelve teeth were carious, eight lesions involved the enamel only, while four had penetrated into the underlying dentine.

In the second experiment eight of the nine animals were affected. Caries was found in twenty-four molars with twenty-one lesions confined to the enamel and three involving the dentine. Thus, by the application of the fluorescent antibody technique it has been possible to

identify and isolate certain streptococci from human caries which are identical morphologically, antigenically and in their biochemical characteristics with streptococcal strains known to be pathodontic for hamsters or rats. These streptococcal strains of human origin have been found to induce caries in hamsters or germfree rats, and also showed an immunological relationship with the organisms originally isolated from these animals.

The results indicate that some strains of streptococci may be found among the micro-organisms present in human dental caries which can induce caries in experimental animals. The possible significance of such organisms in the aetiology of human dental caries is now under investigation.

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## IMMUNOLOGY

### Protective Action of Oestrogen against the Lethal Effect of Endotoxin in the Rat

DURING investigations on the vascular effects of endotoxin in the isolated rat liver<sup>1</sup>, it was noted that the vasoconstrictive response to the administration of bacterial lipopolysaccharide failed to occur if pooled female rather than male rat blood was used as the perfusate, and this prompted an investigation of the value of an oestrogenic compound in prolonging the survival time of rats after the administration of endotoxin. Female albino rats weighing 200–250 g, and male Sprague-Dawley rats weighing 350–400 g, were used. The animals were paired by sex and weight, and each pair was allotted to the treated or control group. Food and water were given freely until the start of the experiment, when they were removed. Conjugated equine oestrogens, expressed as sodium oestrone sulphate, were given intraperitoneally and each rat received 4 mg dissolved in 1 ml. of diluent at various intervals before and after the intraperitoneal administration of purified lipopolysaccharide (*E. coli* 0127:B8, Difco Laboratories, Detroit). The diluent was given at the same intervals to the control animals. The numbers of survivors in the treated and untreated groups were recorded at 18 and 24 h. The few deaths occurring after 24 h were not included in the results, and statistical significance was determined by the method of  $\chi^2$  using Yates correction.

A marked and significant protection of the animals was observed when the oestrogen was given 30 min and 1 h before the endotoxin (Table 1). In addition, the protection was almost statistically significant when given 15 min before the lipopolysaccharide. This protection applied to both male and female rats, but if the oestrogen and endotoxin were administered in the same syringe or the oestrogen was administered after the endotoxin, the animals were not protected. The action of these conjugated oestrogens appears to be short-lived, since no effect was observed if the pre-treatment was given at 2, 4 or 8 h. While the effect of adrenal cortical steroids in protecting against endotoxin is well known, no such action has been previously attributed to oestrogen. The intraperitoneal administration of 10 mg of cortisol 0.5 h before administration of endotoxin in rats has produced a significant increase in survival rate while the same steroid dose given 8 h before or 1 h after the lipopolysaccharide gave no protection<sup>2</sup>.

Table 1. THE RELATIONSHIP OF SURVIVAL TO THE INTERVAL BETWEEN ADMINISTRATION OF OESTROGEN AND ENDOTOXIN

Initial injection	Interval (h)	Endotoxin dose (mg)	Sex	No. of rats	Survivors at 18 h	Survivors at 24 h
Oestrogen	8	2	♀	10	4	2
Control (diluent)	8	2	♀	10	6	3
Oestrogen	4	3	♀	10	3	3
Control (diluent)	4	3	♀	10	3	3
Oestrogen	2	3	♀	10	3	1
Control (diluent)	2	3	♀	10	6	2
Oestrogen	1	3	♀	10	10	10
Control (diluent)	1	3	♀	10	4	4
0.02 > P > 0.01 0.02 > P > 0.01						
Oestrogen	1	3	♂	10	8	6
Control (diluent)	1	3	♂	10	3	2
Oestrogen	0.5	3	♂	10	9	9
Control (diluent)	0.5	3	♂	10	2	2
0.01 > P 0.01 > P						
Oestrogen	0.25	2	♀	10	10	9
Control (diluent)	0.25	2	♀	10	7	5
Oestrogen	0	3	♀	10	1	1
Control (diluent)	0	3	♀	10	1	1
Oestrogen	-1	3	♀	20	8	6
Control (diluent)	-1	3	♀	20	7	7

The mechanism by which these water soluble oestrogens provide protection is not clear. The similarity between their action and that of cortisol raises the possibility of mediation through the adrenal cortex. In man, ethinyl-oestradiol produces a prompt increase in free plasma hydrocortisone<sup>3</sup>, but these increased levels are in a physiological range, and not at the pharmacological levels necessary for such endotoxin protection. Oestrogens have been shown to be reticuloendothelial stimulants of a high order<sup>4</sup>, and endotoxin is inactivated by the reticuloendothelial system. The stimulatory effect of these hormones persists, however, for a prolonged period<sup>5</sup>, and since no protection against endotoxin toxicity could be demonstrated at 2 h or more, stimulation of the reticuloendothelial system would seem an unlikely mechanism. The effect of oestrogen appears to be a direct and immediate one, similar to the action of corticosteroids.

If oestrogens indeed offer the same protection as corticosteroids against endotoxaemia, their use might have a theoretical advantage over the adrenal hormones. There is good evidence that cortisone depresses reticuloendothelial function and lowers resistance to infection<sup>6</sup>, despite its protective effect against the lipopolysaccharides of Gram negative bacteria. Nicol and his colleagues, however, have demonstrated to the contrary that the oestrogen stimulates the body defences against a wide spectrum of infectious organisms<sup>7</sup>. Further work is in progress to investigate the protective effect of female

hormones against endotoxin in other species, and in various states possibly related to endotoxaemia.

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## BIOCHEMISTRY

### Inhibition of Rat Liver Esterases by Organophosphorus Compounds

THE complex family of non-specific carboxylic esterases has been subdivided into different groups, principally on the basis of the results of inhibitor and activator studies<sup>1</sup>. One of the most important substances for this purpose is *E*600 (diethyl-*p*-nitrophenyl phosphate), which has been used extensively—both biochemically and histochemically—to distinguish between the *A* and *C* esterases, which are resistant to organophosphate and unaffected by concentrations of less than  $10^{-3}$  molar *E*600, and the *B* esterases, which are sensitive to organophosphate and are completely inhibited by  $10^{-5}$  molar *E*600 (ref. 2). In a histochemical study of indoxylesterases in rat kidney and other tissues, Hess and Pearce<sup>3</sup> found that 'Mipafox' (*N,N'*-di-isopropyl phosphorodiamidic fluoride) at a concentration of  $10^{-3}$  moles/l. gave a complete inhibition of *B* esterases similar to that obtained with  $10^{-5}$  molar *E*600, allowing the subsequent demonstration of *A* and *C* esterases. In other tissues<sup>4</sup>, however, it has been found that under histochemical conditions a much higher concentration of *E*600 than  $10^{-5}$  moles/l. is required to produce an inhibition comparable with that of  $10^{-3}$  molar 'Mipafox'. Increasing concentrations of organophosphate have a progressive inhibiting effect histochemically, which makes it difficult to distinguish clearly between organophosphate-sensitive and organophosphate-resistant enzyme species in terms corresponding to those of biochemical studies.

Some insight into the relative effects of the two organophosphate inhibitors has been gained from electrophoretic studies of liver homogenates using the technique of Smithies<sup>5</sup>. The starch slabs were submitted to a naphthol *AS* technique for the demonstration of esterase activity, using naphthol *AS* acetate as substrate (0.25 mg/ml. in phosphate buffer pH 7.3 incorporating 3 per cent propylene glycol) and fast blue *BB* (1 mg/ml.) as diazonium salt; incubation was carried out for 1 h at 37° C. Before exposure to the substrate mixture some slabs were treated with varying concentrations of either *E*600 or 'Mipafox' for 30 min at 37° C.

Two regions of the zymograms (labelled 1 and 2 in Fig. 1) are resistant to treatment with  $10^{-5}$  molar *E*600 (compare with the results of Eränkő *et al.*<sup>6</sup> and Niemi *et al.*<sup>7</sup>). Increasing the concentration of *E*600 produces a progressive inhibition within regions 1 and 2, and only the bands in region 1 survive treatment with  $10^{-4}$  molar *E*600. After exposure to  $2 \times 10^{-4}$  molar 'Mipafox' there are again the two regions of persistent activity, with region 1 much more markedly inhibited than region 2. Increasing the concentration of 'Mipafox' to  $10^{-3}$  molar completely abolishes the activity in regions 1 and 2, except for one strongly persistent band in region 2.

It appears, therefore, that there are two regions of organophosphate-resistant naphthol *AS* esterase in zymo-

grams of liver homogenates; one of these is particularly resistant to 'Mipafox' and is more readily inhibited by *E*600, while the other is resistant to *E*600 and more readily inhibited by 'Mipafox'. It might be expected, therefore, that the relative effects of the two inhibitors when used histochemically will vary from one tissue to another, depending on the local proportions of different enzyme species. If enzymes of similar character to those represented by region 1 predominate, 'Mipafox' would be expected to have a much stronger inhibitory effect on the total histochemical reaction than *E*600. Conversely, if enzymes of similar character to those represented by region 2 predominate, *E*600 would be expected to have a much more marked effect. It is possible that the histochemical use of both these inhibitors may permit a more precise identification of individual esterase species than can be attained by using one of them alone.

It is necessary, however, to make some reservations. In the first place, it is likely that the esterases which can be demonstrated histochemically are not identical with those shown in electrophoretograms<sup>6</sup>; the effects of organophosphate inhibitors may therefore be different in the two situations. Second, a paradoxical finding must be recorded, in that the difference between the effects of 'Mipafox' and *E*600 which has been described above for the naphthol *AS* technique, and which was repeated on several occasions with several animals, does not occur when naphthyl acetate is employed as substrate. Here (Fig. 1) *E*600 produces much less effect in region 2 and much more effect in region 1 than occurs with the naphthol *AS* substrate. The most strongly persistent band of



Fig. 1. The zymograms on the left are naphthol *AS* preparations, and show the effects of varying concentrations of 'Mipafox' (*M*) and *E*600 (*E*) compared with the uninhibited control (*C*). Organophosphate-resistant enzymes occur in regions 1 and 2. The two zymograms on the right, showing naphthyl acetate-esterase activity, illustrate the effect of  $10^{-4}$  molar *E*600. (The naphthol *AS* and naphthyl acetate preparations are from different animals; in each case the slots were overfilled (0.05 ml.) so as to provide a stronger residual pattern after inhibition.)

activity appears to correspond with the 'Mipafox' resistant band of the naphthol AS zymograms.

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### Specific Inhibition of Gluconeogenesis by Biguanides

POSSIBLE schemes for the mechanism of the hypoglycaemic action of biguanides have raised considerable controversy in recent years. The possibility that these drugs may act through inhibition of oxidative phosphorylation, which may increase the glucose uptake of peripheral tissues<sup>1</sup>, has been frequently emphasized<sup>2-5</sup>, but experiments on the kinetics of glucose loads<sup>6</sup> as well as the demonstration that no correlation exists between the hypoglycaemic effect of various biguanides and their inhibitory action on oxidative phosphorylations<sup>7,8</sup> did not support this hypothesis. Moreover, it is now well established that the biguanide drugs have no effect on the glycaemia of normal animals and men, and that their action is restricted to the diabetic and fasting animal. Thus it appeared reasonable to assume that they may interfere with one or more metabolic pathways which are widely different in the normal and diabetic organism. One of these is the gluconeogenic pathway, resulting in the synthesis of glucose from metabolites containing three or four carbon atoms, including amino-acids. The possibility that such a mechanism may explain the anti-diabetic action of at least two biguanides (phenylethylbiguanide (PEBG) and dimethylbiguanide (DMBG)) has been investigated.

In the first series of experiments gluconeogenesis was induced in normal rats (200 g) and guinea-pigs (300 g), after a fasting period of 24 h by injection of hydrocortisone hemisuccinate (2 mg/100 g) and estimated 3 h after the injection by measuring the glycogen content of the liver<sup>9</sup>, the blood glucose level<sup>10</sup> and the incorporation of carbon-14-pyruvate into blood glucose. The latter compound was isolated by paper chromatography. Table 1 shows the results of these experiments, either on animals treated with hydrocortisone only, or on animals pre-treated with a biguanide compound 1 h before the hydrocortisone injection. It may be seen that the biguanides strongly inhibit the effects induced by cortisone on the metabolism parameters already mentioned.

In a second series of experiments (Table 2) the specific inhibition by biguanides of gluconeogenesis from various substrates has been determined by the same methods; an additional assay of the level of amino-acids in blood plasma was performed using the ninhydrin method<sup>11</sup>.

Table 1. INFLUENCE OF BIGUANIDES ON THE CORTISONE INDUCED GLUCONEOGENESIS IN THE STARVED RAT AND GUINEA-PIG

Treatment	Glycogen (mg/g)	Blood (g/l.)	Glucose from <sup>14</sup> C pyruvate specific activity c.p.m./mg glucose
(1) Rat			
0	0.075 ± 0.012	0.62 ± 0.08	1,070 ± 102
Cortisone (3 mg)	7.250 ± 0.12	1.19 ± 0.15	2,860 ± 107
Cortisone (3 mg) + DMBG (20 mg)	2.860 ± 0.28	0.72 ± 0.11	1,650 ± 166
(2) Guinea-pig			
0	0.150 ± 0.100	0.95 ± 0.08	3,410 ± 302
Cortisone (5 mg)	10.280 ± 1.200	1.25 ± 0.10	4,990 ± 263
Cortisone (5 mg) + PEBG (10 mg)	1.710 ± 0.210	0.45 ± 0.10	3,870 ± 280

Table 2. INFLUENCE OF BIGUANIDES ON THE GLUCONEOGENESIS INDUCED IN INTACT, STARVED GUINEA-PIGS BY VARIOUS SUBSTRATES

Substrates injected (100 mg)	Biguanide injected	Glycogen (mg/g liver)	Glucose (mg/ml. plasma)	Amino-acids* (mg/ml. plasma)	Glucose specific radio-activity (c.p.m./mg glucose)
(1) 0		0.17	0.77	6.5	
(2) Sodium pyruvate (+ 10 µc. <sup>14</sup> C-pyruvate)	0	4.80	0.61	6.7	2,148
" " " " " " " "	DMBG (80 mg)	2.40	0.54	7.1	1,680
" " " " " " " "	PEBG (10 mg)	1.08	0.13	20.8	1,460
(3) Alanine (+ 10 µc. <sup>14</sup> C-alanine)	0	10.26	0.69	8.5	2,940
" " " " " " " "	DMBG (80 mg)	0.54	0.29	11.2	1,264
" " " " " " " "	PEBG (10 mg)	0.58	0.12	15.3	1,443
(4) Sodium aspartate	0	7.05	0.70	7.2	
" " " " " " " "	DMBG (80 mg)	5.90	0.75	7.3	
" " " " " " " "	PEBG (10 mg)	6.40	0.20	10.2	

\* Estimated against a standard of pure L-alanine.

Control determinations by paper chromatography showed that an increase in ninhydrin positive material reflected the actual amino-acid content of the plasma and not an increase in urea. Both biguanides strongly inhibit gluconeogenesis from pyruvate and alanine whereas only limited effects are obtained when the substrate is aspartic acid. In addition PEBG strongly increases the level of amino-acids in the blood, a result which suggests that its mechanism of action may not be completely identical with the mechanism of action of DMBG.

In an attempt to confirm these results on the gluconeogenesis of an isolated tissue, a third series of experiments has been performed with incubated rat kidney slices by measuring the neoformation of glucose, according to the method developed by Krebs *et al.*<sup>12</sup>. Table 3 shows the results of these experiments in the presence of various substrates: it appears that DMBG preferentially inhibits the conversion of pyruvate and lactate to glucose, with a somewhat less marked effect on alanine and almost no effect on glutamate and oxaloacetate; conversely, PEBG seems to inhibit specifically gluconeogenesis from amino-acids like alanine and glutamic acid and from oxaloacetate. Hence it appears that the inhibitory action of biguanides on gluconeogenesis is perfectly reproducible on an isolated tissue and seems to exhibit a pronounced substrate specificity, according to the inhibitor used.

Two conclusions may be drawn from these experiments. First, it appears that the marked inhibition of gluconeogenesis by all the substrates used, except aspartate, under the influence of biguanides offers a reasonable explanation of the hypoglycaemic action of these drugs, especially since their concentrations throughout this work are near the concentrations which have been used in the diabetic organism<sup>13</sup>. Thus it may be assumed that the inhibition of cortisone-induced gluconeogenesis which has been observed reflects an interference of the drugs with the conversion of endogenous substrates to

Table 3. INFLUENCE OF PHENYLETHYLBIGUANIDE (PEBG) AND DIMETHYLBIGUANIDE (DMBG) ON THE GLUCONEOGENESIS OF ISOLATED RAT KIDNEY SLICES

Substrates (molar conc.)	Biguanide (molar conc.)	Glucose synthesized (mg/g slices fresh weight/30 min)	Inhibition (per cent)
(1) 0	0	0.25 ± 0.1	0
(2) Sodium pyruvate (10 <sup>-3</sup> M)	0	8.05 ± 1.08	0
" " " " " " " "	DMBG (10 <sup>-3</sup> M)	2.02 ± 0.49	80.5
" " " " " " " "	DMBG (5.10 <sup>-4</sup> M)	3.01 ± 0.28	62
" " " " " " " "	PEBG (10 <sup>-3</sup> M)	6.25 ± 1.10	22
" " " " " " " "	PEBG (10 <sup>-4</sup> M)	7.50 ± 0.90	6.9
(3) Alanine (10 <sup>-3</sup> M)	0	1.85 ± 0.20	0
" " " " " " " "	DMBG (2.10 <sup>-4</sup> M)	1.32 ± 0.15	34
" " " " " " " "	PEBG (2.10 <sup>-4</sup> M)	0.30 ± 0.20	84
(4) Sodium glutamate (10 <sup>-3</sup> M)	0	2.40 ± 0.15	0
" " " " " " " "	DMBG (10 <sup>-3</sup> M)	1.90 ± 0.10	20
" " " " " " " "	PEBG (2.10 <sup>-3</sup> M)	1.00 ± 0.26	53
(5) Sodium oxaloacetate (10 <sup>-3</sup> M)	0	3.10 ± 0.18	0
" " " " " " " "	DMBG (10 <sup>-4</sup> M)	3.05 ± 0.315	1.6
" " " " " " " "	PEBG (5.10 <sup>-5</sup> M)	1.56 ± 0.20	49.6



glucose and not an inhibition of the *de novo* synthesis of specific enzymes involved in the gluconeogenesis of liver and kidney<sup>14</sup>. It may be added that the possibility that PEBG interferes with gluconeogenesis had been mentioned by Williams *et al.*<sup>15</sup> after experiments performed with alanine on guinea-pigs.

Second, concerning the specific action of either biguanide on gluconeogenesis from different substrates, it may be premature to conclude from these experiments that these actions are due to the inhibition of specific enzymes, because gluconeogenesis involves a complex regulatory mechanism of carboxylation by pyruvate carboxylase<sup>16</sup> of transamination steps<sup>17</sup> and of an exchange of substrates between mitochondria and cytoplasm<sup>18</sup>. It seems, however, from these results that both biguanides used may provide useful laboratory tools for the study of gluconeogenic pathways at the cellular level.

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### $\beta$ -Xylosidase and $\beta$ -Galactosidase Activities of Mammalian Connective Tissues and Other Sources

It has been shown that the sequence: -glucuronate-galactose-galactose-xylose-serine- serves as a chemical "bridge" between the polysaccharide and polypeptide components in a number of sulphated mucosubstances including heparin and chondromucoprotein<sup>1-6</sup>. A chondropeptide fraction isolated from bovine tracheal cartilage<sup>6</sup> seems to be hydrolysed by a  $\beta$ -xylosidase present in almond emulsin at the point of the carbohydrate-peptide linkage<sup>7</sup>. This communication describes the  $\beta$ -galactosidase and  $\beta$ -xylosidase activities of cartilage, other mammalian tissues, and also in jack beans.

When slices of bovine nasal and rabbit ear cartilage are incubated at 37° C with *o*- or *p*-nitrophenyl- $\beta$ -D-xylopyranoside<sup>7,8</sup> or with *o*- or *p*-nitrophenyl- $\beta$ -D-galactopyranoside

in sodium citrate buffers (0.15 moles/l., pH 4.8) in the presence of 1 mmolar sodium azide (as a bacteriostat), the nitrophenols are slowly liberated. Both enzyme activities are greater in young cartilage (for example, from 6 week old rabbits or 1 year old bullocks) than in cartilage of fully developed and aged animals (2 year old rabbits, 4 year old cattle). These cartilage glycosidases are distinguishable from other endogenous chondrolytic enzyme(s), which behave as proteases and release chondropeptides into the incubation medium<sup>9,10</sup>. The properties of the glycosidases differ from those of the proteases in (i) the lesser enzyme activity at 55° C than at 37° C; (ii) the relative insensitivity to 20 mmolar chloroquine; (iii) the different sensitivity to mercurial inhibitors (see Table 1). Table 1 also shows that the xylosidase and galactosidase activities in a purified fraction<sup>7</sup> of almond emulsin can be distinguished from the corresponding animal enzymes by their relative insensitivity to mercuric ions. Thiol-neutralizing agents (*N*-ethylmaleimide and iodoacetate, 1 mmolar) did not inhibit the mercury-sensitive cartilage glycosidases.

The cartilage and emulsin xylosidases failed to hydrolyse either *o*-nitrophenyl-2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranoside or *p*-nitrophenyl- $\alpha$ -D-xylopyranoside (synthesized and kindly donated by Mr. M. Higham), which indicates specificity for unsubstituted  $\beta$ -xylosides.

Both glycosidases were extracted simultaneously by 0.15 molar sodium chloride from powdered frozen rabbit ear cartilage and remained in the particles and supernatant after centrifugation at 80,000 *g* for 30 min. 'Triton X-100', which at a concentration of 0.1 per cent v/v increases the activity of many lysosomal enzymes, did not affect the glycosidase activities of these uncentrifuged extracts.

The cartilage slices incubated at pH 4.8 at 37° C generally liberated two or three times the quantity of *o*- and *p*-nitrophenol from the galactoside substrates compared with the corresponding xyloside substrates.  $\beta$ -Galactosidase activity also occurred in other tissues where  $\beta$ -xylosidase was found, for example rabbit aorta, beef cornea (approximately equal enzyme activities in the epithelial and stromal fractions), bovine cuspidal and semilunar heart valves, tissue cultures of murine mast cells (kindly donated by Mr. J. F. Wheldrake), mouse skin and many rat tissues (lung, spleen, brain, kidney, heart and liver). Rat serum, on the other hand, contained no detectable  $\beta$ -xylosidase although  $\beta$ -galactosidase was present. Horse serum (used for culturing mast cells) contained neither enzyme.

$\beta$ -Xylosidase and  $\beta$ -galactosidase were also present in particulate fractions prepared by sedimenting (between 3,000 and 20,000 *g* for 20 min) homogenates of beef heart, rat brain, lung, kidney, liver and spleen. The last three tissues also exhibited  $\beta$ -glucosidase activity.

Aqueous extracts of jack bean meal contained  $\beta$ -xylosidase and  $\beta$ -glucosidase of similar activity and a  $\beta$ -galactosidase of greater activity. Other glycosidases have been recently reported to be present in jack bean meal<sup>11</sup>. The  $\beta$ -xylosidase from this source was readily distinguished from the accompanying  $\beta$ -galactosidase because it was not sensitive to mercuric ions at a concentration which abolished the  $\beta$ -galactosidase activity.

With all the animal glycosidases (regardless of tissue source), the galactosidase activity was inhibited by 25

Table 1. COMPARISON OF  $\beta$ -XYLOSIDASE (Xyl),  $\beta$ -GALACTOSIDASE (Gal) AND  $\beta$ -GLUCOSIDASE (Glu), AND CHONDROMUCOPROTEASE (F) ACTIVITIES

pH optimum	Rat liver			Rabbit ear cartilage			Bovine nasal cartilage			Almond emulsin			Jack bean meal		
	Xyl 5.0	Gal 3.4 and 3.8	Glu 5.2- 5.4	Xyl 4.8	Gal 4.8	P 4.8	Xyl 4.4- 4.6	Gal 3.6- 4.2	P 4.6- 5.0	Xyl 4.8	Gal 4.8	Glu 5.4	Xyl 4.0- 4.4	Gal 4.2- 4.6	Glu 5.6- 6.0
Ratio activities at 55°/37° C	—	—	—	<0.2	<0.2	>1.0	0.3	—	1.8	—	—	—	—	—	—
Percentage inhibitions by 20 mmolar chloroquine phosphate	—	—	—	18	10	48	24	18	60	—	—	—	—	—	—
0.5 mmolar mercuric chloride	100	100	100	100	100	10	100	100	0	0	0	0	0	100	0
25 mmolar D-galactono-1,4-lactone	0	100	—	0	85	0	—	—	—	70	80	80	0	100	0
25 mmolar D-glucono-1,4-lactone	100	0	—	90	0	15	—	—	—	100	100	100	20	0	85

Chondromucoprotease activity was measured turbidometrically with rivanol<sup>12,13</sup>. *o*-Nitrophenyl glycosides (2.5 mmoles/l.) were incubated in 0.15 molar citrate buffer (pH 4.8) at 37° C with cartilage slices or homogenates. *o*-Nitrophenyl glycosides (1.5 mmoles/l.) were used with purified almond emulsin extracts<sup>7</sup> at 30° C. The liberated nitrophenol was measured by its light absorption at 416 m $\mu$  after addition of sodium carbonate (0.25 moles/l.). The colour was extracted from turbid solutions into *n*-butanol.

mmolar D-galactono-1,4-lactone and not by 25 mmolar D-glucono-1,4-lactone. Conversely xylosidase activity was inhibited by the gluconolactone and not by the galactono-lactone<sup>12</sup>. The xylosidase and glucosidase activities of rat liver particles (lysosomes) and nasal and ear cartilage could be distinguished by their different pH optima.

The apparent ubiquity of these glycosidases in connective tissues may be of considerable relevance to the turnover of some polysaccharide-protein complexes.

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### **In vitro Anticholinesterase Activity of Certain N-Methylcarbamate Insecticides compared with their N-Acyl Derivatives**

THE insecticidal activity of certain N-acyl N-methylcarbamate insecticides has been reported recently by Fraser *et al.*<sup>1</sup>. Reay and Lewis<sup>2</sup> showed that the N-acetyl derivative of 1-naphthyl N-methylcarbamate (carbaryl) was nearly 100 times less inhibitory towards honeybee head cholinesterase (ChE) *in vitro* than the parent compound (see also Table 1). It is the purpose of this communication to report that the N-acylation of several other N-methylcarbamates leads to a marked reduction in *in vitro* anticholinesterase activity against honeybee head ChE.

The method used for the assay of ChE has already been described in full<sup>3</sup>. The insecticides used were of the general structure I or II, where R' is an aromatic nucleus and R'' is a simple alkyl group.



They were dissolved in 'AnalaR' acetone for addition to the assay mixture, and the activity of ChE in the presence of insecticide was compared with that in the presence of acetone only. The results are summarized in Table 1.

It is clear that N-acylation of all the N-methylcarbamates tested causes a marked drop in their activity against honeybee head ChE *in vitro*. The fall in activity is, in fact, less in the case of carbaryl, already cited<sup>2</sup>, than for any of the others; the factor involved is nearly a thousand in most instances. It is also apparent, however, that acylation with side chains of increasing length, up to C=6, increases the activity against ChE compared with that of the acetyl derivative, though the activity of the parent carbamate is on no occasion fully restored. N-acylation with a dodecanoyl group, however, gives decreased activity against ChE.

Because the lengthening of the nitrogen-attached side-chain from C=2 to C=6 leads to an increase in inhibition

Table 1. *In vitro* ANTICHOLINESTERASE ACTIVITY OF CARBAMATES

Insecticide	IC 50*
1-Naphthyl N-methylcarbamate (carbaryl)	4.75 × 10 <sup>-4</sup>
1-Naphthyl N-acetyl-N-methylcarbamate	4.0 × 10 <sup>-4</sup>
1-Naphthyl N-propionyl-N-methylcarbamate	8.5 × 10 <sup>-7</sup>
1-Naphthyl N-butyryl-N-methylcarbamate	6.0 × 10 <sup>-7</sup>
2-Isopropylphenyl N-methylcarbamate	4.25 × 10 <sup>-7</sup>
2-Isopropylphenyl N-acetyl-N-methylcarbamate	1.4 × 10 <sup>-4</sup>
2-Isopropylphenyl N-hexanoyl-N-methylcarbamate	8.0 × 10 <sup>-6</sup>
2-Isopropylphenyl N-dodecanoyl-N-methylcarbamate	2.2 × 10 <sup>-4</sup>
3-Isopropylphenyl N-methylcarbamate	2.2 × 10 <sup>-4</sup>
3-Isopropylphenyl N-acetyl-N-methylcarbamate	2.0 × 10 <sup>-4</sup>
3-Isopropylphenyl N-butyryl-N-methylcarbamate	8.4 × 10 <sup>-4</sup>
3-Isopropylphenyl N-hexanoyl-N-methylcarbamate	2.3 × 10 <sup>-4</sup>
2-Isopropoxyphenyl N-methylcarbamate	7.5 × 10 <sup>-7</sup>
2-Isopropoxyphenyl N-acetyl-N-methylcarbamate	6.5 × 10 <sup>-4</sup>
2-Isopropoxyphenyl N-butyryl-N-methylcarbamate	3.0 × 10 <sup>-4</sup>
4-Dimethylamino-3,5-xylyl N-methylcarbamate	1.25 × 10 <sup>-4</sup>
4-Dimethylamino-3,5-xylyl N-acetyl-N-methylcarbamate	4.0 × 10 <sup>-4</sup>

\* IC 50 = Concentration (moles/l.) for 50 per cent ChE inhibition.

of ChE, it is clearly not possible to postulate that it is steric hindrance which is responsible for the decrease in ChE inhibition on N-acetylation. Steric hindrance could, however, be an important reason for the loss of activity of the dodecanoyl derivative. It seems more likely that the decrease of activity on acetylation results from the change of electron distribution brought about by the introduction of the electron-withdrawing acetyl —C=O group. This hypothesis is analogous to those proposed by Kolbezen *et al.*<sup>3</sup> and Metcalf *et al.*<sup>4</sup> to explain the activities of certain other substituted carbamates. The withdrawal of electrons from the —O.CO.N— group of the carbamate will result in the formation of a weaker bond between the inhibitor and ChE, because it is this group that has been found to be the point of attachment to the enzyme<sup>5-7</sup>. The increased activity of the longer-chain N-acylated compounds, however, cannot be explained along these lines, because the alkyl group has little electron-shifting capacity and would, if anything, tend to increase the electron-withdrawing capacity of the acyl group (compare Kolbezen *et al.*<sup>3</sup>). This means that some factor other than electron density and steric hindrance must be important in determining the affinity of a carbamate inhibitor for ChE.

I thank Dr. J. Fraser for synthesis of the acyl carbamate insecticides, and many other colleagues for helpful discussion of the results.

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### **Activation of Honeybee Head Cholinesterase by Water-miscible Organic Solvents**

COLHOUN<sup>1</sup> showed that many water-miscible organic solvents increased the activity of cholinesterase (ChE) preparations from the cockroach *Periplaneta americana*. Shatoury<sup>2,3</sup> showed that a similar activation occurred in the case of the housefly *Musca domestica*, though here n-butanol only was used as "activator", and the effect was far less marked. It has now been found that an even larger activation than that recorded by Colhoun can be achieved by addition of certain water-miscible organic

solvents, notably *n*-butanol and *n*-pentanol, to ChE preparations from heads of the honeybee *Apis mellifera*.

The method of assay has been described fully elsewhere<sup>4</sup>. A number of organic solvents have been investigated and a range of solvent concentrations tested in the case of acetone, the solvent most frequently used for *in vitro* investigations of ChE inhibition by insecticides (see, for example, refs. 5 and 6). These results are summarized in Table 1 and Fig. 1.

Table 1. EFFECT OF ADDITION OF VARIOUS SOLVENTS\* TO HONEYBEE ChE ASSAYS

Organic solvent	ChE activity†	Activation (per cent control)
None (control)	30.1	—
Acetone	48.2	158
Methanol	36.0	120
Ethanol	44.2	147
<i>n</i> -Propanol	68.5	228
Isopropanol	63.5	211
<i>n</i> -Butanol	124.0	412
Isobutanol	61.0	202
<i>s</i> -Butanol	93.5	310
<i>t</i> -Butanol	57.2	190
<i>n</i> -Pentanol	136.8	454
<i>n</i> -Hexanol	36.8	122
2-Methoxy-ethanol	49.4	164

\* 0.3 ml. added to assay, 15 ml. total.

† ChE activity measured in  $\mu$ l. 0.01 normal sodium hydroxide/min. at pH 6.95 and 30° C (ref. 4).

It is clear that a number of organic solvents can "activate" honeybee-head ChE preparations, but that maximum activation occurs with *n*-butanol and *n*-pentanol. Branched chain alcohols are less effective and higher alcohols are immiscible with the assay mixture. The amount of acetone added markedly alters the degree of activation up to about 5 per cent of the total volume; thereafter, increasing the concentration has less effect (Fig. 1). The increase in enzyme activity has been shown to be virtually instantaneous (Fig. 2).

Increases in activity up to 120 per cent of the control have also been brought about by the addition of surface-active agents, for example, sodium deoxycholate (compare Shatoury<sup>3</sup>, Ord and Thompson<sup>7</sup>); the addition of organic solvents to an assay mixture treated with deoxycholate resulted in additional activation. Preparations of ChE which have been extracted with diethyl ether showed increased activity when compared with unextracted preparations; this activation, however, may result solely from ether in solution in the aqueous phase.

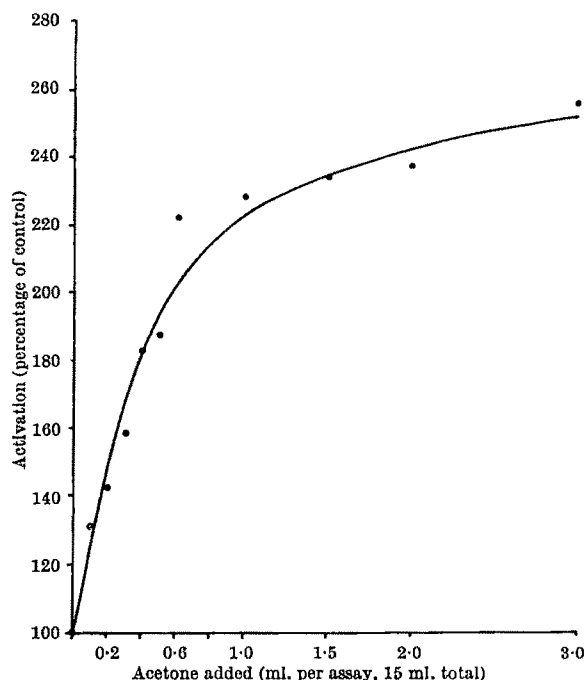


Fig. 1. Activity of bee-head ChE in the presence of increasing concentrations of acetone.

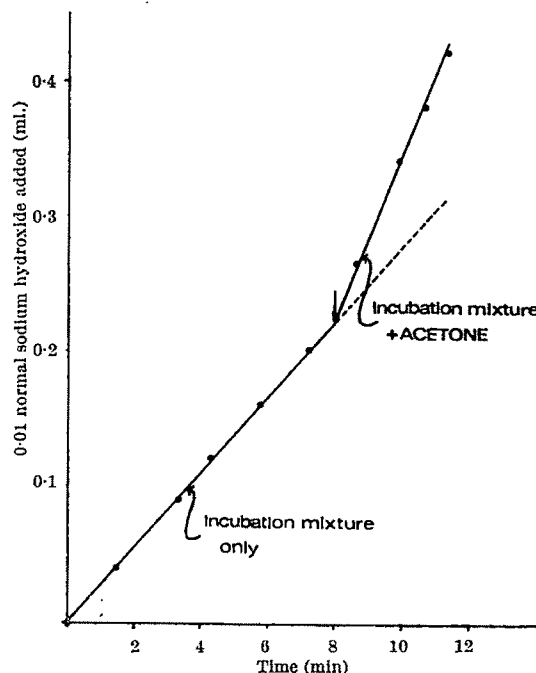


Fig. 2. Activity of a bee-head ChE preparation alone and after addition of acetone (shown thus ↓).

Various reasons for these increases in activity of ChE have been given by other workers<sup>1-3</sup>, but there seems to be no overwhelming evidence in favour of any one theory. One factor which might be important is the lipid-solubility of the "activator". It is possible that ChE exists *in vivo* in a lipoprotein complex, which is severely disrupted by the extraction process, and that this results in loss of activity due to blockage of the active sites of the enzyme with lipid material. The effect of organic solvents and surface-active agents might therefore be explained by solubilization of this interfering material. This theory is not inconsistent with the observation of Metcalf and March<sup>6</sup> that bee-head ChE increases in activity on storage.

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### Relationship between Parathyroid Function and Serum Aminopeptidase A Activity

AMINOPEPTIDASE A, a mammalian peptidase specific for the hydrolysis of N-terminal  $\alpha$ -L-glutamyl and aspartyl residues, has been found in many tissues such as the epithelium of guinea-pig pancreatic duct, islets of Langerhans and human and rat glomeruli<sup>1,2</sup>. It has been found in rat serum as well as in human serum<sup>3,4</sup>, and this serum enzyme activity is markedly activated specifically by calcium.

During investigations of the effects of the parathyroid function on some serum enzyme activities, the activities of aminopeptidase A and alkaline phosphatase in serum were found to have a significant positive correlation with

the parathyroid functional states. On the other hand, similar serum peptidase, leucine aminopeptidase, on which calcium has no effect, showed no correlation with the parathyroid functional states.

Various functional states of the parathyroid glands of rats can be observed histologically during pregnancy, lactation and ablation, and so rats in these states were used as experimental animals, and they were killed at different times. Rats of various ages from the fetus (19 days old) to adult (16 months old) were also used as experimental animals. Adult female or male rats were used as normal controls, and sex differences were not observed. Rats of the Wistar strain were used throughout the experiments. Blood samples were collected by means of heart puncture or decapitation, and serum calcium, inorganic phosphorus and alkaline phosphatase were measured as indicators of the functional state of the parathyroid glands. The functional states of the parathyroid glands were examined histologically, and the morphological changes were compared with calcium, inorganic phosphorus, and alkaline phosphatase activity in the serum. The alkaline phosphatase activity of serum in rats increased markedly during lactation, and this increase could be, in a part, independent of hyperfunction of the parathyroid glands. These values were therefore omitted, and will be reported elsewhere.

About eighty sera of rats with parathyroid glands in various functional states were tested to determine the concentrations of calcium and inorganic phosphorus, and the activity of alkaline phosphatase and aminopeptidase A, with  $\alpha$ -L-glutamyl- $\beta$ -naphthylamide as substrate, and for the activity of leucine aminopeptidase L-leucinamide or L-leucyl- $\beta$ -naphthylamide as substrate.

Microassays using 0.1 ml. of serum were carried out by a modification of the usual methods for calcium<sup>5</sup>, inorganic phosphorus<sup>6</sup>, and alkaline phosphatase<sup>7</sup>. A microassay of  $\alpha$ -L-glutamyl- $\beta$ -naphthylamide or L-leucyl- $\beta$ -naphthylamide-splitting enzyme activity was established by a method based on that of Glenner<sup>2</sup>. The activity of the L-leucinamide-splitting enzyme was measured by a new method based on the isolation by paper chromatography and colorimetric assay of L-leucine liberated from the substrate. Enzyme assay was carried out at 37° C.

The ranges for experimental values and the normal control values with standard deviations (in parentheses) were as follows: calcium, 22–233 (145  $\pm$  39) mg/l. of serum; inorganic phosphorus, 57–228 (118  $\pm$  38) mg/l. of serum; alkaline phosphatase, 23–208 (127  $\pm$  23)  $\mu$ moles of inorganic phosphorus/min./l. of serum;  $\alpha$ -L-glutamyl- $\beta$ -naphthylamide-splitting enzyme, that is, aminopeptidase A, 2.0–29.4 (13.1  $\pm$  6.2)  $\mu$ moles of  $\beta$ -naphthylamine liberated from  $\alpha$ -L-glutamyl- $\beta$ -naphthylamide/min./l. of serum; L-leucyl- $\beta$ -naphthylamide-splitting enzyme, 6.2–42.3 (29.9  $\pm$  8.4)  $\mu$ moles of  $\beta$ -naphthylamine liberated from L-leucyl- $\beta$ -naphthylamide/min./l. of serum; L-leucinamide-splitting enzyme, that is, leucine aminopeptidase, 1.2–76.5 (44.2  $\pm$  8.5)  $\mu$ moles of L-leucine liberated from L-leucinamide/min./l. of serum.

Correlation coefficients (*r*) are shown in Table 1. There is a positive correlation between  $\alpha$ -L-glutamyl- $\beta$ -naphthylamide-splitting enzyme and calcium or alkaline phosphatase activity, as well as between calcium and alkaline

phosphatase activity. There is a negative correlation between inorganic phosphorus and  $\alpha$ -L-glutamyl- $\beta$ -naphthylamide-splitting enzyme or calcium. There is, however, almost no correlation between L-leucyl- $\beta$ -naphthylamide-splitting enzyme or L-leucinamide-splitting enzyme and calcium, inorganic phosphorus, or alkaline phosphatase activity.

The activity of aminopeptidase A was measured in a saturated amount of calcium to obtain the maximum velocity, and so the enzyme activity measured is not influenced by the differences in calcium concentrations in serum samples. It is concluded, therefore that the parathyroid function might exert an influence on the concentration of aminopeptidase A protein in serum. The origin of aminopeptidase A in serum is not yet clear, but it is interesting that the enzyme which needs calcium as co-factor can be regulated by parathyroid function.

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### Separation of a Hydrolase from a Pectic Acid Transeliminase in Cell Extracts of a *Bacillus*

In the past few years it has become increasingly evident that the principal mechanism involved in bacterial breakdown of pectic substances is a transelimination reaction resulting in the formation of unsaturated uronides<sup>1–5</sup>. So far as we know, no one has isolated a true hydrolase from bacteria. The presence of a hydrolase, however, can be deduced from the results obtained by Preiss and Ashwell<sup>3</sup>. Based mainly on the products found, they concluded that the cell extracts of a pseudomonad must have contained both transeliminase and hydrolase activities. These investigators did not isolate the hydrolase.

An attempt was made in this laboratory to isolate a bacterial hydrolase. In preliminary investigations, twenty cultures of bacteria which decompose pectic acid were isolated on a sodium polypectate medium<sup>3</sup> from various soils and manures for a survey of the production of a bacterial hydrolase. The results of this survey indicated that the cell extracts of one strain of an aerobic species of *Bacillus* possessed, in addition to a transeliminase, considerable hydrolase activity. The hydrolase has been separated from the transeliminase by ammonium sulphate fractionation followed by chromatography on a DEAE cellulose column. When the supernatant solution of the cell extract was fractionated with solid ammonium sulphate, all the hydrolase activity was precipitated in the fraction of 0–0.5 saturation, but only 30 per cent of the transeliminase activity was present in this precipitate. The fraction obtained from 0–0.4 saturation was further fractionated on a DEAE cellulose column. After the preparation had been dialysed against water, it was applied to a column of DEAE cellulose, 1.5 cm  $\times$  20 cm, which had been previously equilibrated with 0.05 molar *tris* buffer, pH 7.5. The column was eluted by a stepwise method with a solvent system consisting of 0.1 molar *tris* buffer, pH 7.5, 0.02 per cent cysteine hydrochloride, and increasing concentrations of sodium chloride. The following elution pattern was used: 75 ml. of buffer solution, 50 ml. each of buffer plus 0.1 molar salt, buffer plus 0.2 molar salt, and buffer plus 0.3 molar salt. The trans-

Table 1. VALUES OF CORRELATION COEFFICIENT

Correlation between	<i>r</i>	No. of cases
$\alpha$ -L-Glutamyl- $\beta$ -naphthylamide-splitting enzyme/calcium	0.57	63
$\alpha$ -L-Glutamyl- $\beta$ -naphthylamide-splitting enzyme/inorganic phosphorus	-0.54	65
$\alpha$ -L-Glutamyl- $\beta$ -naphthylamide-splitting enzyme/alkaline phosphatase	0.41	44
L-Leucyl- $\beta$ -naphthylamide-splitting enzyme/calcium	0.20	67
L-Leucyl- $\beta$ -naphthylamide-splitting enzyme/inorganic phosphorus	-0.25	68
L-Leucyl- $\beta$ -naphthylamide-splitting enzyme/alkaline phosphatase	0.10	46
L-Leucinamide-splitting enzyme/calcium	0.12	57
L-Leucinamide-splitting enzyme/inorganic phosphorus	-0.05	54
L-Leucinamide-splitting enzyme/alkaline phosphatase	0.13	38
Calcium/inorganic phosphorus	-0.38	60
Calcium/alkaline phosphatase	0.43	42

eliminase was eluted by the buffer while the hydrolase was eluted by the 0.2 molar salt plus buffer solution. The transeliminase activity was measured by a pectic acid plate method<sup>6</sup> or by measurement of the increase in absorbancy at 232 m $\mu$ , while hydrolase activity was determined quantitatively by measurement of decrease in absorbancy at 232 m $\mu$  with the unsaturated substrates and qualitatively by paper chromatography for both unsaturated and saturated substrates.

The hydrolase of the *Bacillus* species seemed to be quite different from plant and fungal polygalacturonases<sup>7,8</sup> in that the rate of hydrolysis of unsaturated uronides was inversely proportional to chain length. The enzyme attacks from the non-reducing end liberating either unsaturated or saturated monomers depending on the substrate used. In preliminary investigations, it was found also that the enzyme did not require calcium ions for its activity and that its optimum pH seemed to be considerably lower than that of the transeliminase.

Exocellular pectolytic enzymes of the bacterium showed only transeliminase activity. The transeliminase has characteristics similar to that of other bacterial transeliminases in that it has a high optimum pH and requires calcium ions for activity. Paper chromatographic analyses showed that the enzyme attacked higher uronides in a random manner but it did not seem to attack trigalacturonic acid under the same conditions.

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### Activity of Malic Dehydrogenase of the Symbionts and Fat Bodies of a Cockroach

MALIC dehydrogenase is an enzyme of the tricarboxylic acid cycle which catalyses the oxidation of malate to oxalacetate in the presence of NAD<sup>+</sup>. It has been found in the fat bodies of the cockroaches, *Blattella germanica* and *Periplaneta americana*<sup>1-3</sup>, as well as in similar tissues of the locust, *Locusta migratoria*<sup>4</sup>. The purpose of our investigation was, therefore, to determine whether the activity of this enzyme in the fat bodies of the cockroach, *Leucophaea maderae*, is caused solely by the action of these tissues, the symbionts, or both.

Cockroaches were reared according to the method of Pierre<sup>5</sup>, and the symbionts were isolated from the fat bodies according to the methods of Begg and Sang<sup>6</sup>, Pant, Nayar and Gupta<sup>7</sup> and Pierre<sup>5</sup>. Investigation of the activity of the enzyme was carried out on homogenates made from 1-g weights of materials of the symbionts (48-h-old cultures grown on *Neurospora* agar for luxuriant growth), similar weights of the fat bodies of normal adults and nymphs (sixth to eighth instars), and fat bodies of corresponding aposymbiotic insects (devoid of symbionts). The materials were homogenized with a sterile tissue grinder in 2 ml. of 0.03 molar phosphate buffer at pH 7.4. Sufficient of the same buffer and 2 ml. of 0.2 per cent NAD<sup>+</sup> were added to make a concentration of 3.3 per cent, and the homogenates were incubated at 30° C in a constant temperature bath for 0.5 h to oxidize the endogenous substrates. At the end of incubation, they were assayed for the activity of the enzyme.

Determination of the activity of malic dehydrogenase was performed according to the method of Thunberg<sup>8</sup>. The details of this method are as follows: 0.25 ml. of 0.25 molar sodium malate (substrate), 0.5 ml. of methylene blue (1 : 10,000), and 2.35 ml. of 0.03 molar phosphate buffer, pH 7.4, were placed in the main portion of the tube; 0.1 ml. of the homogenate was placed in the side arm. Two standards were prepared; one contained all the ingredients of the reaction tube except the substrate, and 2.6 ml. of buffer instead; and the other was a similar standard the homogenate of which was inactivated by boiling, and which contained 0.1 ml. of methylene blue, and 2.75 ml. of the buffer instead. All the tubes were sealed and evacuated for 5 min. They were then immersed in a constant temperature water bath at 30° C, and the time for 90 per cent of the indicator to be decolorized was determined visually. Accordingly, the activity of the enzyme was expressed as the reciprocal of the time in min required for 90 per cent decoloration of the indicator.

Spectrophotometric determination of malic dehydrogenase was performed with homogenates identical to those already outlined by the Thunberg technique. Assays of the enzyme activity were made with a spectrophotometer, at a wavelength of 340 m $\mu$ , according to the methods of Faulkner<sup>9</sup> and Protá<sup>10</sup>. The cuvettes used contained 0.1 ml. of homogenate, 0.25 ml. of 0.25 molar sodium malate, 0.25 ml. of 0.2 per cent NAD<sup>+</sup>, 0.25 ml. of 1.0 per cent KCN (oxalacetate inhibitor), and 2.15 ml. of 0.03 molar phosphate buffer, pH 7.4. The instrument was set at zero optical density with 3 ml. of all the ingredients of the cuvette except the NAD<sup>+</sup>. Recordings were made at zero time, and then at 5 min intervals for 20 min. The enzyme activity was then expressed as the change in optical density which occurred between zero time and the end of the experiment. Twenty determinations of the activity of the enzyme were performed by each method. This necessitated the use of individual insects, and fresh cultures of the symbionts at each trial.

The results obtained for the activity of malic dehydrogenase investigated by the Thunberg technique<sup>8</sup> in the materials of the symbionts, and those of the normal adults and nymphs, were 0.017, 0.018, and 0.043 respectively. Corresponding activities obtained in the aposymbiotic insects were 0.002 and 0.003 respectively. These activities are expressed as the reciprocal of the time in min required for 90 per cent decoloration of methylene blue. Spectrophotometric results of the activities obtained for materials of the symbionts, and those of the normal adults and nymphs were 0.116, 0.146, and 0.222 respectively; and corresponding results for materials of the aposymbiotic insects were 0.025 and 0.029 respectively. These activities are expressed as the change in optical density of the reaction mixture between readings at zero time and after 20 min. A representation of the spectrophotometric measurements of the activities in materials of the symbionts and normal insects is shown in Fig. 1. These graphs portray the rising change in the optical density of malate as the latter is converted to oxalacetate in the presence of the enzyme.

The results of this investigation have revealed that (a) the highest concentration of malic dehydrogenase is contained in the normal nymphal fat bodies; (b) a higher concentration of the enzyme is found in normal fat bodies than in the materials of the symbionts; (c) despite the high concentration of the enzyme in nymphal tissue, this concentration decreases as adulthood is attained; (d) a very low concentration of the enzyme exists in aposymbiotic fat bodies; and (e) the symbionts contribute almost all of the enzyme found in normal fat bodies.

Dubowsky and Pierre<sup>11</sup> have demonstrated in this laboratory that the malic enzyme of the fat bodies of this cockroach, *L. maderae*, is confined to the action of these tissues. It seems that, when this malic enzyme of the fat bodies reversibly converts malate to pyruvate, the



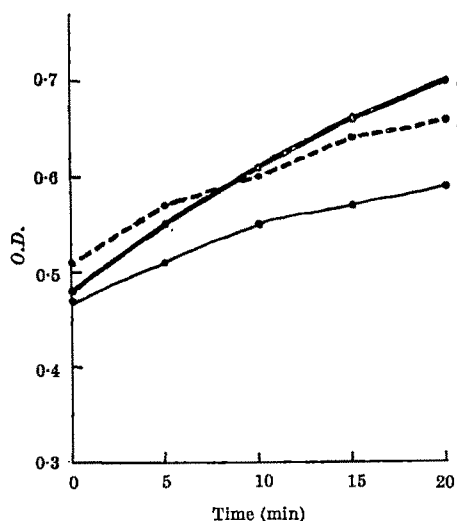


Fig. 1. Graphs showing the activities of malic dehydrogenase from the materials of (a) nymphs; (b) normal adults; and (c) symbionts in the presence of malate.

malic dehydrogenase of the symbionts in turn converts the malate so reformed to oxalacetate.

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### Activity of Isocitric Dehydrogenase in the Fat Bodies of the Cockroach, *Leucophaea maderae* (F)

ISOCITRIC dehydrogenase, an enzyme of the tricarboxylic acid cycle, is responsible for the formation of  $\alpha$ -keto-glutarate from isocitrate. During the reaction, oxalosuccinate is formed (as an intermediate) owing to the presence of two pyridine nucleotide-linked dehydrogenases in biological systems. One dehydrogenase is linked with NAD and the other is linked with NADP. They first catalyse the oxidation of isocitrate, and then its decarboxylation in the presence of the manganous ion. If NADP is used in the reaction, it is reduced and, because of the reversibility of the process, carbon dioxide is fixed in oxalosuccinate and isocitrate. Isocitric dehydrogenase has been isolated from heart tissue of the pig<sup>1</sup>, yeasts<sup>2</sup>, and various animal tissues<sup>3</sup>. This enzyme was discovered in the supernatant fraction of the homogenates of the fat bodies of the cockroach, *Periplaneta americana*<sup>4,5</sup>. Its presence has been reported in the haemolymph of the mealworm, *Tenebrio molitor*, by Protta (see previous communication). Hence, the purpose of this investigation was twofold: to determine whether this enzyme could be detected in normal and aposymbiotic fat bodies of the cockroach, *Leucophaea maderae*; and to determine whether the presence of the

enzyme in this tissue is due to the action of the tissue, the symbionts or both.

Cockroaches were reared, with slight modification<sup>6</sup>, by the methods described in the preceding communication, and homogenates were prepared and enzymes were assayed also in the way described in the previous communication.

Spectrophotometric determination of the activity of isocitric dehydrogenase was performed according to the method of Protta (see preceding communication) with a spectrophotometer at a wavelength of 340 m $\mu$ . The instrument was blanked with 3 ml. of 0.03 molar phosphate buffer at pH 7.4. The cuvettes used contained 0.5 ml. of  $6 \times 10^{-3}$  molar manganous chloride; 1 ml. of 0.2 per cent sodium isocitrate (substrate); 0.1 ml. of homogenate; 0.5 ml. of 1 per cent NADP<sup>+</sup>; and 0.9 ml. of 0.03 molar phosphate buffer, pH 7.4. The optical density of reaction mixtures was measured at zero time, and then at 1 min intervals until the end of the experiment. The enzyme activity was then determined by the change in optical density of the reaction mixture which occurred between the readings at zero time and at the end of the experiment.

Assays of enzyme activity were also performed according to the methods of Thunberg (see preceding article), with materials identical to those used for the first method. One millilitre of homogenate was placed in the side arm of the tube; and 0.5 ml. of methylene blue (1:10,000); 1 ml. of 1 per cent NADP<sup>+</sup>; 1 ml. of 0.2 per cent sodium isocitrate; 0.5 ml. of  $6 \times 10^{-3}$  molar manganous chloride; and 0.5 ml. of 0.03 molar phosphate buffer, pH 7.4, were placed in the main part of the tube. The two standards were: one which contained all the ingredients of the reaction tube except the substrate, which had 1.5 ml. of the buffer instead; and a similar standard which contained 0.05 ml. of the methylene blue instead of 0.5 ml., 0.95 ml. of buffer, and in which the enzyme had been inactivated by boiling. All tubes were sealed and evacuated for 5 min. They were then immersed in a constant temperature water bath at 30° C, and the time in min required for 90 per cent of the indicator to be decolorized was determined visually. The enzyme activity was then expressed as the reciprocal of this time. Twenty determinations of the enzyme activity were carried out by each method. This required the use of individual insects and fresh cultures of the symbionts at each trial.

Spectrophotometric determination of the activity of isocitric dehydrogenase in the materials of the symbionts and those of the normal adult and nymphal cockroaches showed the following results: 0.363; 0.237; 0.227, respectively. Corresponding results for the aposymbiotic adult and nymphal materials were 0.139 and 0.153 respectively. Representations of these activities are shown in Fig. 1. This figure shows the changes in optical density of isocitrate as it is converted to oxalosuccinate and then

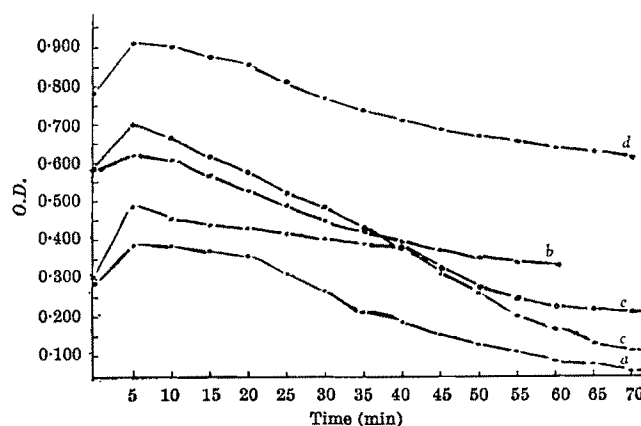


Fig. 1. The activities of isocitric dehydrogenase in the fat bodies of (a) normal adult; (b) normal nymph; (c) aposymbiotic adult; (d) aposymbiotic nymph; (e) symbiont.

to  $\alpha$ -ketoglutarate, in the presence of the enzyme. Results of the enzyme activity determined by the Thunberg technique (see previous communication) were 0.454, 0.014, 0.013, 0.006 and 0.006 for the materials of the symbionts, the normal adult and nymphal fat bodies, and those of the corresponding aposymbiotic insects, respectively.

The greatest activity for isocitric dehydrogenase was obtained in the materials of the symbionts, and so it is obvious that the symbionts play a definite part in the activity of this enzyme in the insects. This fact is further substantiated by the demonstration of small concentrations of the enzyme in aposymbiotic fat bodies. Furthermore, the comparable results obtained for the activity in normal insects, and the comparable results obtained in aposymbiotic tissue, tend to show that the symbionts are definitely responsible for most of the enzyme produced in fat bodies.

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### Sugar Contents of Single Cell Clones of Stem and *Phylloxera* Leaf Galls of the Grape Vine

INSECTS induce galls of many sizes and degrees of complexity in plants. The mechanism of gall induction and why growth finally ceases are not clearly understood. *Phylloxera* induces leaf gall in the grape vine which is typical of such abnormal growth in the sense that it is restricted in its development. Some of the biochemical and physiological changes which occur in these abnormal structures have been investigated by means of the tissue culture technique<sup>1</sup>.

Single cell clones from grape leaf gall and stem calli showed variation in their growth rates, nutritional requirements, biochemical composition, and physiological responses to added sugars in the medium<sup>2-4</sup>. The present investigations were undertaken to determine if there were any differences in the sugar composition of clones of different growth rates of normal and abnormal origin.

Two fast growing single cell (SC) clones (25, 24), one each from grape stems (*GS*<sub>4</sub>) exhibiting medium (81) and slow (18) growth rate, and one each from fast (21), medium (8) and slow (32) growing single cell clones of *Phylloxera* leaf gall (*GP*<sub>4</sub>) of grape were used for these investigations. These clones were grown in the dark at 26° C on "C" medium<sup>4</sup>, transferred every month to fresh medium, and collected after 4 weeks at their fastest growth for analyses. Each experiment was repeated three times.

About 10 g of fresh tissue was washed after all adhering agar had been removed in a Buchner funnel, and the weighed tissue was homogenized with 80 per cent warm ethanol. Tissues were extracted four times and the supernatants were combined. The non-soluble residue was saved for starch analysis. The ethanolic extracts were passed through a 1 x 5 cm bed of 'Dowex 50 x 8' (H form) to take up amino-acids and amides. The filtrate was then passed through a 1 x 10 cm bed of 'Dowex 1 x 10' (acetate form) to take up non-volatile organic acids. The fractions containing sugars passed through a 'Dowex 1' column and were taken to dryness. Pigments and lipoidal substances were removed from the latter fraction by emulsifying in water : chloroform (1 : 3), followed by centri-

fugation and removal of aqueous layers. These extracts were concentrated in 80 per cent ethanol.

Sugars were analysed by one dimensional descending paper chromatography on Whatman No. 3 paper using triple solvent development in *n*-butanol-acetic acid-water (4 : 1 : 5 v/v/v). Guide strips of known sugars and of the tissue extracts were developed with 0.2 per cent  $\alpha$ -naphthoresorcinol in ethanol and phosphoric acid (9 : 1 v/v, 90° C for 5 min)<sup>5</sup>. Corresponding areas were cut from the undeveloped portions of the chromatograms. The spots were eluted with hot water and filtered through glass wool. The eluted materials were dried *in vacuo* and 2 ml. of water were added, followed by 4 ml. of 0.2 per cent anthrone in concentrated sulphuric acid. The contents of the tubes were thoroughly mixed and heated in a steam bath for 10 min and the optical density read at 640 m $\mu$ .

For the starch determination, the residue insoluble in ethanol was collected in a 50 ml. centrifuge tube and made up to 10 ml. with water. Tubes were kept in an ice water bath and 13 ml. of perchloric acid reagent (52 per cent) were added. The contents were occasionally stirred for 15 min. The supernatants were poured into a 100 ml. volumetric flask. Five millilitres of water and 6.5 ml. of perchloric acid reagent were added to the residue, stirred, and cooled as before, and the contents washed into the volumetric flask. Two millilitre portions were taken from the hydrolysed starch extracts, which had been filtered through glass wool, 4 ml. of 0.2 per cent anthrone reagent in concentrated sulphuric acid added, and the optical density values taken for sugars as before and converted to starch by 0.90 factor (ref. 6).

The grape stem and leaf gall tissue cultures contained the same sugars, but in different concentrations depending on the origin and rate of growth of the clones. Extracts of these stem and gall clones contained mainly fructose, glucose, and sucrose. Traces of raffinose and two unidentified sugars were also found. These unidentified sugars had low *R<sub>F</sub>* values and reacted as ketones to various spray reagents. Quantitative differences in the amounts of total and individual sugars were observed between the stem clones and the gall clones and among the clones of different growth rates. The results are given in Table 1.

Table 1. SUGAR AND STARCH CONTENTS OF STEM AND LEAF GALL TISSUE CULTURES OF GRAPE (MG/G DRY WT)

	Growth rates and clone No.						
	25	Stem clones		18	Gall clones		
	Fast	24	81	Slow	21	8	32
		Medium	Medium		Fast	Medium	Slow
Sugar							
Raffinose	3.2	2.7	2.0	2.1	2.3	0.8	2.2
A*	2.9	2.7	2.0	2.3	2.3	0.6	1.8
Sucrose	14.8	15.2	8.0	31.6	46.3	8.2	9.4
B*	2.8	3.0	1.9	0.9	4.7	0.2	2.2
Glucose	17.3	20.5	6.5	17.4	54.3	13.6	30.1
Fructose	0.8	14.3	7.3	29.3	25.3	16.0	27.9
Total	51.3	58.4	27.7	83.6	135.2	39.4	78.6
Starch	33.8	42.3	33.3	38.2	35.3	48.8	40.0

\* A and B, unidentified sugars separated after raffinose and sucrose on the chromatogram.

The differences in the total sugar content between the gall and stem clones of similar growth rates were significant at the 5 per cent level, except for the differences between slow growing gall and stem clones. There were also significant differences between the sucrose, glucose, and fructose, and the raffinose and unidentified sugars A and B. It is not clear why there were significant differences in the total sugar contents but not in the starch contents of the various clones of stem and gall origins.

The sugars isolated from the gall and stem tissue cultures have also been reported in tissues of intact grape plants. For example, sucrose, glucose and fructose were detected in all organs of the grape vine and, of these, glucose was the most abundant sugar<sup>7</sup>. In addition, the stem and leaf gall tissue cultures of grape contained traces of raffinose and two unidentified sugars.

Similarly, agave leaf and leaf tissue cultures contained sucrose, fructose, glucose, raffinose, and six unidentified sugars. In the leaf tissue culture, the dominant sugar was fructose, followed by glucose and finally by sucrose, but in the intact leaf, fructose was present in low concentration<sup>8</sup>. In ginkgo, sucrose predominated in the pollen and glucose in the pollen tissue cultures. Raffinose was found only in the pollen<sup>9</sup>. In rose stem and leaves, xylose was found in addition to the sucrose, fructose and glucose present in stem tissue cultures<sup>10</sup>. These findings indicate that tissue cultures often differ from the intact plant parts with respect to their sugar contents.

In related work from this laboratory, the stem and gall clones varied in amino-acid and total nitrogen contents as compared with intact grape tissues<sup>11</sup>. Stem and gall clones could thus be distinguished from each other by their different nitrogen and carbohydrate metabolism, depending on their growth rates and their gall or stem origins.

All these clones benefited by the presence of dextrose and laevulose in the media, but the fast growing gall clone (21) grew best on sucrose<sup>2</sup>. This suggested that this clone, unlike other clones which depended on dextrose and laevulose, was able to hydrolyse sucrose. This clone had the highest sugar content.

These variations indicate basic differences in the metabolism of the cultures of normal and abnormal tissues. The stem clones were derived from the tissue of normal stem origin, and the gall clones came from induced abnormal leaf growth. The comparison was made between the leaf gall tissue and stem tissue because it was not possible to grow leaf callus. Differences in the contents of sugar and starch in the tissues of different growth rates showed the variability of the clones obtained from single cells of the same mother callus.

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### Free Fatty Acid Metabolism during Embryogenesis of *Periplaneta americana* (L)

EXERGONIC reactions involving predominantly the catabolism of lipid materials<sup>1</sup>, particularly triglycerides<sup>2,3</sup>, provide the developing oviparous arthropod embryo with the energy necessary to sustain the intense metabolic activity occurring during its embryogenesis. The integral metabolism of these lipids should be closely reflected in the pattern of the free or unesterified fatty acids during this period, for these acids ultimately are the oxidizable form of the stored glycerides. It is established from analytical data<sup>2,4</sup> and measurements of the respiratory quotient (R.Q.)<sup>5</sup> that free fatty acids are catabolized to

provide respiratory energy in both invertebrate and vertebrate tissue. The metabolic pattern of these acids was investigated with the developing embryo of the American cockroach, *Periplaneta americana* (L), as the experimental material.

The ova, enclosed in their egg cases (oothecae), were obtained from a controlled culture of mated females and incubated at 25° C and 70 per cent relative humidity. Development within the ootheca was complete in 41–42 days and the nymphs emerged on the forty-third ( $\pm 1$ ) day of incubation. The lipids of the oothecae were analysed at specific times during embryonic development (see Fig. 1). The total lipids were extracted by the method of Folch *et al.*<sup>6</sup>, fractionated by silicic acid column chromatography<sup>7</sup>, and the free fatty acids were then quantitatively isolated on an alkaline silicic acid column<sup>7</sup>.

The fluctuation in the content of free fatty acids in the developing embryo is shown in Fig. 1. For descriptive purposes the curve is divided into three phases. The initial increase (0–14 days) corresponds to the period of rapid cellular multiplication, migration and differentiation—a period of intense metabolism. Measurements of the R.Q.<sup>1</sup> indicate that lipids supersede carbohydrates as oxidizable substrate during this period and later constitute the main source of energy. The initial increase in the amount of free fatty acids reflects this substrate transition during which time it is probable that lipase becomes active and hydrolyses the stored triglycerides. These diminish commensurately and concomitantly<sup>2,3</sup> with the increase in free fatty acids. The ensuing phase (14–40 days), encompassing the period of tissue organization, blastokinesis, and general growth of the embryo, is reflected in a stable pattern of free fatty acids. The triglyceride moiety diminishes while the other lipid fractions remain constant<sup>2</sup>. This indicates that oxidation of free fatty acids is keeping pace with glyceride hydrolysis. The final phase, that is the period of nymphal eclosion, is characterized by a dramatic increase in the content of free fatty acids, which form a rich pool of directly available energy to sustain the nymph during actual emergence and until it commences to feed. The triglycerides show an inverse pattern to that of the free fatty acids, while the mono- and di-glycerides increase<sup>2</sup>. The curve for free fatty acids shown in Fig. 1 reflects a pattern of lipase activity which closely resembles that which occurs in the developing embryo of the silkworm *Bombyx mori*<sup>8</sup>, particularly before hatching.

Aliquots of the free fatty acids were methylated and analysed by gas liquid chromatography<sup>9</sup>. Twenty-eight

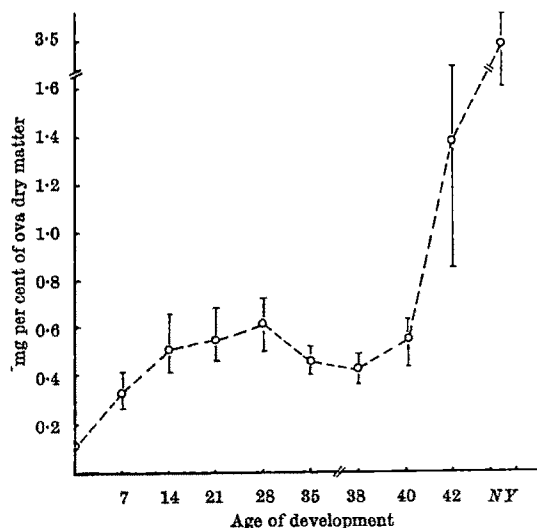


Fig. 1. Content of free fatty acid (mg/100 mg of ova dry matter) at progressive stages of embryogenesis of *P. americana*. Abscissa numbers denote days and NY refers to newly emerged active nymph.

Table 1. VARIATION IN PERCENTAGE COMPOSITION OF FREE FATTY ACIDS DURING EMBRYOGENESIS OF *P. americana*

Fatty acid carbon No.	0	7	14	21	28	35	38	40	42	Nymph
C-14:0	1.6	0.7	0.3	0.5	1.0	1.0	1.7	1.1	1.7	2.0
C-16:0	26.8	27.0	26.5	26.6	25.5	24.5	22.0	21.0	24.3	26.1
C-16:1	2.3	1.6	1.0	2.1	2.2	1.5	1.9	1.9	2.8	1.2
C-17:0	0.3	0.2	0.3	0.1	0.1	0.2	0.3	0.2	0.4	0.4
C-18:0	2.1	3.3	3.4	3.7	3.6	4.6	6.5	6.5	6.4	7.1
C-18:1	43.4	45.0	45.0	47.8	46.5	42.9	42.5	45.0	43.0	46.0
C-18:2	19.2	21.0	24.0	19.0	20.5	19.6	21.0	17.2	17.0	15.0
C-18:3	1.3	0.4	0.4	0.3	0.2	0.5	0.5	0.5	0.2	0.1

fatty acids with six to twenty carbon atoms in the chain were identified, although Table 1 includes only those acids which occurred in measurable quantities. Palmitic (C16), oleic (C18:1), and linoleic (C18:2) predominate in the free fatty acids of the cockroach embryo (in vertebrates they consist mainly of palmitic, stearic and oleic acids<sup>10</sup>). There is no apparent preferential use of any individual acid, though the pattern of linoleic (C18:2) and linolenic (C18:3) acids is compatible with Fraenkel and Blewett's assertion that one or both is essential for eclosion<sup>11</sup>. It is concluded from Table 1 that the various free fatty acids are catabolized in proportion to their quantitative occurrence as reported for the total fatty acids from the embryo<sup>9</sup>, and also for free fatty acids in vertebrate tissues<sup>12</sup>. Thus, the quantitative and qualitative patterns of the free fatty acids provide an indication of the rate and extent of glyceride catabolism during embryogenesis.

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### Polyamide Layer Chromatography of some Synthetic Food Colours

RECENT developments in the preparation of polyamide layers in our laboratory have facilitated the application of polyamide layer chromatography to many kinds of compounds besides phenolic compounds; for example, DNP amino-acids<sup>1</sup>, oestrogens<sup>2</sup>, chloramphenicol derivatives<sup>3</sup>, sulphonamides<sup>4</sup> and indole derivatives<sup>5</sup> have been successfully separated. Here I shall describe its application to the analysis of certain synthetic food colours. All of them have functional groups which form hydrogen bonds with polyamide.

Many analyses of food colours have been made with paper and thin layer chromatography<sup>6</sup>, and thin film electrophoresis has been used for coal tar food colours by Criddle *et al.*<sup>7</sup>. None of these techniques, however, has proved entirely satisfactory.

*R<sub>F</sub>* values obtained with five solvent systems are given in Table 1 and a typical chromatogram is shown in Fig. 1. It can be seen that a sharp resolution of food colours is obtained with polyamide layer chromatography. I found that the addition of sodium *p*-toluenesulphonate is essential to give small and sharp spots. I have observed that the presence of as little as 2 per cent of the salt in the

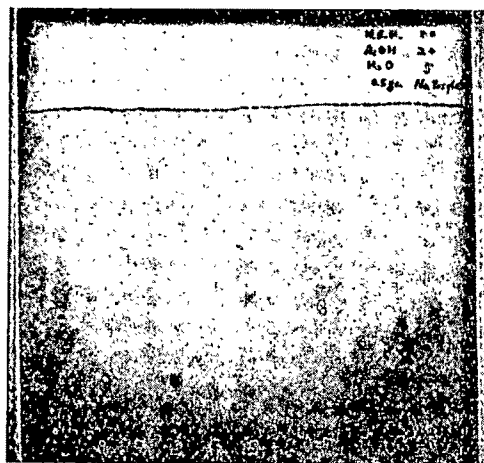


Fig. 1. Solvent: 2-butanone/glacial acetic acid/water/sodium *p*-tosylate v/v (40:40:10:1 g) layer, according to Wang (ref. 8) (Toyo Rayon Co., Amilan CM 1011 (26°)). Loading, 0.5 µg. Photographed by transmitted artificial light. Key: 1, New Cocaine; 2, amaranth; 3, Ponceau SX; 4, tartrazine; 5, orange I; 6, eosin (sodium-salt); 7, naphthol yellow S; 8, sunset yellow FCF; 9, Ponceau 2R; 10, Ponceau 3R.

solvent mixture is sufficient, and that an increase of the percentage of the salt present effects the increase of *R<sub>F</sub>* values observed. This throws open the possibility that compounds with a sulphonic acid group may be efficiently resolved by polyamide layer chromatography with the solvent mixture containing sodium *p*-toluenesulphonate because all the food colours which I have tested have a sulphonic acid group in the molecule except eosin. Eosin shows a circular spot without addition of sodium *p*-toluenesulphonate.

No.	Table 1				
	I	II	Solvents III	IV	V
(1) New Cocaine	0.12	0.03	0.26	0.13	0.12
(2) Amaranth	0.14	0.06	0.43	0.22	0.14
(3) Ponceau SX	0.25	0.16	0.48	0.35	0.16
(4) Tartrazine	0.25	0.13	0.55	0.36	0.26
(5) Orange I	0.40	0.41	0.59	0.49	0.42
(6) Eosin (Na-salt)	0.53	0.64	0.67	0.61	0.54
(7) Naphthol yellow S	0.36	0.44	0.70	0.58	0.35
(8) Sunset yellow FCF	0.46	0.28	0.68	0.49	0.43
(9) Ponceau 2R	0.48	0.30	0.70	0.52	0.45
(10) Ponceau 3R	0.48	0.31	0.70	0.53	0.47

Solvents I, 2-butanone/glacial acetic acid/water/sodium tosylate v/v (20:40:10:1 g); II, 2-butanone/glacial acetic acid/water/sodium tosylate v/v (40:40:10:1 g); III, acetone/glacial acetic acid/water/sodium tosylate v/v (40:40:10:1 g); IV, dioxane/glacial acetic acid/water/sodium tosylate v/v (40:40:10:1 g); V, *t*-butanol/glacial acetic acid/water/sodium tosylate v/v (20:10:5:1 g).

I found 10 cm development was sufficient because the spots were very small and sharp. The time required to ascend 10 cm from spot origin is 2.5–3 h except with the system containing *n*-butanol or *t*-butanol which required 6 h. The colours are very faint because of the small sample size (about 0.5 µg) and therefore they do not give good contrast on photographic paper; however, it is easy to locate the spots by transmitted daylight. Ponceau 2R and Ponceau 3R cannot be separated by these solvent systems probably because of their similarity in structures.

Polyamide layer is very durable and easy to handle. This layer with its sorption character is more suitable for analysing food colours than other adsorbents.

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### Polyamide Layer Chromatography of Indoles

POLYAMIDE was found to adsorb various types of compounds by hydrogen bonding. Carelli *et al.* were the first to report that polyamide powder is an excellent adsorbent for column chromatography of phenols and lower fatty acids, for example, formic acid, acetic acid and propionic acid<sup>1</sup>. Later, Endres<sup>2</sup> found that nitro and sulphonic acid groups are also adsorbed by polyamide powder column. Wang has applied polyamide layer chromatography to several compounds<sup>3</sup> including the separation of lactones<sup>4</sup>.

Here we report the result of our experiment on indole derivatives. In the early stage of development of thin layer chromatography, Stahl<sup>5</sup> showed excellent separation of twenty simple indole derivatives by two dimensional chromatography on silica gel *G* thin layer. More recently, some Russian workers<sup>6</sup> investigated forty-seven basic

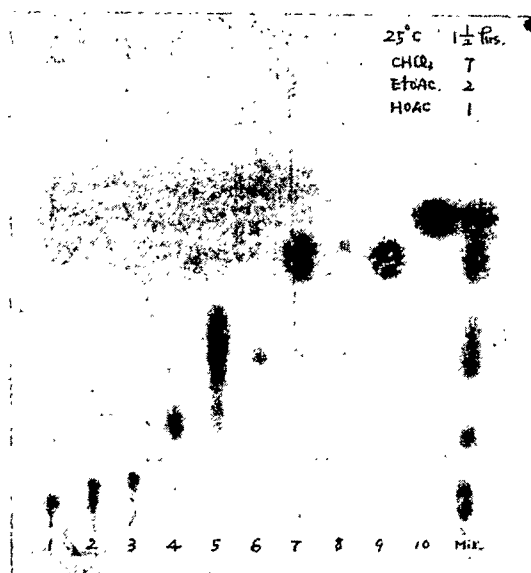


Fig. 1. Solvent system: chloroform/ethyl acetate/glacial acetic acid (7:2:1). Length of run: 10 cm, 1.5 h. Key: Polyamide layer according to Wang (ref. 7) 15 cm x 15 cm. Colour reagent: 1 per cent aqueous ferric chloride + 1 per cent aqueous potassium ferricyanide (v/v 1:1) (ref. 8). (1), 5-hydroxy indolyl-3-acetic acid; (2), tryptophan; (3), serotonin; (4), abrine (*N*-methyl tryptophan); (5), tryptamine; (6), indolyl-3-acetic acid; (7), *N*-methyl tryptamine; (8), melatonin (*N*-acetyl-5-methoxy tryptamine); (9), 5-hydroxy-tryptophan; (10), indole; (Mix.), mixture of above substances.

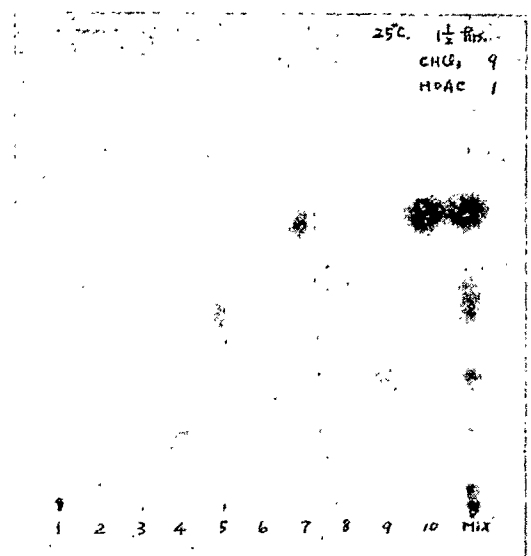


Fig. 2. Solvent system: chloroform/glacial acetic acid (9:1). Length of run: 10 cm, 1.5 h.

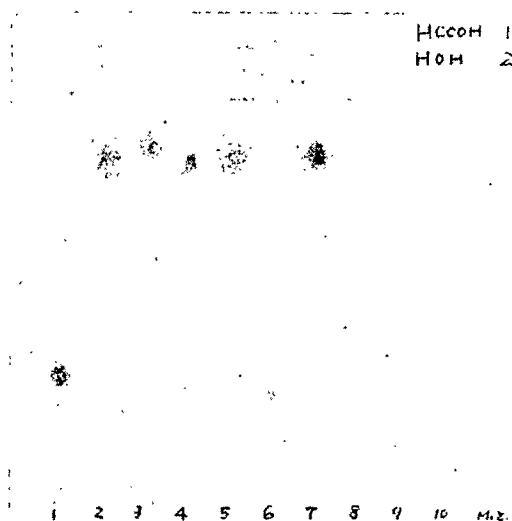


Fig. 3. Solvent system: 80 per cent formic acid/water (1:2). Length of run: 10 cm, 1.3 h.

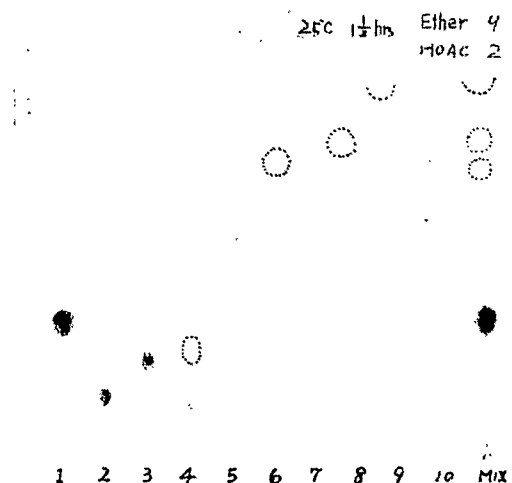


Fig. 4. Solvent system: diethyl ether/glacial acetic acid (9:2). Length of run: 10 cm, 1.5 h.

neutral and acidic indole derivatives and established that the twenty-eight basic and neutral indoles can be separated by alumina thin layer chromatography, and indicated that it is necessary to use paper chromatography to separate the acidic indoles.

In our experiments, ten simple indoles were used and four solvent systems—(1) chloroform/ethyl acetate/glacial acetic acid (7:2:1), (2) chloroform/glacial acetic acid (9:2), (3) 80 per cent formic acid/water (1:2), and (4) diethyl ether/glacial acetic acid (9:2)—were found to be useful for separation (see Table 1 and Figs. 1–4). The system chloroform/ethyl acetate/glacial acetic acid gives especially good resolution of these substances on a 10 cm solvent run.

Indole travels fastest as a spot in solvents 1, 2 and 4, but slowest in solvent 3. The presence of functional groups which can be adsorbed by polyamide was found to affect the  $R_F$  values. Tryptamine tends to show heavy tailing in solvent systems 1 and 2, but not in solvent 3 or 4, while with *N*-methyl tryptamine there is no tailing in any of these solvent systems. It seems that a free amino group is responsible for the non-linear adsorption of tryptamine in solvent systems 1 and 2, because *N*-methyl tryptamine gives a circular spot regardless of the solvent systems used.



Table 1.  $R_F$  VALUES OF INDOLES ON POLYAMIDE LAYERS

Compound	Solvent			
	(1)	(2)	(3)	(4)
(1) 5-Hydroxy indolyl-3-acetic acid	0.07	0.02	0.33	0.35
(2) Tryptophan	0.11	0.09	0.87	0.16
(3) Serotonin	0.13	0.08	0.93	0.26
(4) Abrine ( <i>N</i> -methyl tryptophan)	0.27	0.27	0.89	0.28
(5) Tryptamine	0.55	0.67	0.91	0.58
(6) Indolyl-3-acetic acid	0.45	0.43	0.26	0.77
(7) <i>N</i> -methyl tryptamine	0.73	0.84	0.92	0.72
(8) Melatonin ( <i>N</i> -acetyl-5-methoxy tryptamine)	0.75	0.80	0.38	0.81
(9) 5-Hydroxy tryptophan	0.71	0.41	0.00	front
(10) Indole	0.83	0.85	0.21	0.93

Solvents as in text.

These experiments provide evidence of polyamide sorption of the indole skeleton. It is probable that acidic hydrogen of pyrrole nitrogen is the centre of adsorption. Investigations of other indole derivatives and the application of this technique to indole metabolites in human urine are in progress.

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## SOIL SCIENCE

### New Method for measuring Heat Flux Density at the Surface of Soils or of Other Bodies

Two methods for determining thermal conductivity  $\lambda$ , and heat capacity per unit volume  $C$ , at a soil surface have recently been developed<sup>1</sup>. One method also measures the heat flux density.

A block of an appropriate material, at a uniform temperature, is placed on the soil surface and the temperature of the contact plane recorded. The product  $\lambda C$  and the heat flux density can be calculated. In experiments by myself and by Derksen, Schneider and Belghith, 'Perspex' blocks of  $10 \times 10 \times 4$  cm dimensions were used<sup>2</sup>. The temperature near the centre of the contact plane ( $10 \times 10$ ) was recorded for about 6 to 12 min.

The method might also be useful outside the field of soil science since it possesses the following favourable features: no correction need be made for the presence of the block; no sensing elements need be placed in the soil; a good thermal contact between block and soil surface is not essential.

The temperature near the centre of the contact plane is calculated from the theory of two semi-infinite bodies, each filling a half space, which are suddenly brought into contact along the plane  $z = 0$  at the instant  $t = 0$  (ref. 3). This happened during a certain interval of time in which the contact with the other body affects only a layer of a depth which is much smaller than the dimensions of the body. In the experiments referred to, this depth was about 1 to 2 cm.

If at  $t = 0$  the initial temperature in the block is uniform at  $\vartheta_1$  and in the upper soil layer is at  $\vartheta_2 + Ez$ , where  $z$  is the depth, the temperature in the plane of contact becomes

$$\vartheta(t) = \frac{\vartheta_1 \sqrt{\lambda_1 C_1} + \vartheta_2 \sqrt{\lambda_2 C_2}}{\sqrt{\lambda_1 C_1} + \sqrt{\lambda_2 C_2}} + \frac{2}{\sqrt{\pi}} \frac{E \lambda_2}{\sqrt{\lambda_1 C_1} + \sqrt{\lambda_2 C_2}} \sqrt{t} \quad (1)$$

The indices 1 and 2 refer to the block and the soil, respectively.  $-E \lambda_2$  is the initial heat flux density, since  $E$  is the gradient of the temperature in the soil near its surface. It can be calculated from the slope of the  $\vartheta(t)$  versus  $\sqrt{t}$  curve if  $\vartheta_1$ ,  $\vartheta_2$  and  $\lambda_1 C_1$  are known and  $\lambda_2 C_2$  is found from the temperature immediately after contact.

If two blocks are used at different initial temperatures, the initial surface temperature of the soil  $\vartheta_2$  need not be known, but can be calculated.

Equation (1) holds only for perfect thermal contact, that is,  $\vartheta_1(0, t) = \vartheta_2(0, t)$ , where  $\vartheta_i(0, t)$  denotes the limit of the temperature in body  $i$  for  $z$  approaching zero.

The records show that a more realistic assumption is  $\vartheta_1(0, t) = \vartheta_2(0, t) + \mu H(t)$ , in which  $H(t)$  is the heat flux density pointing from the block into the soil and  $\mu$  a positive constant which indicates the quality of the contact. With this assumption one obtains for the temperature at the contact face of the block

$$\vartheta_1(0, t) = \frac{\vartheta_1 \sqrt{\lambda_1 C_1} + \vartheta_2 \sqrt{\lambda_2 C_2}}{M} - \frac{NE \lambda_2}{M^2} + \left[ \frac{(\vartheta_1 - \vartheta_2) \sqrt{\lambda_2 C_2}}{M} + \frac{NE \lambda_2}{M^2} \right] e^{\frac{M^2}{N^2} t} \cdot \operatorname{erfc} \left( \frac{M}{N} \sqrt{t} \right) + \frac{2}{\sqrt{\pi}} \frac{E \lambda_2}{M} \sqrt{t} \quad (2)$$

$M = \sqrt{\lambda_1 C_1} + \sqrt{\lambda_2 C_2}$ ,  $N = \mu \sqrt{\lambda_1 C_1 \lambda_2 C_2}$ , and  $\operatorname{erfc}$  is the complement of the error function,  $\operatorname{erfc}(x) = 1 - \operatorname{erf}(x)$ .  $\lambda_2 C_2$ ,  $E \lambda_2$  and  $\mu$  can be calculated if  $\vartheta_1$ ,  $\vartheta_2$  and  $\lambda_1 C_1$  are known and the use of two blocks renders a separate determination of  $\vartheta_2$  superfluous. In our experiments the term with  $\operatorname{erfc} \left( \frac{M}{N} \sqrt{t} \right)$  became negligible after an interval ranging from 10 sec to 2.5 min in the individual experiments.

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<sup>1</sup> van Wijk, W. R., *Physica*, 30, 387 (1964).<sup>2</sup> van Wijk, W. R., and Derksen, W. J., *Agricultural Meteorology* (Amsterdam), van Wijk, W. R., and Schneider, T., *Proc. Congress on Ecosystems*, Copenhagen (1965), van Wijk, W. R., and Belghith, A., *Proc. Symposium on Forest Hydrology*, Pennsylvania State University (1965).<sup>3</sup> Carslaw, H. S., and Jaeger, J. C., *Conduction of Heat in Solids*, second ed., chapter 2 (Oxford University Press, 1959).

## AGRICULTURE

### Relation between Mineral Deficiency and Amine Synthesis in Barley

It has been shown that when potassium is deficient, barley—like other species—accumulates the amines putrescine and agmatine<sup>1,2</sup>, which are derived from arginine. The product of decarboxylation, agmatine, is converted to putrescine with the intermediate formation of *N*-carbamylputrescine. Furthermore, when phosphorus is deficient, barley has an increased agmatine content, but there is no corresponding increase in putrescine<sup>3</sup>. In the plants deficient in potassium the production of

agmatine and putrescine is accompanied by an increase in the activity of the enzymes, arginine carboxy-lyase and *N*-carbamylputrescine amidohydrolase<sup>4,5</sup>. It was of interest, therefore, to compare the activities of these two enzymes and the production of amines when phosphorus and potassium were deficient, and also to investigate the concentrations of agmatine and putrescine when some other essential elements were deficient.

Plants of a variety of barley resistant to mildew (H.B. 248-17-4) were grown in sand culture using the calcium type nutrient solution of Richards and Berner<sup>6</sup>. Those plants which were subjected to potassium deficiency received one-sixteenth of the potassium supply of the controls (-K), and those subjected to phosphorus deficiency received one-sixty-fourth of the optimum phosphorus supply (-P).

The plants, grown to the stage of the sixth leaf, in the open air, were sampled in duplicate on three occasions at weekly intervals, and the youngest fully expanded leaf from each tiller was analysed. In addition, other plants were grown on two occasions in water culture in which the concentration of calcium or magnesium had been reduced to one-tenth, or from which sulphur, iron or manganese had been omitted. In these treatments, the latest fully expanded leaves were sampled 2.5 weeks (first occasion) and 3.5 weeks (second occasion) after the start of the treatment.

Table 1. CONTENTS OF ENZYME AND AMINE AS INFLUENCED BY DEFICIENCY OF POTASSIUM OR PHOSPHORUS

Time of sampling	Treatment	Carboxy-lyase (μmoles/g fresh weight/h)	Amidohydrolase (μmole/g fresh weight/h)	Agmatine (μmole/g fresh weight)	Putrescine (μmole/g fresh weight)
1	Control	0.0004	0.32	0.10	Trace (<0.2)
	-P	0.033	0.27	0.32	Trace
	-K	0.134	0.24	1.02	2-8
2	Control	0.035	0.13	0.10	Trace
	-P	0.074	0.14	0.41	Trace
	-K	0.098	0.33	1.05	6-8
3	Control	0.027	0.09	0.13	Trace
	-P	0.058	0.12	0.51	Trace
	-K	0.115	0.23	1.11	6-8
	L.S.D. 5%	0.024	0.08	0.24	
	1%	0.035	0.12	0.32	

Putrescine and agmatine were extracted, purified on ion-exchange resins, and estimated by paper chromatography or colorimetry as described by Sinclair<sup>7</sup>. The activities of arginine carboxy-lyase and *N*-carbamylputrescine amidohydrolase were determined by the methods of Smith<sup>4,5</sup>.

Table 1 shows that significant increases in the activity of carboxy-lyase were induced by deficiencies of phosphorus or potassium. Amidohydrolase activity was increased by deficiency of potassium in two out of three samples, but remained unaffected by deficiency of phosphorus.

Concentrations of agmatine, increased by deficiencies of either phosphorus or potassium, seem to reflect the increases of carboxy-lyase activity involved in its production. Putrescine, the product of amidohydrolase activity, occurred in measurable amounts only in the leaves deficient in potassium.

In the plants raised in water culture, the concentration of agmatine was approximately trebled when calcium or sulphur were deficient; the concentration from magnesium or manganese was approximately doubled, while that of putrescine remained unchanged. Replicate samples of the plants deficient in calcium and sulphur, when analysed for their enzyme content, showed no change in the concentration of amidohydrolase, but did show an increase in that of carboxy-lyase in the samples deficient in calcium. These results suggest that the increased production of agmatine may be associated with deficiencies of several mineral nutrients, whereas further conversion of agmatine to putrescine through *N*-carbamylputrescine<sup>8</sup>

appears to be more specifically related to deficiency of potassium. The possible function of the accumulation of amines as a homeostatic mechanism for the maintenance of ionic balance, when potassium is deficient, has been discussed previously<sup>8,9</sup>.

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<sup>1</sup> Richards, F. J., and Coleman, R. G., *Nature*, **170**, 460 (1952).

<sup>2</sup> Smith, T. A., and Richards, F. J., *Biochem. J.*, **84**, 292 (1962).

<sup>3</sup> Hackett, C., Sinclair, C., and Richards, F. J., *Ann. Bot.*, **29**, No. 115, 331 (1965).

<sup>4</sup> Smith, T. A., *Phytochemistry*, **2**, 241 (1963).

<sup>5</sup> Smith, T. A., and Garraway, J. L., *Phytochemistry*, **3**, 23 (1964).

<sup>6</sup> Richards, F. J., and Berner, Jun., E., *Ann. Bot.*, **18**, No. 69, 15 (1954).

<sup>7</sup> Sinclair, C., thesis, Univ. London (1965).

<sup>8</sup> Coleman, R. G., and Richards, F. J., *Ann. Bot.*, **20**, No. 79, 393 (1956).

<sup>9</sup> Smith, T. A., and Sinclair, C., *Ann. Bot.* (in the press, 1967).

## Pentobarbital Sodium Salt, a Systemic Agent for Control of Powdery Mildew of Cucumber

PHENOBARBITONE (phenobarbital) has been reported to control powdery mildew of marrow<sup>1</sup>. During 1963 and 1964 we have tested a series of barbituric acid derivatives on powdery mildew of cucumber caused by *Sphaerotheca fuliginea* (Schlecht. ex. Fr.) Poll.

Cucumber seedlings, cultivar "Lange gele tros", were grown in pots in the greenhouse until the cotyledons were well developed. These cotyledons were sprayed with suspensions of the chemicals in water with an added surface-active compound. They were then dusted with spores of the fungus by shaking over them cucumber plants heavily infected with powdery mildew. After an incubation period of 10-14 days in the greenhouse the results were assessed and recorded as percentages of the mildew on the cotyledons of unsprayed control plants.

The tests to assess systemic activity were performed in a room maintained at a climate of about 70 per cent relative humidity, and a temperature of 21° C during the 16 h of light (fluorescent light, 3,000-6,000 lux) and

Table 1. POWDERY MILDEW ON COTYLEDONS OF CUCUMBER SEEDLINGS

Sodium salt of	R	Dosage (p.p.m.)				
		Spray test		Systemic test		
		3,000	1,000	100	100	30 10 3
Pentobarbital		3	27	94	0	0 5 100
Amylobarbitol		96	100		13	70
Phenobarbital		26	66	100	0	0 9 70

Results are expressed as percentages of control plants.

18°C during the 8 h of darkness. The cucumber seedlings were grown in the greenhouse on a nutrient solution; 48 h before infection they were moved to fresh solutions to which the chemicals had been added and placed in the adjusted climate; 7–8 days after infection the solution which had been taken up was replaced; infection and assessment of the results 12–14 days later were performed in the same way as in the spray tests.

Our results generally agree with those reported by Zaracovitis: barbituric acid and a number of its derivatives did not show any activity, either after application on to the cotyledons or after addition to the nutrient solutions, whereas phenobarbital (luminal) and its sodium salt did protect the plants after application to the roots (complete control at 30 p.p.m.). Protection at about the same level was obtained by pentothobarbital sodium salt, but the best results were obtained with the sodium salt of pentobarbital (nembutal) which was active not only after root application but also after spraying (Table 1). A closely related compound, amylobarbitol sodium salt (amytal sodium), did not show any activity at all. In the systemic tests the three compounds caused symptoms of slight phytotoxicity: retardation of growth, yellowing of the leaves.

Zaracovitis<sup>1</sup> suggested that phenobarbitone and 6-azauracil could have a similar mode of action. Dekker obtained in a leaf disk test complete antagonism of 0.6 p.p.m. of 6-azauracil by 80 p.p.m. of uracil<sup>2</sup>. We performed a similar test with pentobarbital and phenobarbital. Uracil in a concentration of 500 p.p.m., however, failed to counteract the protective effect of 30 p.p.m. of these compounds.

Spraying with 3,000 p.p.m. of the compounds did not protect apple seedlings against powdery mildew after artificial infection with *Podosphaera leucotricha* (Ell. and Evenh.) Salm.

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<sup>1</sup> Zaracovitis, C., *Nature*, 206, 954 (1965).

<sup>2</sup> Dekker, J., *Meded. Landbouwhogeschool. Opzoekstns Gent*, 27, 1214 (1962).

## APPLIED SCIENCE

### Electrochemical Time Switch

AN electrochemical time switch for a.c. current has been developed. It consists of a copper electrode, two tantalum electrodes with oxide films produced by heating<sup>1</sup> and an electrolytic solution (see Fig. 1). The tantalum plate is 99.9 per cent pure tantalum heated for about 12 sec in an electric oven (enclosed heating element) at 670°C in air to form the oxide film. For convenience, the copper electrode is referred to as the control electrode and the tantalum electrodes are referred to as the main electrodes.

Under the open circuit conditions shown in Fig. 1, there is no d.c. voltage at the control electrode. The path between the main electrodes has an extremely high resistance and there is no current through the load resistance, apart from a negligible small leakage current.

In Fig. 1, the control switch is closed with the result that the control electrode becomes positive with respect to the main electrodes. The current which flows in the

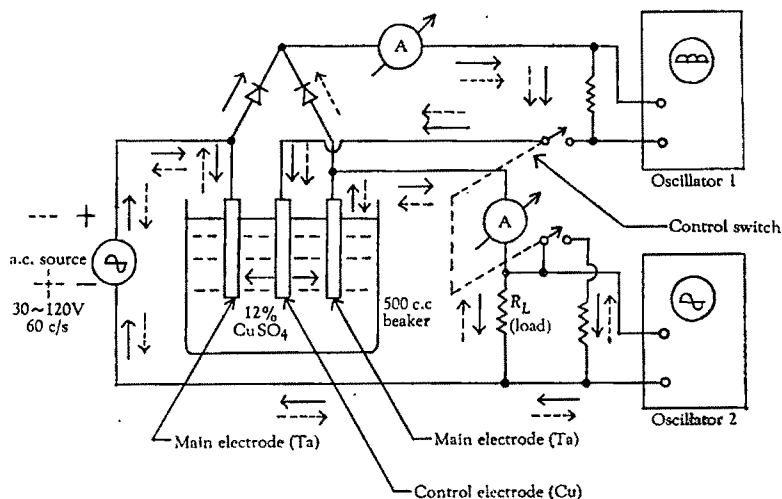


Fig. 1. Experimental apparatus.

circuit is shown by solid and dotted arrows in Fig. 1. Copper is deposited at the surfaces of the tantalum electrodes and the resistance between the main electrodes is decreased, so that the a.c. current through the load resistance is increased.

After the copper is deposited on the main electrodes, the current flows between them even when the control switch is open. The current between the main electrodes maintains its initial magnitude until the deposited copper is removed.

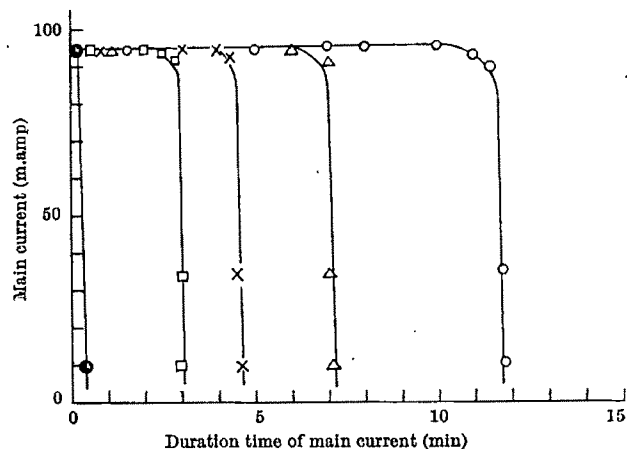


Fig. 2. Time switch characteristics. Temperature of electrolytic solution, 20°C. Time for which control switch keeps closed: ●, 1 sec; □, 2 sec; ×, 3 sec; △, 4 sec; ○, 5 sec.  $R_L$ , 100 V, 10 W lamp;  $R$ , 100 V, 100 W lamp; control current, 0.93 amp; a.c. source, 100 V.

The duration of the current between the main electrodes (main current) depends on the time for which the control switch is closed. Fig. 2 shows the relation between the duration of the main current and the time for which the control switch is closed. Fig. 2 also shows that this electrochemical device has the characteristics of a time switch. This type of time switch is extremely simple to produce in comparison with mechanical time switches.

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<sup>1</sup> Yamaguchi, K., *Oyo Buturi*, 32, 344 (1963).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, January 16

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5 p.m.—Dr. J. R. Norris: "The Use of Insect Pathogens for the Control of Pest Species".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "Laboratory Work Policy" opened by Dr. K. R. Sturley and Mr. A. D. Collop.

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—The Right Hon. Viscount Watkinson, P.C., C.H.: "Industry" (first of four Cantor Lectures of "Some Aspects of the U.S.A. Today").

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Prof. R. Y. Jennings and Dr. P. E. Kent: "Sea Bed Discoveries and International Law".

## Tuesday, January 17

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.20 p.m.—Dr. W. K. Taylor: "The Memory of a Learning Machine".\*

INSTITUTION OF CHEMICAL ENGINEERS, SOUTH EASTERN BRANCH (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Annual General Meeting. 5.30 p.m.—Paper by Mr. E. E. Charlton.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. E. Green: "Assessment of Sensing Channels for High-Integrity Protective Systems".

UNIVERSITY OF LONDON (at the Institute of Child Health, Gullford Street, London, W.C.1), at 5.30 p.m.—Dr. A. D. Bangham: "Lipid Structures and Cell Membranes". (Second of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

UNIVERSITY OF LONDON (at the Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1), at 5.30 p.m.—Prof. O. Eränkö (Helsinki): "Histochemical Aspects of Catecholamines and Cholinesterases".\*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-Acoustics GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "Radio Microphones". Speakers: Mr. M. L. Gayford, Mr. G. R. Pontzen and Mr. R. W. Swain.

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (joint meeting with the Agriculture Group, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "Crop Production for Processing".

## Wednesday, January 18

GEOLOGICAL SOCIETY, VOLCANIC STUDIES GROUP (at Burlington House, Piccadilly, London, W.1), at 2 p.m.—Annual General Meeting.

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 3 p.m.—"The Admiralty Chart".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. J. L. Monteith: "Local Differences in the Attenuation of Solar Radiation Over Britain". Mr. G. Stanhill, Mr. G. F. Hofstede and Mr. J. D. Kalma: "Radiation Balance of Natural and Agricultural Vegetation"; Mr. N. Thompson: "Short-range Vertical Diffusion in Stable Conditions".

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 5 p.m.—Dr. M. Caplin: "The Control of Tuberculosis".\*

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Discussion Meeting on "William Allen Whitworth and a Hundred Years of Probability" opened by Dr. J. O. Irwin.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. M. V. Wilkes, F.R.S.: "Multi-Access Computer Systems".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. G. N. Gould: "Cattle Breeding and Related Diseases".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Dr. B. J. Mason, F.R.S.: "The Generation of Cloud Electricity".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (in the Physics Department, Imperial College, London, S.W.7), at 6.30 p.m.—Mr. L. A. Tysall and Mr. J. Van Westrenen: "Evaluation for Electrodeposition of Recently Developed Water Soluble Epoxy Esters".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "Automatic C.H.N. Analyzers".

INSTITUTE OF FUEL (at Calor Gas (Distributing) Co., Calor Gas House, Key West, Slough, Bucks), at 7 p.m.—Mr. I. Carter: "LPG-Air Plants".

SOCIETY OF DYERS AND COLOURISTS (at the Waldorf Hotel, Aldwych, London, W.C.2), at 7 p.m.—Dr. T. J. Elliott: "Colour in Cosmetics".

## Thursday, January 19

SOCIETY FOR ANALYTICAL CHEMISTRY, AUTOMATIC METHODS GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Meeting on "Computer Applications in Analytical Chemistry".

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. P. M. Harris and Mr. D. V. Jackson: "Investigations into the Recovery of Niobium from the Mirna Hill Deposit"; Mr. L. D. Airey: "Introduction of the Cascade Method of Continuous-Retreat Open Stopping at Mufulira Copper Mines, Ltd., Zambia".

LINEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. J. G. Hawkes: "Botanical Exploration in Argentina".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. G. W. Mackey: "Group Representations and Number Theory".

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 19 New Cavendish Street, London, W.1), at 5.30 p.m.—Prof. W. D. Gill: "Carbon Ratio Theory as Applied to the British Isles".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. H. Nelson: "New Developments in High-Pressure Discharge Lamps".

UNIVERSITY OF LONDON (at the Institute of Child Health, Gullford Street, London, W.C.1), at 5.30 p.m.—Dr. M. F. Oliver: "Control of Hyperlipidaemia in Relation to Ischaemic Heart Disease". (Third of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

UNIVERSITY OF LONDON (at the School of Pharmacy, 29-39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. A. R. Battersby: "Biogenesis of Alkaloids". (Further lecture on January 20.)\*

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. R. Pollock: "The Changing Concept of Organism in Microbiology. I. The Cell as a Molecular Mosaic".\*

CHEMICAL SOCIETY (at the Royal Institution, Albemarle Street, London, W.1), at 8 p.m.—Prof. L. G. Sillén (Royal Institute of Technology, Stockholm): "How have Sea Water and Air got their Present Composition?".

INSTITUTION OF ELECTRICAL ENGINEERS (joint discussion meeting with the I.E.R.E. Computer Group, at the I.E.R.E., 8-9 Bedford Square, London, W.C.1), at 6 p.m.—Meeting on "Practical Applications of Computer Programmes in Electronic Engineering".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDINGS MATERIALS GROUP (joint meeting with the Institution of Highway Engineers, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. C. H. Peters: "The Maintenance of Concrete Roads".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Symposium on "The Clinical and Biochemical Aspects of Thalassaemia" opened by Dr. Ph. Fressas and Dr. E. R. Huehns.

## Friday, January 20

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. R. Pollock: "The Changing Concept of Organism in Microbiology. II. Inter-cellular Exchange of Nucleic Acid".\*

## Monday, January 23

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Mr. M. G. Hall and Mr. D. Weaver: "The Evaluation of Ordinary Shares Using a Computer".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Bryan Robertson, O.B.E.: "The Arts" (second of four Cantor Lectures on "Some Aspects of the U.S.A. To-day").

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 6.15 p.m.—Dr. M. C. Joseph and Dr. G. A. Miller: "The Special Problems of Paediatric Cardiology".\*

INSTITUT FRANÇAIS DU ROYAUME-UNI (at Queensberry Place, London, S.W.7), at 8.15 p.m.—Programme of French Scientific Films.\*

## Monday, January 23—Thursday, January 26

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2)—Conference on "Acoustic Noise and Its Control".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

HEAD (with suitable academic qualifications and preferably some Industrial experience) OF THE DEPARTMENT OF CHEMISTRY—The Clerk to the Governors, Woolwich Polytechnic, Wellington Street, London, S.E.18 (January 20).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT OF COMPUTATIONAL AND STATISTICAL SCIENCE—The Registrar, Liverpool University, Liverpool, 3, quoting Ref. 336 (January 21).

RESEARCH ASSISTANT (graduate in geography or physics and an interest in synoptic meteorology and/or historical climatology) in CLIMATOLOGY in the DEPARTMENT OF GEOGRAPHY—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (January 21).

RESEARCH ASSISTANT (with a good honours degree in geography or a related field and some experience in field surveying and mathematical geography) in the DEPARTMENT OF GEOGRAPHY to work on the bathymetric survey of Loch Leven, Kinross, under the direction of Dr. R. P. Kirby—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (January 23).

UNIVERSITY LECTURER (preferably with some interest in the biometrical use of computers) in the DEPARTMENT OF BIOMATHEMATICS—Prof. M. S. Bartlett, Department of Biomathematics, The University, 7 Keble Road, Oxford (January 23).

ASSISTANT (graduate in veterinary science, pharmacology, pharmacy or other appropriate discipline) in VETERINARY PHARMACOLOGY—Secretary of the University Court, The University, Glasgow (January 27).

LECTURER or ASSISTANT LECTURER (preferably with research interests in analysis) in PURE MATHEMATICS—The Registrar, University of York, Heslington, York (January 27).

LECTURER and ASSISTANT LECTURER in the MECHANICS OF FLUIDS in the FACULTY OF SCIENCE—The Registrar, The University, Manchester, 13, quoting Ref. 254/68/Na (January 28).

SENIOR LECTURER (with special experience of both the clinical and laboratory aspects of haematology) in HAEMATOLOGY—The Secretary, The University, Aberdeen (January 28).

LECTURER or SENIOR LECTURER in PATHOLOGY—The Registrar, The University, Leeds, 2 (January 30).

ASSISTANT LECTURER or LECTURER in the DEPARTMENT OF APPLIED MATHEMATICS AND MATHEMATICAL PHYSICS—The Acting Registrar, University College of South Wales and Monmouthshire, Cardiff (January 31).

LECTURER or ASSISTANT LECTURERS in ELECTRONIC MATERIALS in the SOLID-STATE ELECTRONICS GROUP of the DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, The University of Manchester Institute of Science and Technology, Manchester, 1 (January 31).

**RADIATION PROTECTION OFFICER** (physicist) throughout the University and Hospitals—The Secretary, The University, Aberdeen (January 31).

**SUPERINTENDING TECHNICIAN or SENIOR TECHNICIAN** (experienced in laboratory and workshop practice and supervision, and capable of detail design work and the manufacture and maintenance of laboratory equipment) in the DEPARTMENT OF CIVIL ENGINEERING, Ahmadu Bello University, Northern Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (January 31).

**SENIOR LECTURER** (pure mathematician) in MATHEMATICS—The Assistant Secretary, The London School of Economics and Political Science, Houghton Street, London, W.C.2 (February 2).

**LECTURER** (trained teacher, with an honours or higher degree in science, and successful experience in the teaching of science) in EDUCATION (Science Teaching) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, February 3).

**LECTURER or SENIOR LECTURER in PHILOSOPHY**—The Registrar, The University, Keele, Staffordshire (February 4).

**SENIOR LECTURER or LECTURER** (with a degree in mechanical engineering from a recognized university and experience in teaching or research or in industry) in MECHANICAL ENGINEERING at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 4).

**UNIVERSITY LECTURER** (preferably interested in studies of systematics and variation with reference to genetics, cytology, ecology, plant geography or the origins of cultivated plants) in BOTANY—The Administrator, Botany School, University of Oxford, South Parks Road, Oxford (February 5).

**CHAIR OF APPLIED MATHEMATICS** at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 6).

**READER** (with special interests in mathematical physics or in some other branch of applied mathematics) in MATHEMATICS—The Registrar, Beverley Farm, The University, Canterbury, Kent, quoting Ref. A.31 (February 7).

**LECTURER in CHEMISTRY** (Organic, Physical or Inorganic) at University College, Dar es Salaam, University of East Africa—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 9).

**CHAIR in HUMAN GENETICS in the FACULTY of MEDICINE**—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (February 10).

**LECTURER** (with full training in psychology, special training or experience in clinical psychology, and preferably holding a postgraduate qualification in clinical psychology) in CLINICAL PSYCHOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, February 10).

**CHAIRS (2) OF ELECTRONIC and ELECTRICAL ENGINEERING**—The Registrar, The University, Sheffield, 10 (February 11).

**LECTURER in NUCLEAR PHYSICS, and a LECTURER in THEORETICAL PHYSICS**—The Secretary, University of Lancaster, University House, Bailrigg, Lancaster, quoting Ref. L.197/A (February 11).

**CHAIR OF PSYCHIATRY**—The Registrar, The University, Sheffield, 10 (February 13).

**DIRECTOR** (with special interests in fisheries research) OF THE INSTITUTE OF MARINE BIOLOGY and OCEANOGRAPHY, Fourah Bay College, The University College of Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 15).

**ASSISTANT LECTURERS, LECTURERS or SENIOR LECTURERS in GEOGRAPHY** at University College, Dar es Salaam, University of East Africa—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 16).

**SENIOR LECTURER** (preferably with an interest in device physics, physical electronics or materials science) in ELECTRICAL ENGINEERING at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 17).

**CHAIR OF ANATOMY** at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Tasmania and London, February 28).

**CHAIR OF PHARMACEUTICAL CHEMISTRY or CHAIR OF PHARMACEUTICS** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 28).

**LECTURER in ZOOLOGY** at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (March 1).

**SENIOR LECTURER in GENETICS** at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (March 1).

**LECTURER in CLINICAL BIOCHEMISTRY in the DEPARTMENT of PATHOLOGY**, University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, March 15).

**CHAIRS (3) OF BIOLOGY** in the Research School of Biological Sciences in the Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

**RESEARCH PHYSICIST** (preferably with a higher degree and relative experience) in the PHYSICS DEPARTMENT to undertake investigation of electron scattering using a 400 kV electron diffraction camera with recently constructed energy analysis facility—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

**SENIOR DEMONSTRATOR** (with an honours or master's degree or equivalent qualifications) in the DEPARTMENT of PHYSICS, to take responsibility for the organization and maintenance of first, second or third year undergraduate laboratories—Prof. C. E. Challis, Head, Department of Physics, The University of Calgary, Calgary, Alberta, Canada.

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## SPILLING BLOOD WITHOUT MESS

THE way in which the University Grants Committee has set about rationalizing the schools of agriculture at British universities is an important innovation, but it is not yet time for the defenders of academic freedom to rush to the barricades. Distinguished ex-presidents of the National Farmers' Union may feel themselves threatened, but there is no reason why academics should interpret the plan to do something about agriculture as a sinister plot against the freedom of the universities. On the face of things, at least, the University Grants Committee has a good case. Although by resting the case for a simpler pattern of undergraduate teaching on a report of a committee which finished deliberating more than two decades ago the U.G.C. may be somewhat vulnerable, the size of the demand for graduate agriculturists is not really the crucial consideration. The strongest argument in favour of rationalization is that the twelve schools of agriculture in Britain produce an average of fewer than forty graduates each year, and many of these are labelled diversely as horticulturists, foresters and the like. The chances are high that there is a more efficient way of producing graduate agriculturists.

But if the case for rationalizing in agriculture is strong, it does not follow that the U.G.C. should be cheered on whenever it conceives of a plan to make the organization of universities simpler, cheaper or more efficient. On the contrary, it will need to be watched with extra care now that its efforts to mould the pattern of teaching have gone beyond the discouragement of unwanted innovation to the closing down of unwanted institutions. It is also, however, fair to recognize that the U.G.C. has some difficult problems to contend with. The need to make better use of the facilities which exist—people and plant—has if anything been aggravated by the frequently uncritical acceptance of the assumption of the Robbins Report that a bigger university population must mean more universities on the traditional pattern. In retrospect, there is bound to be a conflict between the reasonable wish of the existing universities, new and old, to teach what they want to teach, and the plain fact that teaching must be on a sufficiently big scale if teachers and students are to get the best out of it. One way out of many present difficulties would be the emergence of a regional pattern of federation for British universities, but that is obviously a distant goal or even just a pipe-dream. Of necessity, the U.G.C. is forced to act where opportunities occur.

But is it acting wisely? Is it sensible to persuade some universities to stop doing things some of them at least want to continue? Might it not be better to take a more positive line, and to encourage the concentration of teaching at a smaller number of centres

by channelling extra funds in the directions that seem most profitable? Experience elsewhere, and particularly in the United States, has shown how the establishment of what are called centres of excellence in particular fields can serve to force the pace of innovation and improvement. But they could also help to concentrate teaching in a smaller number of institutions. In the particular circumstances of higher education in agriculture at British universities, there may be reasons for thinking that the supply of graduates is adequate, but in the nature of things nobody can be sure that there is no room for valuable changes in the character of teaching. In other words, the U.G.C. might have done a service to agriculture and to its own reputation by looking for a more positive way of attaining its reasonable ends.

There remains the question of who should say what ends are worth attaining. In deciding on the rationalization of agriculture, the U.G.C. seems to have acted off its own bat. Prompted, no doubt, by the need to make the best of what funds it has to spend, the committee has found what seems to be a field in which economies are possible. No doubt the subject panel responsible for agriculture has helped enormously with drafting the detailed proposals circulated to the universities affected, but responsibility rests with the main committee of the U.G.C., and cannot easily be questioned. There are several risks in this procedure, not the least of which is that the U.G.C. may sometimes be wrong. In the long run, it should be for the universities as such, and not for the U.G.C., to say how best the pattern of university teaching should be made to meet the reasonable needs identified by the U.G.C. The one who pays the piper may call the tune, but it is not for him to decide just how it should be played. It does not help that the U.G.C. seeks advice from a panel of academics appointed for the purpose. Even if the U.G.C. were to relax its baffling unwillingness to make public the membership of its subject panels, the plain fact is that those who give advice must act as private individuals and not as representatives of the universities. What is needed is some procedure by means of which the U.G.C. could make a case for economy to the universities as a whole, and in which the universities could work out among themselves the best way of responding. This is yet another burden that must eventually be carried by the Committee of Vice-Chancellors and Principals.

## POLITICAL SCIENTISTS

THE activities of science and technology have not become political issues as quickly in Britain as in the

United States, so that there will be a wide welcome for the way in which the *Political Quarterly* has given over its spring issue entirely to a symposium on what it calls the politics of science. Even those who consider that the whole enterprise has somehow fallen short of the declared objective of helping "to reconcile the interests of science with the needs of society" will find pleasure in much of what the contributors have to say. Dr. H. M. Finneston is particularly worth reading on the relationship—or lack of relationship—between the universities and industry; in the present climate, what he has to say will make more friends than enemies. Yet the overall impression left by this symposium is of a miscellany of arguments advanced by different people from different points of view. Only Professor J. D. Bernal comes close to a definition of the kinds of questions on which political decisions can critically affect the health, or otherwise, of science and technology—the scale on which resources should be allocated to research and development, the importance that should be attached to defence research and the allocation of resources to higher education, for example—and he is compelled on several occasions to offer not answers but reasons why answers will be difficult to find. Nobody should be surprised if the readers of the *Political Quarterly* are persuaded by the experience that the concept of the politics of science is nebulous, to say the best of it.

To say this is not to suggest that the political implications of science and technology, and the scientific implications of politics, are unimportant or non-existent, but that the relationship between them is a good deal more complicated than is commonly allowed. Failure to acknowledge the full complexity of the interaction is the root of a good many fashionable fallacies. Thus there is no certain assurance that the best way of obtaining a greater stock of skilled engineers is to introduce more applied science (or more science of any kind) into the secondary schools. Although there is no doubt that science and technology are at the roots of the modern industries with which advanced societies create wealth, it does not follow that more research and development will bring more wealth. In Britain, for example, there is at least a case for saying that the comparatively slow rate of economic growth in the past fifteen years is not a consequence of deficiencies in technology but of a failure to exploit new techniques of management to the full. (On this reading, the brain drain is an empirical proof of a surplus of scientists and engineers, which is not as outrageous an interpretation as it may seem; scientists and engineers would not emigrate in such large numbers if their employers paid them enough to stay at home.) Certainly there is no case for thinking that all research and development is equally valuable, which implies that the gross yardsticks inevitably used for comparing the scale of scientific effort in different places may not be reliable. The *Political Quarterly* itself argues that research aimed at prestige and not economic benefit is a waste of money, which only goes to show that accountants have very little to say about the scale

on which efforts should be deployed. It follows that politicians should not be surprised if their reading of this symposium leaves them with the impression that the politics of science is not a simple monster but a hydra. That is not an illusion but the truth.

## HUNTING THE QUASAR

THE hunt for the quasar goes on. This week (page 239) it is Professor McCrea. Last week it was Professor McVittie, Dr. Stabell (*Nature*, 213, 133) and Dr. Verschuur (*ibid.*, 164). Next week—who knows? The only certainty is that it will be some time before there is a simple answer to one of the most teasing problems for several decades. At least a part of the reason for interest in quasars is that experimental information is so meagre that people are necessarily hard pressed to tell a good answer from one that is merely plausible. It is just within the bounds of possibility that one of these days somebody may point to some well established truth with such conviction that everybody will know what quasars are.

In the meantime, it is only seemly to recall that theorists are rarely so much at a loss as the cosmologists are now when confronted by the observational evidence on quasars. For four years it has been plain that quasars could not be written off as intense radio galaxies, if only because not all of them are radio sources. Their frequently gigantic red-shifts imply great distance, but their variability from year to year and even from month to month implies dimensions which are small (in light years or light months) and therefore, probably, a location somewhere within the local galaxy. How is this apparent conflict to be resolved? In the long run there will be data substantial enough to guide people more certainly to a conclusion. But it is also possible that there may be more to wring out of the observations already on record, which is why there is a continual search for some suggestive irregularity in the data—anisotropy, perhaps, or a significant departure from the relationship between the brightness of quasars and their frequency that would be expected from the hypothesis that the universe is always everywhere the same. This, too, is why people are searching for ways in which comparatively familiar cosmological bits and pieces could be made to behave like quasars. Some theories involve comparatively local quasars, and then have to deploy great ingenuity on an explanation of how spectral lines can be shifted towards the red, presumably as a relativistic consequence of a gravitational attraction, without the celestial object concerned being so dense that it prevents the escape of radiation. Other theories involve more distant objects. But nobody knows which model will succeed, and how soon. And nobody knows how radical a revision of accepted doctrines a decent model of quasars will require. If astronomers should fail to sleep for excitement—or even from anxiety—society should indulge them.

## NEWS AND VIEWS

### What Next at Naples?

A REORGANIZATION of the financial basis of the Naples Zoological Station is now being considered by the learned societies which at present help to support the station by means of annual subscriptions towards the maintenance of "tables" at the laboratory. Hitherto the annual subscription has been \$3,000 a table, for some reason translated into sterling as £1,200. Under the new scheme, subscriptions would be increased by roughly 25 per cent from June 1, 1967. In addition, it is now suggested that those institutions elsewhere wishing to contribute the equivalent of 50 million lire to the annual cost of the station would be entitled to a seat on the Administrative Council which is responsible for the government of the station through its present director, Dr. Peter Dohrn. By all accounts, these proposals are welcomed by the academies principally concerned, partly because it is considered that access to the station would be good value for money even at a higher rate, and partly because a stronger international financial basis of operations would help to ensure the continued international character of the work at Naples.

Altogether, rather more than 60 tables are maintained at Naples by institutions from fifteen countries. Various Italian sources between them make up the largest volume of support (19 tables), with Germany (12 tables) and the United States (10 tables) next in order. The United Kingdom, through the Royal Society, maintains four tables. Although the maintenance of a table is chiefly a way of shouldering a share of the cost of running the station, the academies which contribute funds are also responsible for allocating places at the laboratory to people from their own countries. Naturally enough, marine biologists of various kinds account for much of the demand on facilities at the laboratory, which include an elaborate system of fishing to recover specimens and an ample supply of Mediterranean water, but physiologists and others concerned to work with fresh marine material are also drawn there. So, too, are a number of scientists wishing to make use of the splendid library at the station. Short visits of a month or so are encouraged. The station will provide basic needs, but visitors have usually to bring special items of equipment from their own laboratories. In the past few years, reconstruction of buildings at the station has been supported by government sources in the United States, the Federal German Republic, the United Kingdom and the Italian Government as well as by the Volkswagen Foundation.

Although the proposed increase in contributions is probably well accounted for by increases of costs, it is also in some quarters regarded as a defence against the view, sometimes expressed in Italy, that the zoological station should be allowed to grow into an Italian equivalent of the national laboratories such as those at Plymouth and at Woods Hole, Massachusetts. On the face of things, of course, it is anomalous that one of the most renowned laboratories in Italy should have been founded by a German (the grandfather of the present director) and should be supported by and run

for scientists from a dozen countries. Elsewhere it is argued that the station is an invaluable centre for international collaboration, possibly unique. Its value in this respect is only likely to be enhanced by the siting of an international laboratory of genetics and biophysics at Naples.

The recent history of the zoological station has also been disturbed by what are sometimes described as "labour difficulties" among the technical staff. In part, these probably reflect some of the economic difficulties of carrying out advanced scientific work in the south of Italy, and recent visitors to the station speak well of the quality of the assistance which they have received. Another frequent visitor points out that troubles of various kinds are inseparable from Naples and are "always desperate, never serious".

### Squeeze on Universities

THE University Grants Committee has embarked on a programme of what it calls "rationalization in the teaching of agriculture at British universities". Although it has already provoked a brisk if predictable protest from three members of the House of Lords on the grounds that the national importance of agriculture has not been fully considered, the committee has probably fixed its eyes on a more distant target, and is probably interested to see whether it can work out procedures whereby rationalization could be carried out elsewhere in the academic spectrum.

In a letter to *The Times* on January 16, Lords Abergavenny, Cornwallis and Netherthorpe said that the universities of Leeds and Cambridge had been "invited" to give up their degree courses, and that the schools of horticulture at Reading and Nottingham are similarly threatened. These public signs of pressure seem to be the tips of a substantial iceberg. The U.G.C. says that it wrote before the beginning of the present academic year to three universities at which degree courses in agriculture were considered expendable, to a number of others at which there are schools of horticulture the independent existence of which is not thought to be essential, and to other universities where the present diversity of courses with titles such as "soil science" and "agricultural economics" is considered altogether too rich. As yet most of the universities concerned are still nursing the insult to their pride in silence. According to the U.G.C., all universities are keen on rationalization, but somewhere else than on their own premises.

The case for making a start on agriculture rests, in part, on the Bosanquet Committee which, at the end of the Second World War, considered that the production of graduates in agriculture from British universities might well be greater than the demand. The U.G.C. considers that the few deficiencies which the Bosanquet Committee pointed out have now been made good, but that the broad adequacy of production of graduates in agriculture remains. In any case, the argument goes, the plan is to concentrate teaching in agriculture on "fewer but stronger" schools. According to the U.G.C., there is no intention to reduce the numbers of graduates leaving the universities. Agricultural undergraduates have, however, been a declining proportion of the entire student body in the past decade. In 1964-65 there were 1,701 of them, or 1.5 per cent of the total, compared with 2.6 per cent

of the student population a decade earlier. The number of first degrees works out at around 450 a year, and the U.G.C. says that this is enough to satisfy not only the demand for trained agriculturists in Britain but that from overseas as well.

On the machinery for making these decisions, the U.G.C. says that the main committee must be held responsible for all decisions, right or wrong, although in this case the committee did consult the subject panel responsible for agriculture whose chairman is Sir Harold Sanders, now deputy chairman of the U.G.C. and who was professor of agriculture at the University of Reading from 1944-54 and afterwards chief scientific adviser to the Ministry of Agriculture. According to a spokesman of the U.G.C., the Committee of Vice-Chancellors and Principals has been informed of the scheme for rationalizing agriculture, though it has not been embarrassed with the details.

## Cost of Learning

THANKS to Governor Ronald Reagan, people far from California now know that his state offers its citizens free education at college and university. Not that Mr. Reagan, a fortnight in office, is proud of California's century-old record of free university tuition. He wants to keep his Republican campaign promise to reduce government spending and proposes to cut the university's \$240 million annual budget by 10 per cent. To recoup these losses, he suggests that the university—with nine campuses and 80,000 students—and the network of state colleges—with about 127,000 students—should charge tuition fees of several hundred dollars a year. If this were to happen—and it is by no means certain that it will—the new fees could keep away as many as 20,000 new students next autumn. In turn, the state's admirable Master Plan for Higher Education under which the university and college network expand with the population would be brought to a halt.

To be accurate, higher education in California is not absolutely free of charge. Residents of the state pay about \$240 a year at the university in various required fees. Those from outside its borders pay almost as much as they would at a private university—about \$1,000. The state's Finance Director, who seems to be the Iago of the plot, thinks that perhaps too many Californians are going to university just because it is so cheap. Any graduate of a high school or other secondary school is eligible for the university if he has a *B* average. The state colleges are even more catholic: they take in secondary school graduates with a *C* plus average. These colleges might be harder hit than the university by an imposition of tuition fees, for their intending students presumably are more easily discouraged than the more gifted ones who are hell-bent for university.

With the prospect of political storms on the way, people are already wondering if the university's branch at Berkeley will be able to hold its teaching staff. The mood of the undergraduates at Berkeley, following the celebrated riots of recent years, is said to be restless, even anarchic. The Reagan assault on the university's independence could set off another round of mass demonstrations, sit-ins and strikes which could in turn drive away those young teachers looking for a place to do serious and serene work.

While California's problems are exotic, state-aided colleges and universities throughout the United States are finding themselves forced to raise their traditionally low fees. Some of these are still surprisingly modest, even by British standards. At the University of Texas, a Texan student pays only \$100 a year. Someone from outside the state must pay \$703. New York's prestigious state university at Cornell is more expensive—about \$600 for a New Yorker, about \$1,000 for outsiders. The University of Hawaii generously charges the same fee—\$232.50—to those from the islands and those from the mainland. Tuition charges are relatively low for outsiders at the University of Arkansas (\$470), the University of Ohio at Bowling Green (\$550) and Louisiana State University (\$620). The cost of tuition at Harvard and Yale works out at \$1,800 a year with roughly the same amount for board and lodging. Only the universities of Idaho and Connecticut have somehow been able to resist the temptation—or the necessity—of charging for higher education.

## British Fees Go Up

IN Britain, too, tuition fees are to be increased in the interests of the economy, and, it is claimed, the balance of payments. The Department of Education and Science, which says that the tuition fees for university students are about £70 per year, and for technical college students £30-£40 per year, intends to increase them to £250 and £150 respectively. A furore has been caused because the increase is to apply only to students from overseas. Foreign students already embarked on courses in Britain will not be quite so brutally treated; their fees are to increase by £50 per year.

Critics of the proposals say that more than 70 per cent of the foreign students in Britain come from developing countries, and will have the utmost difficulty in finding the extra money. Some, who arrive without qualifications at British technical colleges to qualify themselves for British universities, will be faced with increases amounting to £760 over five years. Critics also claim that the saving the government hopes to make, £5m, is pure speculation, and that the moves made by the government to offset the additional cost will apply only to a minority of students already in residence, and not at all to those arriving for the academic year 1967-68. A more philosophical point is that the assistance will only apply to students sponsored by governments and government organizations, and not to those who come on their own initiative.

Abroad, the proposals may seem to be a crude way of summarily reducing immigration into Britain and assistance from Britain to developing countries. Certainly the fuss seems hardly worth the putative failing; the government may well be shocked by the intensity of the reaction, which shows no signs of dying down. As in California, students are likely to make the most noise, but they may well be supported by some academics.

## Forward by Degrees

SUBSTANTIAL progress is reported by the Council for National Academic Awards for its second year of operation up to September 30, 1966. The Council has considered 104 courses, and has approved 66, most of them honours degree courses. It has also announced

the regulations for higher degree courses, leading to the M.A., M.Sc., Ph.D. and M.Phil. degrees. The first full-time postgraduate course, for an M.Sc. in Applied Solid State Physics at Brighton College of Technology, has been approved, and others are in the pipeline. The Council has also set up several new subject boards as well as a committee to consider proposals for degree courses in education from colleges of education and technical colleges. It has also adopted its own academic dress, and opens its report with a drawing of its armorial bearings. It is beginning to look like an established institution.

The Council has clearly been thinking deeply about the White Paper setting out the plan for polytechnics and other colleges, but the new report says little about this. It is content to record the assumption that the polytechnics will get the staff, accommodation and equipment they need, and its concern that the polytechnic policy of concentrating resources will not prejudice students following C.N.A.A. courses. The Council has obviously been greatly encouraged by the recommendation of the Committee of Vice-Chancellors and Principals, which recommended that universities should regard holders of C.N.A.A. degrees on the same footing as holders of university degrees.

## More from Medlars

THE United States Library of Medicine has stored on magnetic tape the vast quantity of information which is used each year to produce the *Index Medicus* and has for some months been disseminating this information through the Medical Literature Analysis and Retrieval System (MEDLARS). One way in which this can be done is in the form of specialist bibliographies covering topics of interest to individuals or groups of research workers, and this service has now become available to British scientists through the National Lending Library.

The bibliographies are of three kinds. Some are firmly established, such as the *Index of Rheumatology* and the *Index to Dental Literature*; these are published regularly by the National Library of Medicine and cost money. Others are still at an experimental stage, and are being distributed free to selected groups of scientists in return for 'feedback' in the form of information and criticism. The third type of bibliography is tailored to suit the needs of the individual, who defines his sphere of interest by means of a word profile which can be fed into a computer. The computer then produces a list of titles and bibliographical details, which are set in type by GRACE (Graphic Arts Composing Equipment). The system demands little of the research worker; all he has to do is to produce a word profile (some librarians, trained by the National Lending Library, can assist in this) and send it to the NLL, which will send free of charge a bibliography covering research in his area of interest since 1963.

This open-handedness can scarcely last for ever. The cost of the input end of the system is carried by the Department of Health, Education and Welfare in the United States, while in Britain the running costs of the system are paid by the Science Research Council. When the experimental period ends in 1968, it will have to be decided whether the benefits of the system justify its cost. So far British experience indicates that it is a useful service; one worker said that of the

295 titles selected by the computer 59 were useful to him. To have found these 59 papers without the computer would have meant reading 17,500 references. It also seems that however well read an individual may be, the computer will turn up titles he had not come across before. Whether the costs of the service are justified will only be known when some estimate of its usefulness can be made; British medical research workers are therefore being urged to make use of the service, and then to tell the National Lending Library what they think of it.

## Synonyms for Chemists

CHEMISTS should be less frequently at a loss for words now that the Chemical Abstracts Service of the American Chemical Society has published the word guides which are intended to help those who would extract information from the reels of magnetic tape on which information about the titles of chemical articles in the literature is being stored. The lists now available are more than mere thesauri in the sense of Roget, for the groups of words associated with each main entry in the lists are allocated to categories intended to give some sense of the character of the relationship between words in a pair. Two word guides are available, one for use with the *Chemical Titles* tapes and one with the magnetic tapes called *Chemical-Biological Activities* on which are stored the titles of articles in some 600 periodicals and short abstracts as well.

As well as straightforward synonyms for words, the guides include terms which are both broader and narrower, together with some which are merely close relations. For example, the chemist who looks up "Hamster" will find that the terms "Animal" and "Rodents" are both broader terms, that "Chinese Hamster" is a narrower term, that "Rat" is a related term and "*Cricetus cricetus*" is a synonym. The importance of the word guides stems from the way in which machine retrieval systems require the potential user to specify the area of his curiosity by means of words or word stems. Computers will pick out titles in which the specified words appear, either singly or in combination with others. One obvious difficulty is that of anticipating at the beginning of a search the variants which may be substituted for particular words by different authors. Such is the enthusiasm of the Chemical Abstracts Service for machine retrieval that it is presumably only a matter of time before the word guides themselves are incorporated into the machine programmes. In the meantime, etymologists will be glad to see that the word guides take a stern line on a great many malpractices. Thus those who would use the word "level" instead of "concentration" will get no encouragement from the first editions, at least.

## British Abortion Law

MR. DAVID STEEL's Medical Termination of Pregnancy Bill, 1966, which reached the committee stage of the House of Commons this week, seems assured of getting through the House in one form or another. The Bill is not quite as radical as some of its opponents (and, indeed, some of its supporters) have maintained. It does, however, extend somewhat the grounds for abortion that are already recognized in case law. It would legalize abortion if, in the opinion of two



doctors, the pregnancy endangers the life, health or well-being, whether physical or mental, of a pregnant woman, taking account of the patient's total environment. Other grounds for abortion in the Bill are substantial risk of deformity in the child, severe overstrain of the pregnant woman's capacity as a mother, and her mental deficiency, or pregnancy as a result of rape.

A number of medical organizations raised various doubts about the Bill in its original form. The British Medical Association objected to the use of the word "serious" to qualify the risk to life and of "grave" qualifying the injury to health in the original form of the Bill. Both these words could provide lawyers with talking points and lead to unforeseen interpretations. These objections were accepted by the sponsors of the Bill, who also inserted the phrase "account may be taken of the patient's total environment, actual or reasonably foreseeable". The BMA also wanted to alter some of the clauses relating to the method of notification of abortions, but the sponsors of the Bill have not taken these into account as yet. Doctors themselves are not entirely happy that the Royal College of Obstetricians and Gynaecologists is pressing for abortions to be carried out only by consultant gynaecologists. The BMA does not entirely agree with the RCOG because there are parts of Britain where consultant gynaecologists are not readily available, but it does want the operation to be performed by a consultant, or a doctor of equivalent status.

Most of the concern about the present form of the Bill centres around the "doctor's dilemma" that it might introduce. It is thought in many quarters that the Bill as it stands asks doctors to decide whether or not to abort using criteria that fall outside their special competence. This is the case when they are asked to consider the woman's "capacity as a mother" apart from her life and health. In practice these clauses may prove difficult if not impossible to put into effect, and as has been pointed out the ethical, not to speak of the legal, problems that could arise might well be considerable. Other doctors think that their role is to decide on social questions as well as on purely "medical" matters, but there is clearly considerable disagreement in the profession. One obvious pitfall to avoid is that which the Swedish law on abortion has fallen into—there the process of obtaining an abortion is so wrapped up in red tape that pregnancy is frequently well advanced before a potential mother can get permission. Difficulties like these are probably unavoidable as long as decision making rests with society and not the pregnant woman.

## Ministry Magazine

As if to celebrate the bloodless take-over of the Ministry of Aviation, the Ministry of Technology has produced the first issue of a new publication. Called *New Technology*, the magazine is intended to reach a monthly circulation of 100,000, and will be sent out free to industrialists, technologists and other interested parties. Launching the new magazine, which will cost the ministry £22,000 per year, Minister of Technology Anthony Wedgwood Benn said that it was hoped to improve communications between the ministry and those on whom its work impinges. It in

no way implied a criticism of the existing technical press, he said, and they might be happy to use it as a press service. Those interested in receiving the magazine should apply to the Central Office of Information.

The minister also spoke of the need to focus the attention of the universities on to industrial problems. This could be done through the institutes of advanced technology which were being set up in the universities; the first, in machine tool technology, is at the University of Strathclyde. Another is expected to be established quite soon, but it is not yet known where it will be.

## Civil Service Promotions

THIS is the season at which the procedure in the British civil service for deciding which scientists should receive special merit promotions gets under way. Very soon, directors of establishments will be invited to recommend the names of those senior people likely to be successful candidates for special promotion. Later in the year names will be sifted by government departments and their chief scientists and by the Civil Service Commission. Eventually a short list of candidates will be interviewed by a panel under Sir Frederick Brundrett and with members from outside the civil service. Twenty or thirty people will be promoted to a higher grade on the strength of their scientific attainments and in the hope that they will continue creative work within the public service. This year this procedure is likely to be looked at with especial care because the Fulton Commission on the civil service is bound among other things to be concerned with the way in which scientists function in public life.

This special merit promotion scheme in the British civil service goes back to 1947 and is a consequence of the Barlow Report on the civil service. At that point it was recognized by the Treasury to be imprudent that scientists could be promoted only by being shouldered with the extra administrative responsibility normally associated with higher rank. Merit promotions can now be awarded to civil servants with the rank of principal scientific officer and above, and the men and women concerned are carried on the books of the establishments outside the normal complement agreed with the Treasury. In practice, those who hold special merit promotions are expected to continue actively with scientific work, and indeed the Civil Service Commission takes care to see, by means of a review every five years, that establishments do not use the device as a means of increasing the number of senior administrators on their books.

Over the past two decades, something like a score of scientific civil servants a year have been awarded special promotions. More recently, other governmental organizations not strictly within the civil service, such as the Agricultural Research Council, the Atomic Energy Authority and (now) the General Post Office, have been included in the scheme, so that the number of special promotions has increased to something like thirty a year. Surprisingly there seems to be no danger that the promotions will be institutionalized and made to seem to civil servants as automatic accolades for senior people. At least a part of the explanation is the care with which candi-

dates are sifted. The fact that outside assessors, many of them academics, help in the selection of candidates gives the system objectivity. The awarders seem also to have been fortunate in the people chosen so far, who have been acknowledged by their colleagues as outstanding research workers. For the civil service one important consequence is that the scientific establishments are able to compete on more even terms with the universities for the services of bright people.

## Ritual and Behaviour

WHEN spiders are given lysergic acid they construct webs of more than usual regularity; they become, like man in a similar situation, withdrawn from external stimuli so that their perceptive awareness is reduced, and they cease to adjust their webs to the irregularities of the surroundings. This example is used by Sir Julian Huxley in his introduction to *A Discussion on Ritualization of Behaviour in Animals and Man* (*Phil. Trans. Roy. Soc., B*, 251, 247; 1966) to illustrate the importance of perception in ritualized behaviour. Psychedelic substances, as well as schizophrenia, distort perception to the extent that contact with external objects is prevented.

Ritualization can be described broadly as the adaptive formalization of behaviour through the influence of natural selection. It seems to have occurred in animals as a means of improving signalling to other individuals; to serve as a more efficient stimulator of more efficient patterns of action in others; to reduce intra-specific damage, and to serve as sexual or social bonding mechanisms. Sexual display and threat activities act as signals to other animals, and stimulate a particular pattern of reactions. Threat can reduce damage; in some lizards threat is expressed by size-exaggeration and fighting never occurs. The antlers of deer are so constructed that fights are rarely fatal, and are so large that combatants are often deterred. Social bonding rituals include grooming, particularly in non-human primates. In fishes, too, the removal of harmful growths from one species by another benefits both. In many mammals, play is a socializing activity, for example in young monkeys, and in lions where both adults and young are involved. During the evolution of vertebrates, ritualization has tended more towards the maintenance of efficient bonding and ceremonies have become more elaborate, with individual learning playing an increasingly important role. Particularly in the primates, ritualized behaviour resembles to some extent that of humans.

## Graduates in Industry

by our Special Correspondent

A FREQUENT criticism of British industry is that it fails to attract graduates in science and technology, or to make good use of those it does attract. The Swann and Willis Jackson Reports (*Nature*, 212, 227; 1966) on scientific manpower implied that the universities are to blame for absorbing too many of their own graduates instead of dispatching them into the world with a determination to use their talents in a directly productive way. One reason is certainly the rapid expansion of the universities, which has enabled them

to find places for increasing numbers of graduates before producing a comparable increase in their own output, but another may be that industry is unable to present itself as an attractive alternative. *Nature* has recently been talking to twelve science graduates (all men) from the universities of Oxford and London with one or two years experience of industry, and asking them why they joined, and whether they regret their decision. The names were supplied by the two university appointments boards, and the companies included breweries, computer and chemical companies, and companies in the motor industry. The only proviso is that most of the companies were large.

Very few of the group questioned admitted to any regrets. "Industry is far more interesting from the inside than it looks from the outside" was a typical comment. An Oxford chemist compared the flexibility of industry with the rigidity and absence of a defined career structure in university life—"In a big company there is bound to be a job one enjoys doing", he said. None of them expressed any desire to return to academic life, although opinions of universities varied. One graduate, working for a computer company, said that he thought academic life too dedicated, but another, an Oxford graduate also in a computer firm, remarked that "university centres around fun, industry around work". In general they neither praised nor criticized university life, and seemed not to have felt the conflicting claims of industry and the universities. For some, of course, the university option was closed because their degrees were insufficiently good. Academic life, often seen as the prize awarded for a first class degree, is unlikely to be considered by those who feel, rightly or wrongly, that they are going to get a third. Industry, which cares least what degrees its recruits have, is likely to be the beneficiary; this may explain why so few seemed to have felt a conflict. Discouraged early in their university careers, people are likely to see their degree courses as a way of qualifying themselves for something else as soon as possible.

Most of those questioned believe that scientists have as good a chance of reaching the top in industry as anyone else. The widely canvassed claim that British industry discriminates against scientists found no support. While this is unsurprising from a group of industrial scientists, there is some evidence that their optimism is justified. A study carried out in north-west England by D. G. Clark of the University of Manchester Institute of Science and Technology<sup>1</sup> shows that 80 per cent of all graduates in managerial positions are scientists. There is, in fact, a broad correspondence between the proportions of graduates from different disciplines going into industry and the proportion in managerial positions, since an earlier report by P.E.P. (ref. 2) showed that at the beginning of the fifties about 78 per cent of the graduates entering industry were scientists or technologists. A more legitimate criticism might be that industry fails to employ enough graduates generally (only 35 per cent of the managers in the Manchester study were graduates). It may be that scientists are still under-represented in the boardrooms, either because the wave of scientific managers has not reached this level yet—there are more scientists among younger managers than among older—or because of the complicating factor of background. Public schools, for instance, produce fewer scientists but more managers.

Nothing in the replies given by graduates casts doubt on these findings. Most graduates thought scientists could get to the top, but as one put it, "It depends how you define the top". He added that as a scientist he preferred to be managed by scientists, even by those who were not particularly good managers. Several expressed approval of a flexible management structure, enabling research scientists to move into management or to stay in research without loss of salary if they preferred.

The graduates were also asked why they thought more scientists did not enter industry. Two factors were blamed—prejudice in the universities, and the failure of industry to do more to improve its image. None thought it would be easy for industry to correct the situation. One man, a research chemist in a chemical firm, suggested that people in universities underestimate the quality of the work done in industrial laboratories because the companies are reluctant to publish results in scientific journals. This also makes it harder for an industrial scientist to gain recognition for his work, a most important consideration. Another, working for a computer company, seemed to feel that the task of presentation was hopeless. "The only way to find out about industry is to join it", he said. If it is true that students decide long before their final year to join industry, industry might be better advised to spread its recruiting campaign rather than concentrating on the final year students. What is necessary is to reach students at the point of decision, and the assumption that this occurs in the last year of a university course may not be justified.

One point which did emerge strongly was the importance of interviews. "It's the first interview that counts", said one London graduate in the motor industry. Companies clearly make no impression by sending unimpressive personnel to conduct interviews. Appointments boards, too, have an important role to fill. Several graduates admitted that their choice of company was decided entirely by what the appointments board had said. "At the time I thought they were wrong", said one Oxford graduate, "but now I think they were right."

<sup>1</sup> Clark, D. G., *The Industrial Manager, His Background and Career Pattern* (Business Publications, London, 1966).

<sup>2</sup> Political and Economic Planning, *Graduate Employment* (George Allen and Unwin, Ltd., London, 1956).

## Helix Stability

by a Correspondent in Molecular Biology

THE fact that helical RNA is more stable than DNA, and the similar effects observed in their synthetic analogues, have been puzzling questions for some time. A substantial new study by Ts'o, Rapaport and Bollum (*Biochemistry*, **5**, 4153; 1966) presents many new data and offers a general explanation of the stability differences. Bollum's preparations of polydeoxyribonucleotides have made possible a direct comparison of their optical properties and thermal stability with those of the corresponding ribo-polymers, and the generality of the stability differences has been confirmed.

The properties of single stranded poly-dA, poly-rA, poly-dC and poly-rC have been examined. Poly-dC, by all the criteria which can be applied, shows negligible structure in solution at neutral pH, while poly-

rC is known to be substantially in a conformation involving stacking of the bases on one another. The acid forms of the two polymers (thought to be two stranded) also show differences, particularly in their pH stability: the transition to the single stranded form occurs at lower pH by 1.5 units in poly-rC, and a difference in this respect is also found in the polyadenylic acids. Again, whereas poly-rT is known to enter an ordered (most probably two stranded) conformation under the right conditions, very little structure is observed in poly-dT.

Striking differences in optical rotatory dispersion between the various single and double stranded polymers are observed. The prominent system of Cotton effects seen in neutral poly-rA in the 260–280 mμ region are all but missing in poly-dA, although at shorter wavelengths the discrepancies are less. The hypochromicities of the two polymers are moreover comparable. The acid forms also show large differences. From the theory of optical activity in interacting systems of chromophores it is possible tentatively to infer that the bases in the ribose polymer are more skewed relative to their neighbours. Ts'o *et al.* draw a similar conclusion from optical rotatory dispersion studies of the four possible base-paired complexes of ribo- and deoxyribo- poly-A and poly-T. The distortion in the ribose containing strands is attributed to hydrogen bonding between the 2'-sugar hydroxyl (missing in deoxyribose) with the 2-carbonyl group of cytosine and uracil, and the N-3 ring nitrogen of adenine, and in particular it is suggested that this is also the origin of the differences in stability. Ts'o *et al.* have collected a considerable mass of supporting evidence in favour of such a scheme, including, for example, consideration of the positions available for attack by methylating reagents in native and denatured DNA and poly-dA, and many data indicating the existence of a similar hydrogen bond in mononucleotides.

At the same time, it is important that other types of hydrogen bond have been suggested as stabilizing agencies in polyribonucleotide helices. Models of helical RNA have been reported as showing that the 2'-sugar hydroxyl group can form good hydrogen bonds either with the ether oxygen of the neighbouring ribose or with the adjacent phosphate group. Arnott *et al.* have, however, recently stressed that no intramolecular hydrogen bond from the ribose hydroxyl is compatible with the X-ray data on crystalline helical RNA. The absence of X-ray data on single stranded helices leaves the issue in some doubt but, unless there is a configurational change during the transition from the crystalline state to dilute solution, there must be some other source of stabilization of ribo- relative to deoxyribo-helices.

Recent work by Brahms and Sadron (*Nature*, **212**, 1309; 1966) advances evidence based on the behaviour of polyinosinic acid, and the aggregation of monomeric 5'-guanylic acid, that hydrogen bonding, at least in these systems, involves the ribose hydroxyl and the phosphate group. The best evidence to implicate the latter derives from the rather narrow pH range, centred on the  $pK_a$  of the secondary phosphate group, over which helical guanylic acid aggregates will form. X-ray analyses of polynucleotide structures, especially those involving single strand stacking, will be awaited with interest.

## University News:

## University College of South Wales and Monmouthshire

DR. F. A. ROBINSON, managing director of Twyford Laboratories, has been appointed to the honorary post of professorial fellow in the Department of Biochemistry. This is the first post of its type to be created within the university, and it follows the recommendations made by the Robbins Committee on Higher Education for developing co-operation between industry and the universities.

## Appointments

THE following have been appointed vice-presidents of the Royal Society for the year ending November 30, 1967: LORD FLECK, treasurer of the society and formerly chairman of Imperial Chemical Industries, Limited; SIR ASHLEY MILES, biological secretary of the society and director of the Lister Institute; PROFESSOR M. J. LIGHTHILL, physical secretary of the society and Royal Society research professor at Imperial College of Science and Technology, London; PROFESSOR H. W. THOMPSON, foreign secretary of the society and professor of chemistry in the Physical Chemistry Laboratory, Oxford; PROFESSOR G. E. BLACKMAN, Sibthorpian professor of rural economy at the University of Oxford and director of the A.R.C. Unit of Experimental Agronomy, Oxford; DR. S. G. HOOKER, technical director (aero), British Siddeley Engines, Limited; DR. R. D. KEYNES, director of the Institute of Animal Physiology, Babraham, Cambridge; DR. N. KURTI, reader in physics, Oxford.

MR. R. H. A. SKJÖLDEBRAND, technical assistant to the managing director, and project leader of the Oskarshamn Nuclear Power Group, Stockholm, has been appointed special assistant to Dr. S. Eklund, director general of the International Atomic Energy Agency.

## Announcements

THE Trustees of the Lady Tata Memorial Trust invite applications for fellowships and scholarships for research on leukaemia, in the academic year beginning October 1, 1967. Candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukaemia will be eligible for consideration, and the awards are open to suitably qualified investigators of any nationality, working either in their own institutions or in other centres abroad. Further information and forms of application can be obtained from the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London, S.W.3. Applications must be submitted before March 31.

THE U.S. Atomic Energy Commission has approved 64 educational assistance grants totalling \$517,431 to 57 colleges and universities through its Division of Nuclear Education and Training. Twenty-one grants amounting to \$109,310 are in the life sciences group, and 43 grants totalling \$408,121 are in physical sciences and engineering. The primary purpose of the awards is to assist educational institutions in their advanced science curricula.

THE Geological Society of London has made the following awards for 1967: *The Wollaston Medal* to Sir Edward Bullard; *The Lyell Medal* to Professor W. Q. Kennedy; *The Murchison Medal* to Professor T. S. Westoll; *The Bigsby Medal* to Professor F. H. T. Rhodes; *The Wollaston Fund*, an award of approximately £30, to Mr. A. H. Stride; *The Murchison Fund*, an award of approximately £30, to Mr. F. W. Dunning; a moiety of the *Lyell Fund*, amounting to approximately £10, to each of the following: Mr. G. A. Goodlet, Mr. A. C. MacL. McKinlay and Mr. D. J. Shearman.

DR. W. T. DEAN, of the British Museum (Natural History), has been awarded the silver medal of the Liverpool Geological Society, in recognition of his contributions on the

geology of the Ordovician of the Welsh Borderlands, North West England, and Montagne Noire, France.

THE U.S. Atomic Energy Commission has presented its Distinguished Service Award to the following members of its headquarters staff for their contributions to U.S. nuclear energy programmes: E. J. BLOCH, deputy general manager; D. L. CROWSON, director of the Division of Military Application; G. F. QUINN, assistant general manager for plans and production.

MR. M. W. JENSEN, manager of engineering standards and chief of the NBS Office of Weights and Measures, has been awarded the Edward B. Ross Award, presented annually by the National Bureau of Standards, U.S. Department of Commerce, for outstanding achievement in the development of standards of practice.

DR. L. M. BRANSCOMB, chief of the NBS Laboratory Astrophysics Division at the Joint Institute for Laboratory Astrophysics in Boulder, has been awarded the 1966 Samuel Wesley Stratton Award by the National Bureau of Standards, U.S. Department of Commerce. The award, which consists of a bronze plaque and a \$1,500 honorarium, was presented to Dr. Branscomb in recognition of his "outstanding contributions and sustained leadership in the field of atomic and molecular physics".

DR. HELEN S. HOGG, professor of astronomy and research associate in the University of Toronto's David Dunlap Observatory at Richmond Hill, has been awarded the Rittenhouse Silver Medal.

THE University of Liverpool has arranged a series of safety lectures to be given on Wednesdays during the Lent term, at 2.30 p.m., in the University Lecture Theatre Building. The lectures will deal with general safety principles as well as their application to specific types of laboratory.

A SYMPOSIUM on "Structural Analysis of Polymers" will be held in the University of Bradford during February 24-25. Further information can be obtained from The Registrar, University of Bradford, Bradford 7.

A SYMPOSIUM on "Modern Developments in Food Preservation" will be held at the Grimsby College of Technology during March 20-21. Further information can be obtained from Dr. B. J. Boffey, Grimsby College of Technology, Nuns Corner, Grimsby, Lincolnshire.

A SYMPOSIUM on "Nuclear Magnetic Resonance—A Design for the Future" will be held at the Hatfield College of Technology during March 1-2. Further information can be obtained from Dr. F. J. Swinbourne, Department of Chemistry and Biology, Hatfield College of Technology, Hatfield, Herts.

ERRATUM. In line 17 of the article "Variation in a Strain of Classical *Vibrio cholerae*", by K. N. Neogy and A. C. Mukherjee, in *Nature*, 212, 303 (1966), the words polymyxin (50 µg/disk) should read Polymyxin (50 U/disk).

## CORRESPONDENCE

## Stonehenge

SIR,—I have read with much interest a number of articles on Stonehenge as a possible computer. It occurs to me that it might be just possible that the circle of Aubrey Holes is a notation indicating a FORTRAN IV "DO-LOOP", the number of holes being the terminal value of the index.

Yours, etc.,

JOHN STANLEY

Professor of Population Dynamics,  
McGill University,  
Montreal 2, P.Q.

(For *Night Sky* in February see page 229)

# The Stereotypical Scientist

by  
L. HUDSON

Research Centre, King's College, Cambridge

Do schoolboys regard the scientist as dull? A recent study may be relevant.

At the age of 15 or 16 most able English schoolboys face a choice between the arts and sciences. Boys who specialize in science are known to differ both intellectually and in personality from those who specialize in the arts<sup>1,2</sup>. Scientists, for example, have been found to be reticent in their expression of emotion<sup>3</sup> and to deal with personal relationships in an inhibited fashion<sup>3</sup>.

Further evidence suggests that there exist certain stereotypical beliefs about the personal characteristics of arts men and scientists. American students see the scientist as highly intelligent and masculine, but lacking in sensitivity and concern for people; as gaining satisfaction from his work, but having a shallow personal life<sup>4</sup>. Previous research on this topic, however, suffers a central weakness. It does not indicate whether such attitudes are shared by potential arts and science specialists alike.

The first part of the present enquiry was based on the "semantic differential"<sup>5</sup>. The individual rates a number of typical figures (for example, Mathematician, Poet) on pairs of adjectives (such as exciting/dull, dependable/undependable), each rating being on a seven-point scale (for example, "extremely exciting" to "extremely dull"). The sample ( $n=390$ ) was limited to 13, 15 and 17 year old boys of high academic ability, drawn from two schools, one public boarding and one grammar.

The present data confirm the American result. The typical physical scientist emerges as entirely distinct from typical figures in the verbal arts. Table 1 shows one contrast, representative of the data as a whole: that between the Physicist and the Novelist (of twenty mean ratings given, eighteen are statistically significant:  $P<0.05$ ). The semantic differential also includes a number of typical women and, again, American findings are borne out. Compared, for example, with the wife of the Novelist, Doctor or Barrister, the wife of the Research Scientist is seen as dull and plain.

The sample was then broken down by academic speciality, and two sub-groups formed: (i) arts specialists, taking a sixth form course in English, history or modern languages ( $n=75$ ), and (ii) physical science specialists, studying mathematics, physics and chemistry ( $n=67$ ). Their responses were then compared.

Fig. 1 shows the contrast between Novelist and Physicist, as seen by arts specialists and by young physical scientists. Despite "haloes" of approval for members of their own speciality, the attitudes of the two groups are strikingly similar. Both groups agree that the Novelist is the more imaginative, warm and exciting; the Physicist the more dependable, hard and hard-working. (Of differences plotted, nineteen deviate significantly from

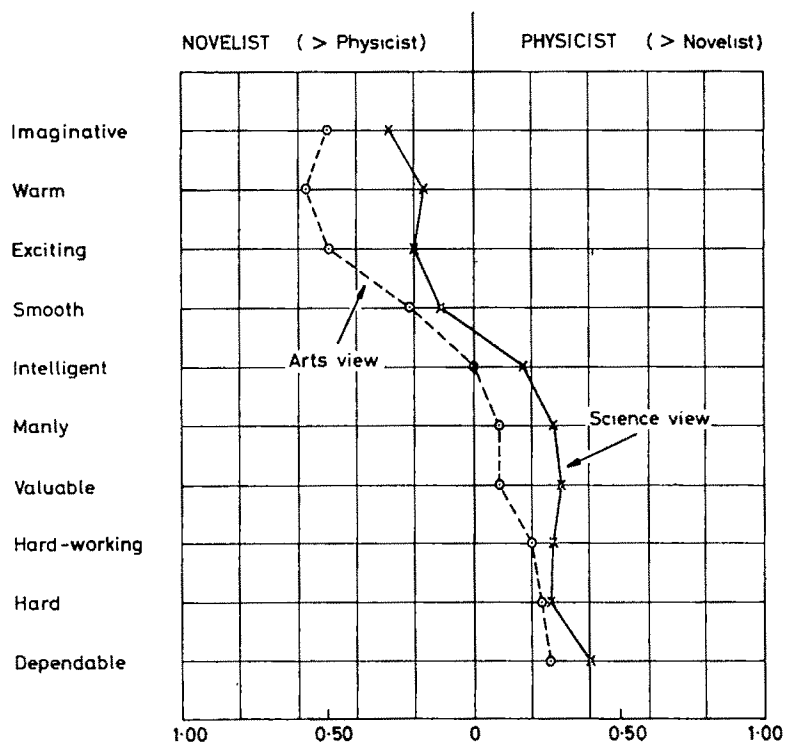


Fig. 1. Differences between "the Novelist" and "the Physicist", as seen by young arts specialists ( $n=75$ ) and by young physical scientists ( $n=67$ ). Each point represents a difference between two mean ratings on the semantic differential—one for "Novelist", the other for "Physicist". Differences are converted to a scale from zero to 1.00.



Table 1. QUALITIES ATTRIBUTED BY SCHOOLBOYS ( $n=390$ ) TO TWO FIGURES: "THE NOVELIST" AND "THE PHYSICIST"

Novelist		Physicist	
Imaginative	0.86	Valuable	0.72
Warm	0.48	Intelligent	0.58
Intelligent	0.41	Hard-working	0.51
Exciting	0.38	Dull	0.32
Valuable	0.31	Dependable	0.31
Undependable	0.30	Cold	0.29
Smooth	0.19	Hard	0.28
Soft	0.19	Manly	0.27
Feminine	0.12	Rough	0.13
Lazy	0.04	Unimaginative	0.03

Mean ratings on the semantic differential converted to a scale from zero to 1.00, where zero represents the absence of a given quality, and 1.00 the maximum possible rating upon it.

zero:  $P < 0.05$ ). Furthermore, the two groups agree closely in their rank ordering of discriminative adjectives from "warm" to "dependable" for the arts group, and from "imaginative" to "dependable" for the young scientists:  $r_s = 0.88$ ,  $P < 0.01$ .

Analogous results emerge from the contrast between the wife of the Novelist and the wife of the Research Scientist. Both groups agree that, of the two, the wife of the Novelist is significantly the more exciting, feminine, soft, imaginative; while the wife of the Research Scientist is the more dependable. (In each case,  $P < 0.05$ .) Agreement in the rank ordering of adjectives is again high:  $r_s = 0.91$ ,  $P < 0.01$ . Substantially, the relations shown in Fig. 1 hold true for boys of all academic specialties, and for all typical figures relevant to the arts/science choice. Furthermore, such stereotyped attitudes are found as pronounced among unspecialized 13 year olds as among specialists of 17.

A clear implication of these data is that adult scientists are seen by both future arts and science specialists as leading dull personal lives. It was to explore this particular inference that a "typical graduates questionnaire" was devised. A typical male arts graduate and a typical male science graduate are compared in the light of thirty characteristics, some general (such as "competitive with others") and others specific (such as "wears fashionable clothes"). Responses are on a five-point scale, ranging from "arts graduate much more likely" to "science graduate much more likely". The sample used was of similar composition to the first. Each item in the questionnaire yielding a significant rating for both arts and science specialists is given in Table 2 ( $P < 0.05$ ). These results suggest that both groups see the typical arts graduate as the more pleasure-seeking and irresponsible figure, and the typical science graduate as the more puritanical. The agreement between the rank ordering of items is again high: for all thirty items,  $r_s = 0.82$ ,  $P < 0.01$ .

It might be protested that such evidence is trivial: arts and science specialists may differ in the value they

Table 2. COMPARISON OF QUALITIES ATTRIBUTED TO THE "TYPICAL ARTS GRADUATE" AND THE "TYPICAL SCIENCE GRADUATE" BY YOUNG ARTS SPECIALISTS ( $n=79$ ) AND BY YOUNG PHYSICAL SCIENTISTS ( $n=59$ )

Young arts specialists' view			Young physical scientists' view		
Arts	Wears fashionable clothes	0.62	Wears fashionable clothes	0.57	
Graduate	Sociable	0.61	Gets divorced	0.39	
>	Likes wife to look		Panics in emergencies	0.39	
Science	glamorous	0.52	Flirts with his secretary	0.38	
Graduate	Flirts with his secretary	0.51	Sociable	0.34	
	Gambles	0.49	Gets into debt	0.30	
	Likes expensive restaurants	0.42	Gambles	0.28	
	Gets into debt	0.36	Likes expensive restaurants	0.25	
	Gets divorced	0.30	Likes wife to look		
	Has fast car	0.23	glamorous	0.23	
	Panics in emergencies	0.18			
Science	Faithful to wife	0.19	Embarrassed (for example,		
Graduate	Embarrassed (for example,		about sex)	0.18	
>	about sex)	0.24	Has fast car	0.23	
Arts	Competitive at work	0.25	Faithful to wife	0.31	
Graduate	Works long hours	0.58	Competitive at work	0.43	
			Works long hours	0.70	

Mean ratings on the "typical graduates questionnaire" converted to a scale from zero to 1.00, where zero represents the absence of a given quality and 1.00 the maximum possible rating upon it.

attach to such adjectives as "warm" and "cold". Further evidence from the semantic differential refutes this. Arts and science specialists agree overwhelmingly in attributing the adjectives intelligent, imaginative, exciting, warm, dependable and valuable to figures defined in the test as "good" (for example, Good Father, Good Teacher, Good Friend).

It seems, in summary, that whatever their speciality, the attitudes of boys towards the arts and sciences are influenced by a common set of preconceptions. Psychologically, this finding is intriguing. Large numbers of boys choose careers in the physical sciences, believing as they do so that the personal life of the adult scientist is unexciting. This choice may represent a reluctant compromise, but previous research suggests that it will frequently be made gladly, and even with a sense of relief<sup>2</sup>. Such stereotyped preconceptions may also help to explain why, contrary to predictions of a historical or economic nature, the proportion of able children recruited to the physical sciences in Great Britain has failed to increase since 1960 and may indeed have decreased<sup>3</sup>. It remains unclear, however, where boys' stereotyped ideas originate and to what extent they are open to change.

These results arise from a programme of research supported by the Nuffield Foundation.

<sup>1</sup> Hudson, L., *Brit. J. Educ. Psychol.*, **33**, 120 (1963).

<sup>2</sup> Hudson, L., *Contrary Imaginations* (Methuen, London, 1966).

<sup>3</sup> Roe, A., *Psychological Monogr.*, **67**, No. 352 (1953).

<sup>4</sup> Beardslee, D. C., and O'Dowd, D. D., in *The American College* (edit. by Sanford, N. (Wiley, London, 1962).

<sup>5</sup> Osgood, C. E., Suci, G. J., and Tannenbaum, P. H., *The Measurement of Meaning* (University of Illinois, Urbana, 1957).

<sup>6</sup> Phillips, C. M., *Times Educational Supplement* (November 19, 1965).

(Continued from page 227)

## THE NIGHT SKY IN FEBRUARY

All times are in Universal Time

MOON		CONJUNCTIONS WITH THE MOON	
New Moon	9d 11h	Venus	11d 09h, 3° N.
Full Moon	24d 18h	Mars	28d 15h, 2° N.
		Jupiter	21d 23h, 4° S.
		Saturn	12d 13h, 1° N.

PLANETS		Times of rising (R) and setting (S) during the month					
Name	R/S	Beginning	Middle	End	Mag.	$D_p$ (10 <sup>6</sup> miles)	Zodiacal position
Mercury	S	17h 20m	18h 55m	18h 15m	-0.5	92	Aquarius
Venus	S	18h 25m	19h 20m	20h 05m	-3.3	141	Aquarius
Mars	R	23h 40m	23h 05m	22h 20m	+0.2	90	Virgo
Jupiter	S	7h 20m	6h 20m	5h 20m	-2.1	408	Gemini
Saturn	S	21h 00m	20h 05m	19h 20m	+1.3	961	Pisces

$D_p$  is the distance of planet from the Earth on the 15th of the month.

### OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
98 Tau	D	19d 01h 50-0m	+5.6
76 Gem	D	21d 18h 43-3m	+5.4

(D, disappearance; R, reappearance)

### OTHER PHENOMENA

25d 20h Uranus 3° S. of Moon

# Marion and Prince Edward Islands

Early in January 1965 a scientific expedition to the isolated Marion Island and its small companion Prince Edward Island in the Southern Ocean was launched by the Republic of South Africa. The following article is a preliminary report of the early investigations undertaken of the biology and geology of the two islands.

## Biological Studies

A CONCERTED effort has been made to study the animal and plant life of the two small sub-antarctic islands, Marion and Prince Edward, which are situated at 46° S. In their geographical position these islands can be compared with Crozet and Kerguelen Islands. An expedition was sponsored by the South African Government under the supervision of the South African Council for Scientific and Industrial Research and consisted of four biologists and two geologists.

The party stayed on Marion Island from January 4 until March 17, 1965, and on Prince Edward Island from March 18 to 22, 1965. Two members, B. J. Huntley and E. M. van Zinderen Bakker, jun., then returned to Marion Island for a further stay of 12 months, while the others left for Cape Town on the research ship the *R.S.A.*

After arrival the biological team organized a field laboratory which was provided with apparatus for research in ecology, cytotaxonomy, palynology, pedology, hydrobiology and for general collecting activities. Electric power was available for this work and an existing small greenhouse proved to be very suitable for experimental work.

## Systematics and Endemism

The first aim of the expedition was to make representative collections of all the taxa which are to be found on the island and in the intertidal zone. A number of specialists have consented to investigate the material. The flora and fauna, especially those of Marion Island, should be well known when this work is completed. Special attention was directed to endemism and the problem of long distance dispersal in connexion with the isolated position of the islands. The list of the limited number of flowering plants has so far been extended with two species of *Juncus* and one of *Limosella*. An extensive investigation of the chromosome patterns of the flowering plants and ferns was carried out in order to obtain information about the populations of plants which occur. Special attention was also directed to problems of the speciation of certain groups of animals, such as the wingless butterfly *Pringleophaga*, wingless flies, fresh water Crustacea, Curculionidae and others of which interesting material had been collected.

The systematic and biogeographic investigations cover, besides the two plants already mentioned, the Musci, Hepaticae, Lichens, macroscopic fungi, and the fresh-water and marine algae. A great variety of lower invertebrates were collected with the aid of Berlese funnels and nets and also by squeezing sap from plant material.

In the intertidal zone about 250 different animals were collected together with 200 samples of marine algae. A 'Neoprene' 'wet-suit' was used for diving, which enabled the marine biologist to stay in the water for up to an hour at a time. Fish and plankton were regularly collected.

The ornithological programme was directed by Professor J. M. Winterbottom, director of the Percy Fitzpatrick Institute of African Ornithology at the University of Cape Town. By early 1966 twenty-four species of birds had been observed. An extensive study was made of the distribution of the birds, especially of the large

numbers of rockhoppers, Gentoo, Macaroni and King penguins, in the rookeries.

## Ecology

The ecological research was concentrated on different fields. The botanist studied the plant communities and their micro-climate with the aid of a battery-operated six-point temperature recorder. These investigations revealed interesting differences between the habitats and especially the degree of protection provided by the cushion growth form which is so common on the islands. The study of the great influence of seals, penguins, night birds, gulls and the extensive rookeries on the vegetation may provide an explanation for the vegetation pattern and succession in the coastal region.

Special attention was paid to the extensive swamps which occur on the island and which develop in an oligotrophic direction. The peat contains well preserved pollen, and some borings, which were made on a reconnaissance expedition to Marion Island in 1963, revealed an age of about 5,000 years for some of these swamps. More borings were made this time and recent pollen material has been collected. At a later stage borings will be made on Prince Edward Island.

The ornithological programme paid special attention to the aetiology of the sooty albatross and the Gentoo penguin, both of which occurred in fairly large numbers near the expedition's base. A 16 mm cine film was made to show the behaviour of these birds and the temperature recorder was used for studies of their breeding habits. The population dynamics of the royal albatrosses was also studied, as these birds occur in large numbers on the islands. An extensive banding programme, for which bands of the U.S. Antarctic Research Program were used, was undertaken. Special attention was also paid to the night birds and protein samples were collected for serological work.

The ecology of the intertidal flora and fauna was studied at five different stations situated at various points around Marion Island and at two stations on Prince Edward Island. The transect method was used. Temperature and chemical determinations were regularly made and some experiments on lethal temperatures were conducted with molluscs.

After much experience had been gained on Marion Island, the short stay on Prince Edward was extremely important, as the latter island is inhabited mainly by the same species, but very interesting differences exist in the flora and fauna, and especially in the general ecology and the age of these two islands. From the biological point of view it is estimated that Prince Edward is the older of the two with a more advanced drainage system and consequently a different ecology.

**Patterned soil.** Some interesting types of polygons and stone lines were discovered on both islands and will be studied in detail. The temperature recorder was used on these sites to assess the microclimate which is responsible for these freezing-thawing effects.

**Conservation.** The conservation of the ecosystems of the islands and of their flora and fauna was an important consideration in the scientific programme. The South African Department of Transport, which is responsible

for the permanent Weather Station of Marion Island, is interested in the preservation of the wild life of the island and will see that the disturbance of the vulnerable flora and fauna will be as small as possible. So far eight different weeds have been found on Marion Island. The vegetation on Prince Edward is practically undisturbed.

The results of this expedition are being worked out in co-operation with colleagues working on similar islands and in contact with the U.K. Scientific Committee on Antarctic Research. It is intended to publish the records of the expedition in the form of a monograph.

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## Geological Studies

A BRIEF note<sup>1</sup>, emphasizing the lack of geological knowledge about Marion and Prince Edward Islands, appeared in *Nature* two days before we set foot on the larger island in order to commence its first systematic geological survey. The field work, which entailed topographic and geological mapping, volcanological observations and sampling for geochemical, petrographical, geochronological and palaeomagnetic purposes, was completed between January and March 1965. This included a 5 day visit to Prince Edward Island of which no reliable map or any scientific information has yet been published. The generalized geological

maps presented here (Figs. 1 and 2) are based on triangulation, Royal Air Force air photographs corrected by radial line plotting, and field sketches. Petrographic and other investigations are still in progress and will eventually be published in the final report of the expedition.

## Marion Island

Marion is a sub-antarctic island situated about 900 nautical miles south-east of Cape Town (lat.  $46^{\circ} 54' S.$ , long.  $37^{\circ} 45' E.$ ). It is near the junction between the Atlantic-Indian and Crozet Ridges, occupying a similar position with respect to sub-oceanic ridges as the Tristan da Cunha island group much farther west. The island represents the top of a volcanic cone rising steeply from the ocean floor at a depth of about 12,000 ft., but has a low dome-like profile above sea level. There is no central cone or crater, and no evidence of previously existing ones. The summit, Jan Smuts Peak (alt. 3,890 ft.), is one of numerous scoria cones dotted about the island.

The island is roughly oval in outline and is some 115 square miles. It measures 15 miles from east to west and 10.5 miles from north to south. The western half is exposed to strong westerly trade winds often reaching gale force. Above 1,000 ft., this area is shrouded in almost perpetual cloud while the summit area from Jan Smuts Peak westwards carries a permanent or semi-permanent snow cover.

Physiographically the island can be divided into (a) a coastal plain, (b) an inland slope, and (c) the summit plateau.

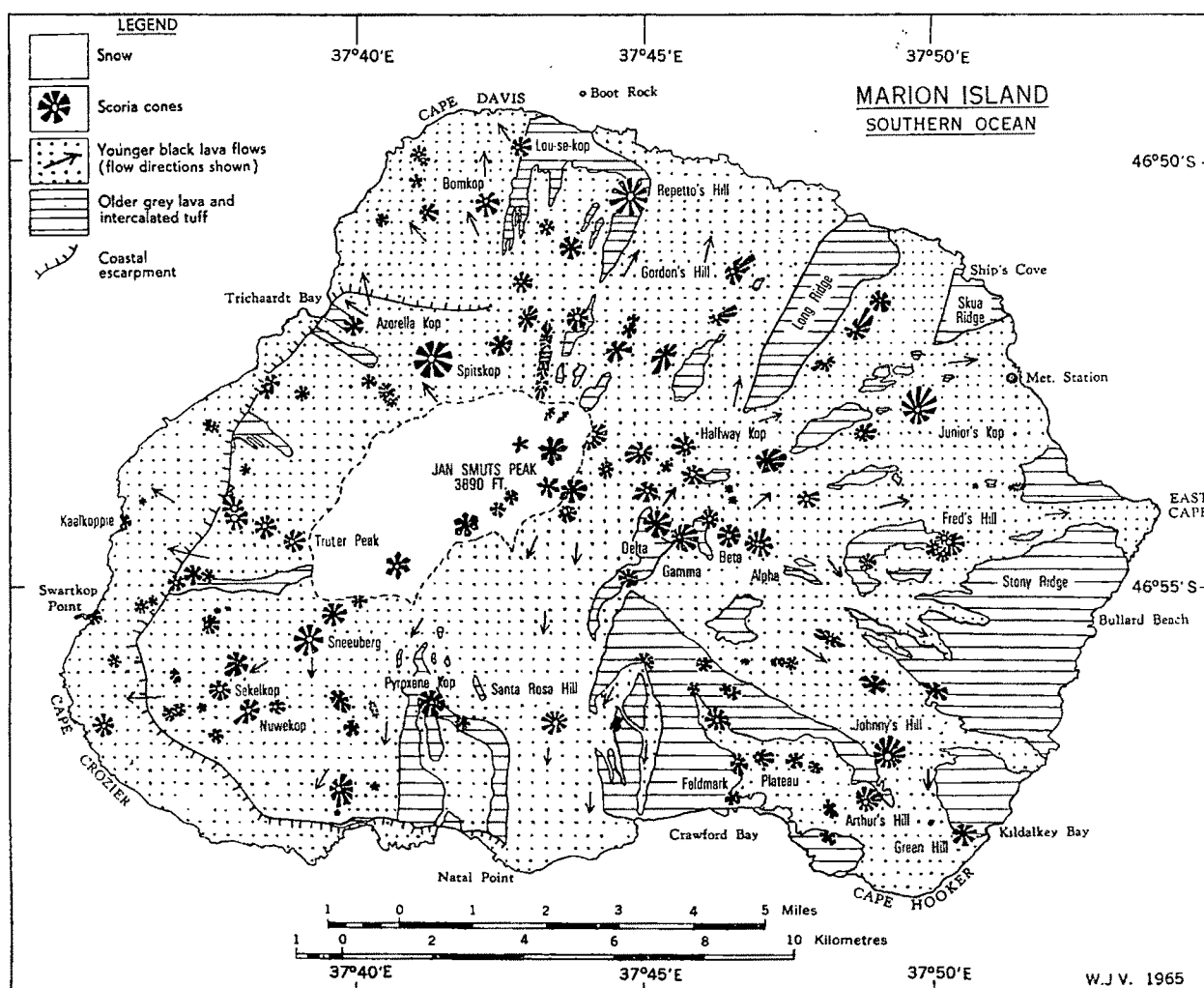


Fig. 1.

The coastal plain is only present along the western and south-western periphery of the island. It varies in width from 0.5 to 1.5 miles, and has an average altitude of 120 ft. above mean sea level. The generally flat, marshy surface is diversified by a few younger cones and lava flows. It is separated from the inland slope by an escarpment, 600–1,200 ft. high, trending parallel to the coast. Although the escarpment obviously represents a former coastline, the coastal plain is not considered to be a raised wave-cut terrace. It is built of lava that flowed over the escarpment on to an eroded shelf which is still submerged. The entire coastline of Marion combines the characteristics of retreat due to marine erosion and advance by seaward flow of lava. Low-lying areas which can perhaps be regarded as portions of a coastal plain are also found in the north-eastern half of the island, for example, between Repetto's Hill and Long Ridge and at East Cape. These areas, however, have tectonic boundaries and merge gradually with the inland slope; they are therefore different from the south-western coastal plain, both in shape and origin.

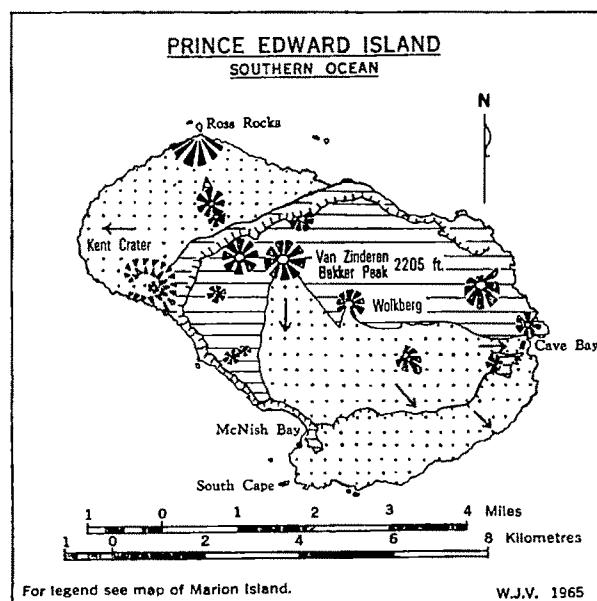


Fig. 2.

The inland slope comprises the greater part of the island. Despite a fairly smooth profile, the slope is by no means an even surface. Conical hills rise up to 750 ft. above it, and the eastern half of the island consists of roughly triangular plains and plateaux arranged around the central peaks in alternating fashion. Long Ridge and Feldmark Plateau are two of the most prominent highlands, adjoined on both sides by broad valleys that can be ascribed to relative depression. Stony Ridge is part of a segment with intermediate elevation.

The summit plateau is a narrow strip above 2,500 ft. extending for a distance of about 4 miles from Alpha Kop westwards. Two conspicuous cone lines, one from the south-west and the other from the south-east, meet here. Vast quantities of younger black lava were poured out from the summit plateau on to the lower slopes.

Geologically, two stages in the evolution of the island can be distinguished. The first stage is represented by a succession of grey lava flows (trachybasalt?) with interbedded tuff and agglomerate. The lava flows dip at low angles seaward and vary in thickness from a few to about 100 ft. They are vesicular but very seldom scoriaeous. Most grey lava is massive and often it is columnar jointed. Megascopically, several petrographic types appear to be present, some of them porphyritic. Gabbroic

or ultrabasic inclusions have not been encountered. The areas built of the grey lava succession strongly contrast with the younger black flows and protrude as isolated inliers through the latter. The grey succession is thought to be much older because its topography is smoothly rounded, well established water courses exist, minor irregularities of the coastline have been erased, and prolonged mechanical weathering has resulted in surfaces strewn with rock slabs.

The second stage again gave rise to effusive and pyroclastic products, the latter being restricted to the mostly unconsolidated cone-building scoriae already mentioned. The lava belonging to this stage is black basalt with olivine but individual flows contain different proportions of feldspar, olivine and pyroxene phenocrysts. The two analysed specimens described by Abbott<sup>2</sup> undoubtedly belong to the younger suite, even though he referred one of them to the older grey series. The numerous flows that must have succeeded each other over a long period of time can be distinguished. Some are covered with vegetation on the lower slopes of the island, while others are bare and appear to be no more than a few hundred years old. Some scoria cones are partially buried by the latest lava flows. The most striking difference between the black lavas of the second stage and the older grey succession is that the former still shows all the primary features of an uneroded volcanic landscape, for example, lava tunnels, lava channels, lava levees, tumuli, spatter cones, craters, aa and pahoehoe. The stream pattern is determined by the contacts between adjacent flows, while the previously established drainage has been disrupted at many places by black lava flows following pre-existing valleys. Where outbuilding flows of black lava form the coastline, initial irregularities have been accentuated by wave erosion.

There is considerable evidence in favour of tectonic movement between the two stages. The high plateaux are invariably built of older grey lava and intercalated tuff, to a greater or lesser extent concealed by younger lava flows. In the intervening plains and valleys, however, the grey succession is buried beneath great thicknesses of younger black lava. The plateaux are often bounded by straight lines of cliffs extending radially from the central highland. Moreover, some scoria mounds are also aligned along such radii. It is concluded that the major features of the inland slope are fault blocks; the valley containing Santa Rosa Hill, for example, is best described as a rift valley. Variable tilting of individual blocks during radial fracturing would also explain, in part, the asymmetric development of a coastal escarpment.

We found no fumaroles or other signs of recent volcanic activity.

### Prince Edward Island

Prince Edward Island is 12 nautical miles north-north-east of Marion and represents a subsidiary peak of the same volcano. Two stages in the geological evolution corresponding to those on Marion have been recognized. The older group of grey lava and tuff builds mainly the central, high part of the island bounded on the north-west and south-west by steep coastal escarpments, up to 1,500 ft. high. During the second stage of volcanism, scoria cones were built up on the older succession and black basaltic flows extended the island both to the north-west and south-east. The black lava flows are all covered with vegetation, which seems to suggest that volcanic activity on Prince Edward came to an end earlier than on Marion.

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<sup>1</sup> Truswell, J. F., *Nature*, 205, 64 (1935).

<sup>2</sup> Abbott, D., *Ann. Geol. Surv. S. Afr.*, 2, 89 (1963).

## BOOK REVIEWS

## BEAU TEMPS

Les écrivains français et la météorologie de l'âge classique à nos jours

By L. Dufour. Pp. 122. (Bruxelles: Institut Royal Météorologique de Belgique, 1966.) n.p.

METEOROLOGY has been called by some the physics of the atmosphere and by others the science of the weather. As soon as we bring in that splendid word, with its Norse affinities, we are conscious of a subject that touches our emotions; something we have to live with. Earlier French poets, like the British, were thrilled by the simple emotions of springtime; Charles d'Orleans was writing his rondeaux when William Langland was enjoying his May mornings. Sixteenth-century Caliban's instinctive knowledge led him to roar: "All the infections that the sun sucks up on Prosper fall". Attitudes change; four centuries later, the meteorologist prefers to examine the extent to which limited convection can develop in the lower layers of the essentially stable atmosphere surrounding a small island in early summer, bearing particulate matter for but a short distance. From the seventeenth century onward, literary expression has seen the rise of logical prose at the expense of emotional poetry; but within this frame, the literature of Western Europe has continued to abound in expressions of those responses that the variable westerly weather that we all share continues to provoke.

It is entertaining to find a work such as this appearing from an official meteorological service, but, after all, meteorology and Molière came in together. M. Dufour is a doctor of science whose agreeable purpose is to discuss one aspect of the relationships between science and literature. He concludes by suggesting that the authors who have best described the phenomena of the atmosphere are not the scientists, but those who have been moved to do so by physiological, aesthetic or other considerations. Here he begs the question. What, after all, is satisfactory description? Can feelings be adequately conveyed in the words of a scientist to anyone other than a scientist?

M. Dufour has much to say about Bernardin de Saint Pierre and his imaginative descriptions of the weather at sea, no doubt influenced by accounts of the voyage of Bougainville. We can counter with Coleridge and Cook. He finds the first lively comments on French weather in the letters of Mme. de Sévigné; we have to seek Evelyn's diary, or wait for Horace Walpole's prolific output. Early in the nineteenth century Chateaubriand reveals a lively regard for the weather at a very proper period, when Gay-Lussac was ballooning above Luke Howard's clouds. Dorothy Wordsworth and Stendhal displayed their sensitiveness to the moods of the weather at very much the same time; and perhaps we might offer Meredith as a counter to Victor Hugo. We are brought down to the descriptions of Saint Exupéry, who was an air pilot before he took to writing.

Thoughtful scientists who like to observe the phenomenon of literature, as well as liking it, will find the author's comments and extracts interesting, for France and England have shared many fashions in common. Meteorologists will see in them that background of regard for the weather that the countries of Western Europe must share; even as the "ciel lumineuse" of the Ile de France, the clear light of East Anglia and the grand mobile skies of Holland towards the edge of the prevailing track of Atlantic

depressions have enlivened their painters. Englishmen can reflect on how long they have continued to express so much of their feeling in poetry. Is this because too many Frenchmen have been led to seek the town, while we still battle to keep our hold on the land, our little patch of suburban garden in Surrey or Cheshire where the west wind can still be felt?

GORDON MANLEY

## PROBABILITY OR SUPPORT?

Logic of Statistical Inference

By Ian Hacking. Pp. ix+232. (London: Cambridge University Press, 1965.) 40s. net; \$7.50.

THIS book is a philosophical discussion of statistical inference, better informed than most others on the subject by professional philosophers, and written in a lively and provocative style.

The book is based on a notion close to that of subjective probability but which the author calls "support", by analogy with Carnap's equally misleading use of the word "confirmation". He says (page 221) that he has not defined "support" but has used it in its familiar English usage. He does sometimes, and the double meaning leads him to deny himself one of the axioms of (partially ordered) intuitive probability. Therefore, as he says (page 208), his theory cannot reach as far as existing theories of (either logical or) subjective probability. He cites (pages 32-33) some axioms of intuitive probability due to Koopman, calls them "axioms of support", and says they are indispensable to the rest of the work, but cites one of them incorrectly (as he has since noticed). The argument at the foot of page 33, in which he disputes one of Koopman's axioms, is wrong when the axioms are concerned with intuitive probability, as Koopman intended them to be.

On page 41 Nelson Goodman's riddle is said to "combine precision of statement, generality of application, and difficulty of solution to a degree greater than any other philosophical problem broached in this century". The riddle is: "Let 'blight' mean 'black until the end of 1984 and white thereafter', . . . Goodman argues that every shred of evidence which supports the claim that most balls in an urn are black . . . equally supports the claim that most are blight . . .". The reviewer thinks that, from the point of view of subjective probability, there is no riddle: the initial probability of blight is very much less than that of "black" and, therefore, so is its final probability. A riddle does arise if "support" in the sense of probability is confused with its English meaning, best explicated by "weight of evidence", defined as  $\log \{O(H|E)/O(H)\}$ , where  $O$  denotes "odds",  $H$  a hypothesis, and  $E$  an experimental result, evidence, or event.

In the discussion of fiducial probability the author tries to salvage something from Fisher's fallacious arguments. I find the discussion obscure here, but, as far as I can understand it, it seems to me to depend on an assumption (page 141) that relative support is proportional to relative likelihood. Since the author's interpretation of "support" is also that of  $P(H|E)$ , this assumption is essentially the same as a principle of indifference, in spite of protestations (page 207) to the contrary.

From the standpoint of the sociology of philosophy it is interesting that so many philosophers should have been misled by the misuse of the single word "confirmation"—albeit by a famous philosopher. The author, in spite of his undoubted intelligence, is among these, although, to distinguish his work from that of Carnap, he uses the word "support". Inherently lucid in style, he is driven into complications and obscurities in his attempt to defend a position that is basically untenable. "If the first button is buttoned wrongly, the whole vest sits askew."

Although he starts on the wrong foot or button, there are many stimulating passages, and even some opinions



which I have often supported. For example (page 32), the importance of partial ordering of intuitive probabilities is emphasized: and (on pages 212–213) Hacking expresses simultaneous admiration and dissatisfaction with de Finetti's explanation of physical probabilities in terms of subjective probabilities. He has privately apologized for misrepresenting me on page 110. I. J. GOOD

## PLANNING AHEAD

### Manpower Planning

Operational Research and Personnel Research. (A Conference under the aegis of the NATO Science Committee, Brussels, 17–20 August 1965.) Pp. v+291. (London: English Universities Press, Ltd., 1966.) 55s. net.

THERE is much of interest to manpower planners, at the level both of the company and of the national economy, in this volume of conference papers. The authors describe a part of the military experience of a number of member countries of NATO in dealing with problems of recruitment, training and the efficient use of human resources. It is not for the first time that war—or the preparation for war—has meant that a particular management problem has been taken seriously; and it is not surprising that a quarter of the conference papers should be concerned with the operational research techniques themselves, rather than with the subject matter to which they might be applied.

Two of the discussions at the conference centre round the human element in personnel planning. The selection of an individual for employment, or enlistment, often occurs before any judgment can be made about the successful fitting of man to job. No personnel or recruiting officer can avoid applying tests, whether objective or subjective, at the interview. Co-operative investigation by psychologists and operational researchers should in the future indicate the attributes of human personality which ought to be tested and the criteria that should be used for later performance—questions to which, at the moment, only partial answers may be possible.

The training of the individual to a pitch of proficiency required by the job is the second subject discussed. This is something to which military manpower planners apply quantitative techniques, and it is from this subject that programmed learning stems. For those to whom the idea of such a technology of training is still novel, there is a detailed and fascinating comparison in cybernetic terms between a closed loop system (in which the performance of the trainee feeds back to determine the training stimulus) and the open loop system characteristic of conventional training courses. Training officers in industry could well take note, if they have not done so already, of the principles that emerge from this idea: rigorous job analysis, specific objectives to any training course, a variable training period and a fixed end-result (rather than fixed training period and variable end-result).

The employment of human resources, considered as a group rather than as individuals, poses two types of problems for the manpower planner, one of which is discussed explicitly. This is the natural or induced rates of participation in a certain form of employment, in this case membership of the armed forces; and the geographical and hierarchical mobility required or made possible by the strategic location and command system of the armed forces. Solutions to the analogous industrial problems are suggested here: the industrial labour market may, for example, yield similar measurable supply curves which indicate pay-setting procedures effective in obtaining adequate labour.

The conference was almost entirely silent on the other main problem of manpower planning, though there were signs that it had not been neglected. This is the estimation of future needs for various types of men. The in-

redients for a solution must be: future military budgets, the technology of future weapon systems, and the needs that these imply for servicemen of different ranks and skills. Clearly a considerable integration of the personnel and technological planning activities takes place—you do not design a warship without detailing its crew's accommodation or an electronic tracking system without its maintenance staff—and this consideration greatly simplifies the problem of forecasting the manpower needs of future techniques in a military context. The need for military secrecy may hide the details of how such tasks are carried out, but the lesson for civil manpower planners is the need to co-ordinate economic, technological and manpower information relating to the future.

Finally, it should be said that the price of this volume seems excessive. It is adequately but by no means elegantly produced, being in fact offset reproduction from unjustified typescript. It is a conference volume, and so it seems likely that royalty considerations would not loom very large. And it is sponsored by an international organization which, in organizing conferences, must be mainly concerned with the exchange and dissemination of ideas.

RICHARD STONE

## BODY FLUID CONTROL

### Electrolytes, Fluid Dynamics and the Nervous System

By Joseph Henry Cort. Pp. 228+3 plates. (Prague: Publishing House of the Czechoslovak Academy of Sciences; New York and London: Academic Press, 1965.) \$10.

THIS is an unusual book on fluid and electrolyte metabolism. The chemical structure and routine analysis of the body fluids are not dealt with—no matter as there are many excellent texts available—but what is unique is the comprehensive and detailed consideration of the control of the fluids and electrolytes of the body and especially mechanisms which may be involved in the control of extracellular fluid volume. The important chapters are Chapter 3, "A Selective Review of Those Parts of the Nervous System thought to be involved in the Regulation of Body Fluids" (eighteen pages); Chapter 4, "The Innervation of Separate Organs in relation to Their Interactions with the Extracellular Fluid" (thirty-one pages); and Chapter 7, "'Volume' Reflexes, or the Relationship of Haemodynamics to Na Balance" (seventy-nine pages).

One of the crucial observations considered in Chapter 7 is the rise in water and sodium excretion which occurs when both common carotid arteries are occluded. Cort believes that this natriuresis depends partly on afferent impulses arising in the thorax, as the response is reduced after cervical vagotomy. The fact that vagotomy also augments the pressor response to carotid occlusion makes it unlikely that it is the rise in filtration pressure which causes natriuresis. Furthermore, the posterior nucleus of the hypothalamus appears to be involved in processing the afferent nerve impulses from the various baroreceptors, because after destruction of this region an animal goes into negative sodium balance and the natriuretic response to carotid occlusion is abolished. The onset of the natriuretic reflex is rapid and the reflex can be elicited after adrenalectomy; it can also be obtained during infusion of aldosterone, vasopressin or angiotensin in amounts which are large compared with the amounts produced endogenously; finally, denervation of the kidneys or administration of dibenzylamine does not seriously affect the response. Thus the efferent signal to the kidney is probably humoral, though neither aldosterone, vasopressin, angiotensin nor the catechol amines are involved. As a working hypothesis Cort suggests that the natriuretic response involves "1. inhibition of release of an anti-natriuretic factor (? vasotocin) related to inhibition of

posterior hypothalamic activity and 2. release of an oxytocin-like peptide or its precursor". In all this he does not understate the possible importance of aldosterone in the long term regulation of extracellular fluid volume.

Rather less satisfactory is the treatment of acid base balance and osmoregulation, mainly because these sections are so brief, but also because some of the statements made are open to question. It is not accurate to say that the anti-diuretic hormone osmoreceptors normally respond to rate of change of osmolality rather than the absolute level: Verney showed twenty or more years ago that these receptors do not adapt when exposed to sustained small increases in osmotic pressure. Osmoreceptors may give transient responses, but these would not be particularly helpful in the long term regulation of osmotic pressure where some sort of proportional control is required.

Cort is deliberately polemical, for he believes that an unopinionated book seldom makes good reading, and I think many would agree with him. Running through the text is the argument that in classical physiology we usually study an isolated mechanism under rigidly defined conditions and often under anaesthesia. We quite naturally interpret the behaviour of the intact conscious animal in terms of this mechanism, often without taking into account the possibility that there are other unknown and perhaps more important mechanisms which may supplant and certainly modify the original mechanism. Several completely different types of mechanism may function in parallel towards the control of some important variable the stability of which is essential for life. As examples of this we may note that the adrenalectomized rat will maintain reasonable health and sodium balance if provided with saline to drink, and that the serum osmotic pressure in diabetes insipidus is close to normal despite the greatly increased turnover of water. Even in experiments in which we are apparently at pains to study the responses of an animal with all its mechanisms functioning, as in balance studies, we may be deluding ourselves because the animal is usually placed on a constant intake while the renal regulation of some variable such as osmolality or volume is studied. We must remember that the central nervous system plays a part in both sides of the balance equation, that there is often a change in voluntary intake to a given stress as well as a renal response and that intake and excretion normally interact and modify each other. Cort asks the somewhat enigmatic question "... is the whole, working, biological system a sum of its parts, i.e. mechanisms, or does it represent a qualitative quantum jump over the individual regulating mechanisms?"

In short, this is a most stimulating book that covers every aspect of the control of body fluids. It is easy to disagree with many of the ideas that are advanced, but they are put forward with the intention of arousing interest, which they do, and of provoking research, which they will.

J. T. FRIZSIMONS

## BETA DECAY IN REVIEW

### The Theory of Beta Radioactivity

By E. J. Konopinski. (The International Series of Monographs on Physics.) Pp. x+403. (Oxford: Clarendon Press; London: Oxford University Press, 1966.) 75s. net.

### Beta Decay

By C. S. Wu and S. A. Moszkowski. (Interscience Monographs and Texts in Physics and Astronomy, Vol. 16.) Pp. xiv+394. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 120s.

THE story of the development of the theory of  $\beta$  decay and from this of the theory of weak interactions in general

is one of the most fascinating in physics. It began with a puzzle, the continuous  $\beta$  spectrum, solved by Pauli by the introduction of the neutrino in 1931. Then, after a period of gradual progress initiated by Fermi in 1933, came that extraordinary *volte face* in 1956, the breakdown of parity conservation. This was first proposed by Lee and Yang and subsequently verified experimentally by Miss Wu and her collaborators in 1957. At once, through the two component theory of the neutrino, came major advances in understanding of  $\beta$ ,  $\pi$  and  $\mu$  decay and a satisfactory formulation of low energy weak interaction processes.

A detailed account of this stage in the development is now the subject of a book by one of the early pioneers of the theory of  $\beta$  decay—Professor Konopinski. This spells out step by step each stage in the rather complicated calculations including all effects which might be expected to give a measurable contribution. The basic assumptions of the theory are compared with experiment wherever possible and the reader is provided with all those tools of the weak interaction trade appropriate to the low energy domain (although  $\beta$ -circularly polarized  $\gamma$  correlation theory is omitted).

More recently the interrelation of weak interaction theory with other branches of particle physics has led to many important developments. The present fashion for current-current commutation relations has its roots in the work of Goldberger and Trieman in connexion with  $\pi$  meson decay, and the ideas of unitary symmetry have, through the Cabibbo angle, explained why even small differences in coupling constants which occur in the theory of transitions conserving strangeness are nevertheless significant. Perhaps most important of all has been the suggestion by analogy with electrodynamics that the weak interactions are mediated by an intermediate vector boson. This, together with the availability of high energy neutrinos from the big accelerators and in the cosmic ray flux, has led to what is now another branch of high energy physics—neutrino physics.

The volume by Wu and Moszkowski is a review of the subject to this point. With the advantage of a much broader canvas, they have presented an extremely readable account of both the theory and the more recent experiments. The formal details required for a reasonable understanding of the processes by which the relevant numbers are arrived at are reserved for appendixes, a historical introduction is provided and so also is a final chapter on recent developments written, as the authors put it, "with great emotion and enthusiasm". The book is completed by an excellent bibliography.

It is only to be expected, in this field in particular, that nature should have the last word. In 1964, experiments on the decay of neutral  $K$  mesons by Christensen and collaborators demonstrated unexpectedly the apparent failure of time reversal invariance, a rather curious echo of the " $\tau$ - $\theta$  puzzle", presented by the decay of the charged  $K$  mesons, which led to the developments associated with the breakdown of parity conservation. If certain recent conjectures by Lee are verified, and the experimental evidence is at present conflicting, our understanding of the basic invariance properties of the interactions of elementary particles must shortly undergo another major upheaval.

G. N. FOWLER

## PUTTING TO SLEEP

### Animal Anaesthesia

By Melchior Westhues and Rudolf Fritsch. Vol. 2: General Anaesthesia. Translated by A. David Weaver. Pp. xxi+458. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 95s.

THIS is the second of two volumes entitled *Die Narkose der Tiere*. The first volume is concerned with local

analgesia and this second work is devoted to general anaesthesia. Dr. A. David Weaver has now made an excellent translation of both volumes. The subject matter of the present book is well presented and is divided into four parts with an introductory chapter on the history of anaesthesia. Part I deals with the principles of anaesthesia including the application of physics and physiology. Part II considers drugs which may be used in anaesthesia and Part III describes techniques and accidents and complications associated with anaesthesia. Part IV is concerned with the application of anaesthesia to different species including, in addition to the domesticated animals, small laboratory ones, wild or captive creatures, birds, fish and frogs.

The authors have attempted a comprehensive coverage of the subject of general anaesthesia in animals, and since the volume is of moderate size it is not surprising to find that many aspects are dealt with briefly. It is perhaps unfortunate, however, that this should have been the case with such important topics as the general preparation and post-anaesthetic care of the patient. Some of the techniques, such as endotracheal intubation are poorly described, while others, notably those describing the use of barbiturates in horses, could lead to serious trouble if carried out literally. In addition, some surprising statements are to be found, for example: "As a direct result of its unphysiological breeding, the pig is a very poor subject for general anaesthesia". Terminology is sometimes at variance with that generally accepted among anaesthetists, for example, no distinction is made between ventilation and respiration and the term "forced ventilation" is used instead of "controlled ventilation".

The pharmacology of anaesthetic agents is well described and the appendix contains a useful list of drugs. The bibliography is extensive, but selection of a particular reference is tedious since they are listed together at the end of the book. This book contains much useful information and is well illustrated, but the general standard of the text is variable.

BARBARA M. Q. WEAVER

## INTEGRAL EQUATIONS

### Integral Equations and their Applications

Vol. 1. By W. Pogorzelski. (International Series of Monographs in Pure and Applied Mathematics, Vol. 88.) Pp. xiii+714. (London and New York: Pergamon Press Ltd.; Warszawa: PWN-Polish Scientific Publishers, 1966.) 120s.

THIS book is a translation from the Polish of three separate volumes which appeared between 1953 and 1960, and so divides naturally into three parts. The aim of the book is to present the general theory of integral equations, together with their applications. Special techniques, such as the use of Fourier transforms, which lead to explicit solutions in certain cases, are not considered.

The first part gives an account of the theory of Fredholm integral equations. The Fredholm theory for equations of the second kind and the Hilbert-Schmidt theory for symmetric kernels are given in some detail. Other topics treated here are Fredholm equations of the first kind and Volterra equations. The treatment in this part is classical, little use being made of functional analysis. This section could well serve as an introduction to the theory of linear integral equations, and may be read without previous knowledge of the subject.

The second part is chiefly concerned with applications of the integral equations studied previously to the theory of linear ordinary differential equations and to second order linear partial differential equations of all three types. The treatment of partial differential equations is given in considerable detail with discussions of the reduction of many of the classical boundary value problems to inte-

gral equations. This part also includes a treatment of non-linear equations by use of functional analysis and the Banach and Schauder fixed point theorems.

The third part deals mainly with strongly singular linear integral equations, that is, equations containing an integral which converges only as a Cauchy type limit. These equations are then applied to several problems in potential theory. There is also a section on strongly singular non-linear equations.

As this description of the contents shows, the range of topics considered is wide. The book should be useful as a reference work with a broad coverage for applied mathematicians, physicists and engineers, and in particular to those interested in the treatment of partial differential equations by integral equations.

V. HURSON

## SOVIET MAMMALS

### Mammals of the U.S.S.R. and Adjacent Countries

Mammals of Eastern Europe and Northern Asia. By S. I. Ognev. Vol. 4: Rodents. Translated from the Russian by Jean Salkind. Edited by O. Theodor. Pp. x+429+61 tables. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1966.) 142s.

THIS volume, the fourth on rodents in the series of *Mammals of the U.S.S.R. and Adjacent Countries*, deals with the pikas, hares, rabbits, squirrels and chipmunks. Although basically a systematic work, it is a great deal more besides. The disparate nature of systematic accounts and general biological works are blended together in such a way that the bare bones are well clothed. Details are given of morphology, taxonomy, ecology and behaviour as well as valuable information not readily available on the role of these rodents as agricultural pests. There are accounts of the cyclic nature of hare populations and of epizootics in fluctuations. Not least is the detailed zoogeographical information which is one of the chief contributions of this volume and of the series as a whole. Like its predecessors, this book is a mine of information of the greatest value to the mammalogist.

The text is generally easy to read. Many of the field observations are verbatim accounts by the original observers and this makes for some pleasant changes in style. There are many tables and these are clear and comprehensive.

The small single page maps of other volumes have been replaced by larger detachable folding maps. Increase in size has unfortunately not been matched by increase in clarity—the shading in some cases is coarse and obscures geographical detail. The book is well illustrated by a variety of contributors. Many of the drawings are delightful, although a few are of more artistic than scientific value. Some of the original colour plates, now in black and white, have been so darkened that they serve little purpose and could have been omitted without detriment. These are, however, minor matters and do little to detract from the great value of the volume and the series.

G. I. TWIGG

## ELECTRON MICROSCOPY COMPENDIUM

### Techniques for Electron Microscopy

Edited by Desmond H. Kay. Second edition. Pp. xiv+560. (Oxford: Blackwell Scientific Publications, 1965.) 84s. net.

MY first reaction to the second edition of this book is critical, in that metallurgists are forced to pay (handsomely) for a great deal of information on the preparation

of biological specimens, and microbiologists for information on metallurgical techniques. A glance at the table of contents will show, however, that those pages useless to either type of microscopist comprise less than a quarter of the whole, so that this particular compendium of articles appears to be well justified.

The second edition differs from the first chiefly by the addition of an article by Howie on the interpretation of micrographs of thin crystals. This article is the best short account of the subject now available. Some of the other articles do not reconcile so well the conflicting roles of elementary exposition of theory and up to date description of advanced techniques. This difficulty could be alleviated to some extent by the inclusion of a list of standard books in which the interested reader can find treatments of the various theoretical topics at a suitable level. (I have in mind such topics as the scattering of electrons by atoms, general principles of diffraction, etc.)

A casual reading of the book reveals very few errors. The book is also quite up to date, although inevitably some recent developments (particularly those concerning ancillary apparatus and special attachments) are not included. A casual reading also reveals those areas in which further technical advances would be most welcome; in the measurement of magnification, for example.

The book is certainly essential for any laboratory using electron microscopy. It is an extremely good introduction to experimental techniques for research students, and for other research workers trying unfamiliar methods.

L. M. BROWN

## CARBANIONS

### Carbanions in Synthesis

(Oldbourne Chemistry Series.) By D. C. Ayres. Pp. viii + 207. (London: Oldbourne Press, 1966.) 52s. board; 25s. limp.

Clearly, a considerable degree of condensation and compression is required to deal with such a far-ranging topic as the role of carbanions in synthesis in a book of 207 pages. This is most noticeable in the first main chapter of *Carbanions in Synthesis*—forty-two pages on the formation, stereochemistry and reactions of the metal alkyls—in which the selection of the material covered seems a trifle capricious, to say the least. In the section entitled "Stereochemistry of Metal Alkyls", for example, the only stereoisomers of metal alkyls mentioned are the optical isomers of substituted cyclopropyl derivatives of lithium and magnesium; there is no reference at all to the widely investigated secondary butyl compounds of mercury, although work on these latter compounds has led to a much better understanding of mechanisms of reaction in organometallic chemistry.

The succeeding chapters, 152 pages, making up the bulk of the book, form a somewhat separate section or classical organic syntheses. Here Dr. Ayres seems to be much more at ease, and deals convincingly with established topics such as the synthetic uses of acetylides, malonic esters, diazomethane, and the various carbonyl condensation reactions, as well as with more recent developments in the form of the Wittig reaction, arynes, carbenes, and so forth.

Reaction mechanisms are considered wherever appropriate and, indeed, help to unify the treatment on the "carbanion" theme. As illustrations of the various general reactions and methods, specific examples, often from natural product chemistry, are invariably quoted, and each chapter ends with a useful selection of problems together with the original sources of the problems. Second-year and third-year undergraduates should find the book, in general, both interesting and informative.

M. H. ABRAHAM

## ALGORITHMS

### Introduction to Cybernetics

By Viktor M. Glushkov. Translated by Scripta Technica, Inc. Translation edited by George M. Kranc. Pp. x + 322. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 94s.

THIS book is really an introduction, not to cybernetics as it is known in the West, but to the theory of algorithms. As such it covers mathematical logic, from the propositional calculus to the Gentzen formalization of the predicate calculus and the decision problems, Post, Turing and Markov algorithms, Boolean algebra, switchings nets, and the elementary theory of finite sequential automata, and some aspects of the theory of self-organizing or self-improving systems. There is also a chapter on Algol programming, as an example of a universal language for representing algorithms on a general-purpose digital computer.

In general, this is not a book which is suitable for the uninitiated. Most of the chapters are too concise, and there are not nearly enough examples to work through. The sections dealing with Boolean algebra applied to switching nets, and the theory of finite automata, leave much to be desired. There is no treatment of threshold logic, which has so many applications in the construction of actual automata, nor of the algebra of finite automata, centred around the Rabin-Scott/Schutzenberger introduction of finite semi-groups and the subsequent Kron-Rhodes canonical decomposition theory.

The book is, however, well worth reading for its main chapter—that dealing with self-organizing systems. The well known Perceptron is analysed in detail, and it is clearly shown that there are severe disadvantages in the use of randomly connected nets for pattern recognition, even if there is an external trainer. It is also shown that if there is no external trainer, the Perceptron is the least likely automaton to achieve correct pattern recognition. In this respect this volume will be useful to those interested in artificial intelligence, but not necessarily to those interested in the wider aspects of control and communication in animals and machines.

J. D. COWAN

### Engineering Materials

Selection and Value Analysis. Edited by H. J. Sharp. Pp. 428. (London: Heywood Books, for Iliffe Books, Ltd.; New York: American Elsevier Publishing Company, Inc., 1966.) 85s. net.

THIS book is intended to serve two purposes: it provides, for the benefit of designers and materials engineers, a concise body of factual information about the properties of commercially available constructional materials, and it also seeks to expound the philosophy of "value analysis" applied to materials selection as a means of finding the cheapest material to fulfil a precisely defined function adequately. The first purpose is served better than the second; nevertheless, the importance of economic considerations is emphasized throughout the book, and information on comparative costs is listed in many places.

The chapters on various categories of materials are uneven in quality. The best is that by P. C. Thornton on constructional steels, which can serve not only as a useful source of data, but also as a lucid primer on the principles of selection and heat treatment of steels. Several other metallurgical topics, such as bearing alloys, are also well treated, and indeed the book has a strong metallurgical bias, in spite of the fact that the editor contributes a useful summary of the properties of modern plastics. The editor also contributes a critical survey of materials processing methods. Engineering ceramics and glasses and composites (other than fibreglass) receive no attention.

Apart from these omissions, and the more general criticism that there are no detailed case histories of material selection and associated design improvement, this book can be recommended as a useful concise source book for designers, and a refresher for metallurgists who wish to be reminded of the uses of their science.

R. W. CAHN

### Advances in Enzymology and Related Subjects of Biochemistry

Vol. 28. Edited by F. F. Nord. Pp. v+547. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 115s.

VOLUME 28 of this series contains seven chapters. Laylin K. James and Leroy G. Augenstein discuss the adsorption of enzymes at interfaces. This is timely, because biochemists are increasingly conscious that the dilute aqueous solution in which they study enzymes is an artificial environment compared with the gels in which those enzymes occur in nature.

E. R. Stadtman deals with the allosteric regulation of enzyme activity. His article is restricted to a consideration of those regulatory processes that involve reversible metabolic activation or inhibition of the enzyme activity. Tsao E. King considers the problem of the reconstitution of the respiratory chain, but limits himself to the mitochondria of animal tissues.

Nathan Citri and Martin R. Pollock review the biochemistry and function of  $\beta$ -lactamase (penicillinase). Alan T. Bull and C. G. C. Chesters deal with laminarin and laminarinase and focus attention on the biosynthesis and depolymerization of  $\beta$ -1,3-glucans. Von Ed. Hofmann and Gg. Hoffmann review (in German) the estimation of biological activity in soil using enzyme assays. Henry Z. Sable gives an account of the biosynthesis of ribose and deoxyribose excluding those studies which attempt to assess the relative proportions of glucose metabolized by the glycolytic and pentose cycle pathways. The volume maintains the standard to which biochemists have long been accustomed in this series.

T. J. BOWEN

Dissatisfaction with students who only wanted a qualification from his course, repugnance for biological work, and the wish to run his own department encouraged Hodkinson to accept the post of professor and head of the Department of Physics at Virginia State College. He was the first full-time white faculty member to be appointed. During his two years in this Negro college the benefit to the students was immense. He gave them first class visiting lecturers and a Federal research grant, revived his department, and, revealing a musical and poetic talent, contributed to the artistic life of the college. He was, however, impatient with the college administration and criticized it for its acceptance of low academic standards, an authoritarian rule and the assignment of funds to architectural features rather than to learning. When last year he decided to take a sabbatical year he was dismissed from his post. He went to Sweden, and it was while sailing alone in his yacht that he died.

C. N. DAVIES

### Isaac Pomeranchuk

ISAAK YAKOVLEVICH POMERANCHUK, who died on December 14 aged 53, was one of the outstanding theoretical physicists of the Soviet Union. Born in 1913, he graduated from the Polytechnical Institute in Leningrad in 1936, and then did research in several institutes of the Academy of Sciences. His last appointment was in the Institute of Experimental and Theoretical Physics. He was awarded the Stalin Prize, and since 1953 had been a corresponding member of the U.S.S.R. Academy of Sciences.

His interests ranged widely over the whole of theoretical physics. His earliest known contributions concerned the theory of solids and in particular the problem of the thermal conductivity of dielectrics, for which he pointed out a number of important facts which had been overlooked. He worked also on the related problem of the absorption of sound waves, and did some early work on the scattering of neutrons by crystals. Other papers relate to such diverse subjects as the properties of liquid helium, the "Delbrück scattering", and the effect of the emission of radiation in limiting the energy of primary cosmic ray electrons reaching the Earth. In recent years his work was mainly concerned with the physics of elementary particles and in particular with the theory of collisions at high energy. His best known result in this field is the proof, surprisingly simple and powerful, that collision cross-sections at high energy, if they reach finite limits for infinite energy, must become the same for particles and antiparticles on the same target. Another important result is contained in joint work with Gribov, which showed that scattering amplitudes regarded as analytic functions of angular momentum may contain not only poles ("Regge poles") but also essential singularities which invalidate some of the conjectures fashionable at the time. These are a few examples of a prolific output of work, which was broken only by his final and fatal illness.

His approach was always to emphasize simple physical arguments and to pick out the general features of the type of situation which could throw light on the physical problems, without becoming involved in too much detail. He was capable of carrying through a sophisticated mathematical argument when this was necessary, but he never regarded this as an aim in itself. In this respect his attitude showed very much the influence of L. D. Landau, with whom he collaborated on many occasions.

As a person he was held in deep affection by all his colleagues. He was modest to the point of self-deprecation and careless about appearances. The figure in a crumpled suit who always managed to look as if he had last shaved two days ago, but who always had something sensible and relevant to say, will be sadly missed at all gatherings of physicists.

R. E. PEIERLS

## OBITUARIES

### Dr. J. Raymond Hodkinson

RAY HODKINSON was drowned in the Baltic on August 24, 1966. He had planned to spend a year in Sweden and write a book about the measurement of airborne dust.

From the Grammar School he went to the University in Manchester and graduated in physics in 1949. He worked briefly with Associated Electrical Industries and at Jodrell Bank, before his national service in the Royal Air Force. He entered the Safety in Mines Research Establishment at Sheffield in 1952, and here he rose rapidly. His work was concerned with dust physics and the use of radioactive tracers to investigate the ventilation of mines. On his promotion to principal scientific officer he was allowed to spend nearly three years at the London School of Hygiene to undertake research into the scattering of light by particles. Some of his ideas he put into effect on his return to Sheffield.

In 1962 he obtained his Ph.D., and in the same year he went to the Department of Radiation Biology at the University of Rochester, to take charge of aerosol physics. He stayed there until June 1964. During this time he travelled extensively in the United States and Canada to visit laboratories and speak at scientific meetings. His knowledge of the optics of particles was useful to the U.S. Public Health Service, for whom he became a consultant, and he went to Canada with an eclipse expedition. With Judith Greenfield he used a computer to calculate the response of optical particle counters using a variety of light systems.



# A Mechanism for Radio-galaxies and Quasars

by  
W. H. McCREA  
University of Sussex

Gravitational collapse and thermonuclear processes, operating through the formation of many temporary stars, can provide a basis for a model of radio sources. The model may explain some of the properties of the sources and provide a unifying theory for quasars and radio-galaxies

THIS article presents what seems to be a possible explanation of the phenomena of radio-galaxies and quasars. While the theory is speculative at present, it does nevertheless succeed in correlating many empirical properties without invoking any hitherto unknown processes or states of matter.

Lynds and Sandage<sup>1</sup> and others have found direct evidence for the occurrence of violent events in the central regions of certain galaxies. Less direct, but accumulating, evidence tends to show that when such an event occurs, the galaxy concerned may become a strong radio-galaxy or a quasar, and that it may also become a strong X-ray source and possibly a cosmic-ray source. Thus these events seem to underlie and to correlate a wide range of features in the large scale behaviour of certain galaxies. A satisfactory theory of any of them seems, therefore, to require the understanding of the physical nature of such an event.

The predominant characteristic of an event of the kind considered is that it releases an enormous amount of energy in a cosmically short time. It seems that the radiation rate is of the order of  $10^{46}$  ergs/sec during an interval of from  $10^3$  to  $10^6$  years, and that the total energy requirement is about  $10^{50}$  or possibly as high as  $10^{52}$  ergs. The only known processes which can release large quantities of energy, and in which the material taking part is in some state or another known to physics and astronomy, are those which release gravitational energy or nuclear energy. The gravitational energy released in forming stars and galaxies is not enough for the present requirement. On the other hand, if the material of a galaxy of  $10^{11}$  solar masses was originally mainly hydrogen, and if all of it were converted into helium and heavier elements with the release of energy equivalent to about 1 per cent of the mass, then the total energy released would be more than  $10^{52}$  ergs. There is, therefore, an ample source of energy; this has long been appreciated, and the problem is to discover how the source can be made use of. Two processes are essential to the solution I propose.

**Gravitational contraction.** Consider a mass  $M$  of material, mainly atomic or molecular hydrogen, occupying a cylindrical volume of height  $2h$  and diameter  $2R$ ; numerically, take  $M=10^8 M_\odot$ , where  $M_\odot$  is the solar mass,  $h=R=10$  parsecs. Let the initial density be roughly uniform, and let any pressure gradient be small compared with the force of self-gravitation of the material. Thus the material is effectively in a state of free fall under its own gravitation. Let any initial motion of the material be small, but let it be such that the material is falling towards its equatorial plane. We can then treat the subsequent motion as being mainly towards this plane, because this tends to flatten the distribution and the flatter it becomes the more predominantly the attraction tends to be towards the equatorial plane. If  $t$  is the time for the material to fall the distance  $h$  from either side to the equatorial plane, we find  $GMt^2=hR^2$  approx., where  $G$  is the gravitation constant;  $t$  is the characteristic time for the process we are considering. In our example,  $t$  is about  $5 \times 10^4$  years. The gravitational energy released in the passage to a highly flattened state is of the order of

$\frac{1}{2} GM^2 h/R^2$ , which is  $10^{56}$  ergs using the numbers suggested. Material that falls the full distance  $h$  reaches the equatorial plane with speed about  $(2 GM h/R^2)^{1/2}$ . In the example, this is about 300 km/sec, and the escape-speed from any point on the outside of the initial configuration also has about this value, while the escape-speed from the centre after the system has become highly flattened is about 400 km/sec.

The material will thus tend after about the time  $t$  to form a thin disk of radius about  $R$ . But a thin disk some 10 parsecs in radius would obviously not be a stable state, and before the state is reached the material will therefore fragment into many portions. Each of these portions will proceed to collapse gravitationally on to itself, or will at least tend to do this.

**Temporary stars.** The only fate which we know for a body of material such as we are considering is that most of it should go to form stars. Also we know that no

normal star can have mass greater than some value  $M_{\max}$ , where  $M_{\max} \approx 50 M_\odot$  according to the usual estimate. Consider then two fragments of the sort mentioned at the end of the last section, fragment  $A$  having mass  $M_A$  well above  $M_{\max}$ , and fragment  $B$  having mass  $M_B$  well below  $M_{\max}$ . By definition,  $A$  and  $B$  both start on similar courses of gravitational collapse. In the case of  $B$  this leads to a comfortable career as a normal star. In the case of  $A$ , we know in advance that the material cannot form a single normal star and so that it must sooner or later disintegrate. But the material itself does not know in advance that it cannot form a star. It can discover this only by trying to form a star; it will then discover that its mass is too great, and its only resort is to break up. The material cannot be said to have tried to form a star until it has begun to release energy. It will scarcely discover that it cannot form a star by releasing only gravitational energy, because the gravitational energy released by such a contracting body is not sufficient completely to disperse a large part of its mass. We conclude that at some stage before contraction is halted, nuclear energy generation will start near the centre.

Thus we get a "star" with large mass  $M_A$  and with thermonuclear energy generation proceeding in its central part. Because of the large mass, the rate of energy generation is very great, and as already inferred, the star can have only a temporary existence and will break up. Because of the rapid generation of energy, we infer that the break-up will be explosive.

An estimate which seems plausible is that the explosion will occur after the hydrogen has been used up in a central region of mass about equal to the Chandrasekhar limiting mass  $M^*$ , which is about  $1.2 M_\odot$ . There is no reason for expecting any catastrophe before that, while there is the possibility of catastrophic processes after that. On this estimate, the energy released by the temporary star in its brief career would be about  $0.01 M^* c^2$ , or about  $2 \times 10^{52}$  ergs.

It is natural to compare the inferred explosion of a temporary star with the observed explosion of a supernova. Ginzberg and Syrovatskii<sup>2</sup> quote the energy generated by a number of supernovae as having exceeded  $10^{50}$  ergs and even reached  $10^{52}$  ergs. The nuclear processes preceding a supernova outburst are presumably not the same as those preceding the explosion of a temporary star, but the fact that my estimate shows somewhat more energy to be associated with the explosion of a body of somewhat more mass lends plausibility. It also indicates that the effect of the exploding temporary star should be generally similar to that of a particularly powerful supernova.

*Fragmentation of contracting system.* Suppose that we are dealing with a case in which the fragments of the gravitationally flattened system first formed mostly exceed  $M_{\max}$  in mass. Most then become exploding temporary stars. Some of the material thrown out by the earlier explosions will become enmeshed by the collapsing material in the system, and it will fall back with this material. This is because the energy released in any one explosion is inadequate to check significantly the collapse of any large part of the whole system. Consequently, much of the material may participate more than once in the explosion temporary stars.

As a rough estimate of the total number of explosions  $N$  we shall take  $N \approx M/M_{\max}$ . This is the optimum number of first-time explosions. Although we do not expect to get all these explosions first time, this will be compensated by the fact that some of the material goes through the process more than once. In our example,  $N \approx 2 \times 10^6$ , so the total energy emitted would be about  $0.01 N M^* c^2$ , which is  $4 \times 10^{58}$  ergs for the figures quoted. This would be emitted in time of the order of  $t$ , say  $2t$  in order to make allowance for material taking part in more than one explosion. This gives a mean rate of emission of about  $0.01 N M^* c^2 / 2t$ , or about  $10^{46}$  ergs/sec for the figures chosen. In this example, the values  $N \approx 2 \times 10^6$ ,  $2t \approx 10^6$  y give about 20 explosions a year, so that there would be fluctuations of brightness of the system rather more than once a month on the average.

*Proposed model.* The suggestion is that a violent event in a galaxy is produced by the formation and explosion of a large number of temporary stars in a contracting mass of gas near the centre of the galaxy.

It is further suggested that while the eruptions are in progress the system forms a quasar, and the optical emission is mainly that coming directly from the rapid generation of energy in the temporary stars. It is suggested that the system subsequently becomes a radio-galaxy. In both stages, the radio-emission would be produced by relativistic electrons ejected by the explosions and continuing to radiate for some considerable time afterwards. Thus, at any particular epoch, the optical emission from a quasar would come from a few explosions, perhaps with only one predominating, that happened to occur near that epoch. The radio emission, on the other hand, would come from the relativistic electrons produced in many explosions, perhaps all those that have already taken place up to that epoch. Remembering that we envisage the explosions occurring in a region of the order of magnitude of only 10 parsecs across, it is seen that the electrons from the various explosions will rapidly become intermingled in some sort of cloud enveloping the whole system. The subsequent behaviour of this cloud must depend on the ambient features of the particular galaxy, principally, no doubt, its magnetic field.

*Properties of the model.* Evidently a model of the sort proposed can produce the required rate of energy release over the expected time interval. This is shown by the numerical example, and similar results would be obtained from other starting conditions.

We have also seen that the model gives about the observed frequency of fluctuations in the optical emission of quasars. These fluctuations are produced by the succession of peaks of emission from the succession of exploding temporary stars, with a more or less random distribution in time. If the explosions do show similar peaks of emission to supernova explosions, it seems that the model could give the observed amplitude and the frequency of fluctuations. On the other hand, if the peaks were somewhat less pronounced, or the frequency somewhat greater, then the fluctuations could easily become blurred. So the model is not contradicted by quasars which show no detectable fluctuations.

It seems that supernovae of a given type have a fairly standard pattern of behaviour; the same might be expected of the temporary stars we are considering. If at any epoch the optical luminosity of a quasar is produced predominantly by one such "star", it follows that the peak luminosity of a quasar may be a fairly standard quantity. There is evidence that this agrees with observation<sup>3</sup>.

From the account of the radio emission given in the preceding section, it is seen that in the case of a quasar the fluctuations in the radio emission would not closely match those of the optical emission. In fact, we should not expect to find short lived fluctuations. Also, because of the additive effect of successive explosions, during much of the career of a quasar we should expect a fairly steady increase in radio emission. I believe that all these inferences have some observational support.

According to our model, in a radio-galaxy as distinct from a quasar there are no fresh injections of relativistic electrons, so we expect only a slow, and probably undetectable, decline in power.

The inference that radio sources within the Milky Way Galaxy like the Crab nebula are ex-supernovae is now generally accepted. So, neglecting the foregoing discussion, we might simply assert that a radio-galaxy is so similar to an enormously powerful Crab nebula that, had a whole battery of supernovae been fired off, instead of just one, we should have produced a radio-galaxy. Moreover, remembering that the maximum optical brightness of a supernova may exceed that of the entire galaxy in which it occurs, we can see that during the outbursts we should have something very like a quasar. For we know that it is the excessive optical emission that mainly distinguishes a quasar from a radio-galaxy. Also, if the supernovae were fired off in rapid succession, we should reproduce the rapid optical fluctuations of a quasar.

G. R. Burbidge<sup>4</sup> advanced such ideas at one stage. There is no known way, however, in which supernovae could trigger one another to produce the battery effect needed. By contemplating temporary stars rather than supernovae we appear to secure all the merits of Burbidge's scheme while also finding a natural way in which many eruptions may be associated.

If then we may think of a quasar as consisting of a gigantic "crab nebula", then, by comparison with the actual Crab nebula, we infer that the material of the "nebula" is expanding outwards. It is indeed part of our suggestion that the material seen to be ejected from, say, the galaxy M82 is produced by the same mechanism. Actually, observers have recently reported absorption features in the spectra of quasars that indicate the presence of material moving outwards at very high speed (about 1,000 km/sec), thus giving further support to the model (see, for example, Bahcall, J. N.<sup>11</sup>).

Another essential feature of the model is that the main processes occur in or near a disk. This means that the resultant outward flow would tend to be normal to the disk. This would account in principle for the axis of some sort that so many radio sources possess. There must be a magnetic field if there is to be synchrotron radiation; if the field is strong enough, the outflow would be along the lines of force giving the jets that characterize some sources.

We infer that the emission features in the spectra of quasars arise in the gas in the immediate vicinity of the temporary stars. We have seen that the gas falling into this region has a speed of several hundred kilometres per second, so we should predict considerable Doppler widths for these features; again this is observed. In other respects we should expect the optical spectrum of a quasar to be generally similar to that of a supernova, and this seems to be the case. Furthermore, in our model, while the optical emission of a quasar comes directly from the explosions, the radio emission depends on the conditions that develop in the system as a whole. In particular, if the magnetic field in the system is too weak, we should not get detectable radio emission, but the optical emission would be unaffected. This could account for "quasi-stellar galaxies" that give no detectable radio effects.

If a violent event is produced in the manner suggested, there is no reason why a particular galaxy should not be the scene of several such events at various epochs. Actually, the concept of a radio-galaxy as the scene of multiple outbreaks has been suggested by several detailed studies made by Ryle and his colleagues<sup>5</sup>.

*Concept of exploding stars.* Ebert<sup>6</sup> gave a highly instructive treatment of gravitational contraction of material under external pressure. I then found<sup>7</sup> that the amount of material that could be made to collapse by Ebert's process, in the conditions that he envisaged in interstellar space, would have to be some hundreds of stellar masses. I then suggested that this quantity of matter might form an exploding temporary star, to the fragments of which Ebert's process could be applied afresh to produce normal stars. I published an account of only the first part of this work because I became convinced that the "angular momentum difficulty" defeated the second part; I eventually proposed to overcome this difficulty in a different way<sup>8</sup>. Nevertheless, I mentioned the original idea at the Solvay Conference of 1958 and at that time T. Gold expressed similar views.

A recent stimulating discussion with Dr. D. Lynden-Bell and Dr. I. W. Roxburgh reminded me of these ideas. In the context of the present work, much greater bodies of material are concerned than in the earlier work. The conversation with my colleagues led me to think of contraction towards a disk as a first stage, and to think of its fragmentation as a second stage to which my former ideas might apply. There may still be an angular momentum difficulty, but this may perhaps be resolved by the operation of whatever magnetic field is present.

Observers do have evidence of material flowing into the nucleus of a galaxy, and they do also see material that has been violently ejected. So the availability of material and the occurrence of violent events are not speculative. Also, it is scarcely speculative to say that if the material is present it must ultimately either form normal stars or disperse. Star-formation demands

fragmentation of the material. If at some stage some fragments are more massive than  $M_{\max}$ , then the consequences we have been discussing seem to be almost inevitable. It even seems that the particular masses of the initial fragments (so long as some exceed  $M_{\max}$  are not of great importance; for, as we have said, if some are greatly in excess of  $M_{\max}$ , their material will compose temporary stars more than once.

Thus the operation of the mechanism is qualitatively plausible, while our discussion has shown that the consequences qualitatively and quantitatively appear to be in good agreement with observation. As to the quantitative realization of the starting conditions, we are clearly dealing with rather rare experiences in the history of a galaxy, for at any epoch perhaps only one galaxy in  $10^8$ – $10^9$  can be a strong radio source or a quasar; less spectacular occurrences of similar phenomena may, of course, be quite common.

An attractive feature of these ideas is that they would closely link the phenomena of radio-galaxies and quasars. Nevertheless, there are at present few positive observational links, and there is even an absence of links that might be expected. Crucial tests of the ideas might be had from further observational examination of possible connexions. Theories of quasars that invoke some energy-source other than nuclear energy all seem to require some process or state of matter not yet known to occur. A mechanism requiring a new physical process is that discussed by Hoyle and Narlikar<sup>9</sup> depending on the non-conservation of baryons. A mechanism requiring a special state of matter is any that ascribes the energy production of a system to the mutual annihilation of matter and antimatter. Any mechanism that appeals to the release of gravitational energy in the required quantities leaves the material concerned in a state the existence of which has not been verified. This applies whether we consider energy release from a single very large mass, or from a compact cluster of many bodies, so long as the subsequent release of nuclear energy is not invoked. A theory that does appeal to nuclear processes is that of Wyller<sup>10</sup>.

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## Synthesis of Dolomite

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When the solubilities of calcium and magnesium carbonates approach one another, dolomite will precipitate directly from solution. In sea water this happens at a minimum salinity of four to six times that of normal sea water and when the pH after effective abstraction of carbon dioxide is 8–9

WORK carried out in the past on the synthesis of dolomite under conditions prevailing in marine sedimentary environments has been completely unsuccessful<sup>1</sup>, although synthesis has been achieved at high temperatures and pressures. The experiments carried out by Zeller *et al.*<sup>2</sup> and by Siegel<sup>3</sup>, although using mild or relatively mild conditions, were

not representative of a sedimentary environment because of the peculiar composition of the solutions used in these experiments.

My working hypothesis for the formation of marine dolomites is based on three fundamental postulates. (a) Precipitation of a crystalline compound from an

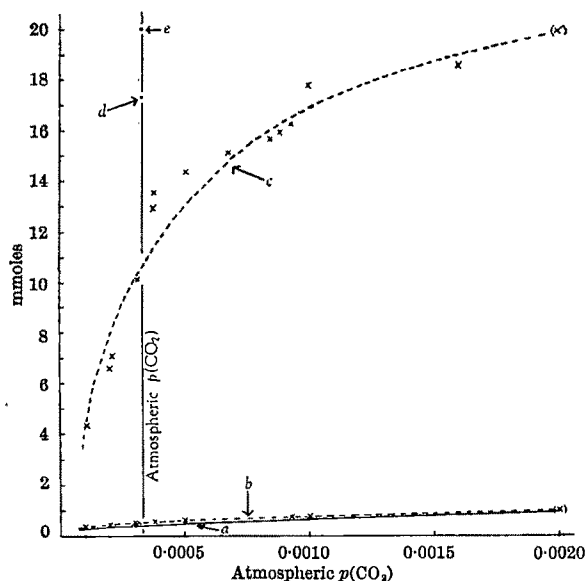
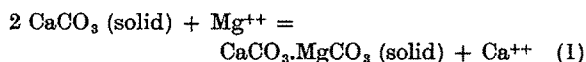
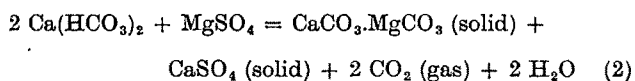


Fig. 1. Solubilities of calcium carbonate and magnesium carbonate in pure water and in sea water at 25°C as functions of carbon dioxide partial pressures. (Data for magnesium carbonate in sea water only available for atmospheric  $p(\text{CO}_2)$ .) *a*, Calcium carbonate in sea water (ref. 10); *b*, calcium carbonate in pure water (refs. 7 and 10); *c*, magnesium carbonate in pure water (ref. 8); *d*, magnesium carbonate in sea water (ref. 11); *e*, magnesium carbonate in sea water (ref. 4).

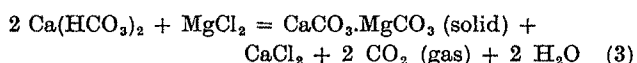
aqueous solution implies that the compound must be in true ionic solution at the moment of precipitation. The same principle applies to any double or multiple salt and the often quoted equation for the dolomitization reaction



is not strictly correct. Assuming that the magnesium ions are supplied by sea water, the dissolved magnesium sulphate will be used up first and then the magnesium chloride, as indicated by the schematic equations



and



(b) If dolomite or any other double or multiple salt is to separate out from solution as a solid, its solubility must be lower than the solubility of its constituents. In the case of dolomite this condition is easily satisfied because its solubility is much lower within a wide range of partial pressures of carbon dioxide, salinities and temperatures than of either magnesium carbonate or calcium carbonate. (c) The relative solubilities of the constituents of the double or multiple salt should be equal or nearly equal at the moment of co-precipitation; that is, in the case of dolomite, the molar solubilities or the solubility products of calcium carbonate and magnesium carbonate should be approximately equal before precipitation starts.

The calculation of the solubilities of calcium carbonate and magnesium carbonate in hypersaline sea water from the equilibrium constants encounters serious difficulties because activity coefficients have only been published for these compounds in normal sea water. Apart from this, one must expect that the various interionic relationships will become more involved as interionic distances decrease and that relevant data for normal sea water<sup>4,6</sup> will have

to be modified and supplemented to become applicable to hypersaline sea water.

Instead of attempting such an approach, use was made of previous solubility determinations<sup>6-13</sup>. The results of these determinations have been assembled in the form of four graphs (Figs. 1-4).

Fig. 1 shows that an increase in the partial pressure of carbon dioxide causes a gradual, although diminishing, divergence of the solubility curves, magnesium carbonate always remaining more soluble in pure water than calcium carbonate. The same certainly applies to the solubilities in sea water, although solubility of magnesium carbonate has only been determined at atmospheric pressure of carbon dioxide.

Fig. 2 confirms the theoretical demand that the solubility of calcium carbonate in pure water should be higher than in a solution saturated with respect to calcium sulphate. It also shows that the difference in solubilities is almost the same over a wide range of  $p(\text{CO}_2)$ . Because of the nearly identical solubilities of calcium carbonate in pure water and in sea water (Fig. 1), it may be concluded that the removal of calcium sulphate from sea water will cause an increase of the solubility of calcium carbonate which is of the same order as the one shown in Fig. 2.

For the study of the influence of salinity of sea water on the solubility of calcium carbonate, data are only available which extend up to 60 parts per thousand salinity. For magnesium carbonate none could be found at all, only solubility determinations in sodium chloride solutions of varying concentrations (Fig. 3). Use was made of these, however, because it may be safely assumed that the behaviour would be similar in sea water, even if the absolute values differ slightly. It can be seen from the graph that at approximately 110 parts per thousand salinity two events take place which seem to contribute to a definite approach towards equal solubilities. At this point the solubility of magnesium carbonate reaches a peak and starts to decrease at a fast rate, while the solubility of calcium carbonate, after the start of precipitation of calcium sulphate, should increase sharply. Although it is highly hypothetical because of the lack of more precise information, the configuration suggests that the two solubility curves will meet eventually, possibly at a salinity of approximately six times the salinity of normal sea water.

In Fig. 4 the firm lines represent the solubilities of calcium carbonate and magnesium carbonate in sea water in relation to pH. No information seems to exist on the influence of pH on the solubilities of the carbonates in hypersaline sea water. Hypothetical curves with dotted lines have therefore been drawn to show in a schematic way the expected changes, that is, the increased solubility of calcium carbonate after the start of precipitation of calcium sulphate and the increased solubility of magnesium carbonate (in the range of salinities from normal to five or six times the normal salinity of sea water).

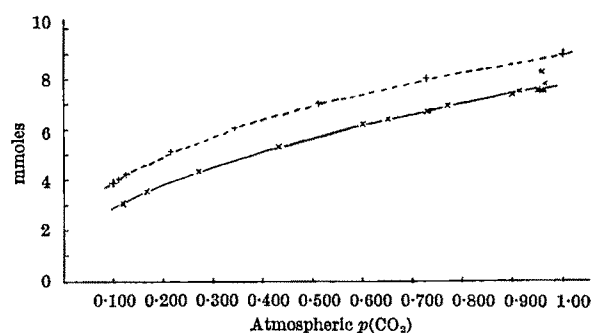


Fig. 2. Solubilities of calcium carbonate in pure water at 20°C (+---+) and in a solution saturated with respect to calcium sulphate at 25°C (x---x), as functions of carbon dioxide partial pressures (ref. 7).

Also shown is the highly probable nullification of this effect by an increase of  $pH$ . Both sets of curves lead to the same target, namely, a meeting point in the region of  $pH = 9.0$ .

The hypothesis which forms the basis of the experimental work has been formulated as follows. The formation of dolomite is a two-step process: the first step consists of the dissolution of calcium carbonate deposits by hypersaline sea water and the second step in the co-precipitation of calcium carbonate and magnesium carbonate. The second step can only take place if the three fundamental postulates listed before are satisfied.

The dissolution of calcium carbonate is furthered by low temperatures, high partial pressure of carbon dioxide, and low  $pH$  apart from such factors as increased salinity and higher pressure. The co-precipitation of the carbonates is promoted by elevated temperatures, low partial pressure of carbon dioxide, and high  $pH$ , apart from other less important factors. The first set of conditions may prevail during cool periods or at night, whereas the second set of conditions will be prevalent during warm periods of the season or in the daytime generally. Photosynthetic activity of certain algae able to survive high salinities may play an important part by abstracting

carbon dioxide from the solution in daytime. This activity will be at rest during the night, while increased carbon dioxide tension promotes the dissolution of calcium carbonate. Decay of organic matter on a large scale will result in the production of carbon dioxide and ammonia, the decay being caused by the mass death of marine organisms swept into the restricted coastal area by the inflowing sea water and immediately killed if the salinity of the water exceeds 70 parts per thousand. There are not many organisms which tolerate higher salinities.

The aim of the laboratory procedure was the synthesis of dolomite under conditions which appeared characteristic for coastal areas with restricted water circulation during periods of aridity. The necessary acceleration of the natural process of dolomite formation was achieved by a strict regulation of the two alternating steps and by helping the dissolution of calcium carbonate in every possible way within reasonable limits.

Using reagents of analytical quality, artificial sea water was made up to the following formula:

	(g)
Sodium chloride	26.83
Magnesium chloride	3.21 (using $MgCl_2 \cdot 6H_2O$ )
Magnesium sulphate	2.24 (using $MgSO_4 \cdot 7H_2O$ )
Calcium sulphate	1.23 (using $CaSO_4 \cdot 2H_2O$ )
Potassium chloride	0.76
	34.27

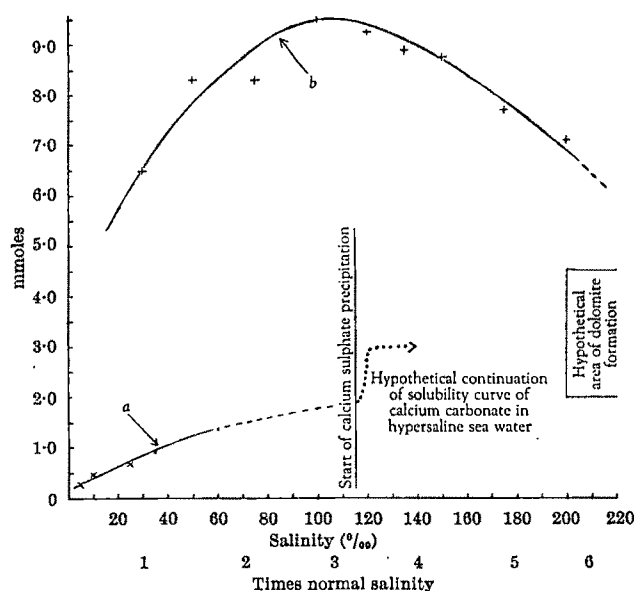


Fig. 3. Solubility of calcium carbonate in mixohaline, normal and hypersaline sea water at 25°C (a, refs. 10 and 13) compared with solubility of magnesium carbonate in sodium chloride solutions of varying concentrations at 23°C (b, ref. 6). (Comparable data for solubility of magnesium carbonate in sea water not available.)

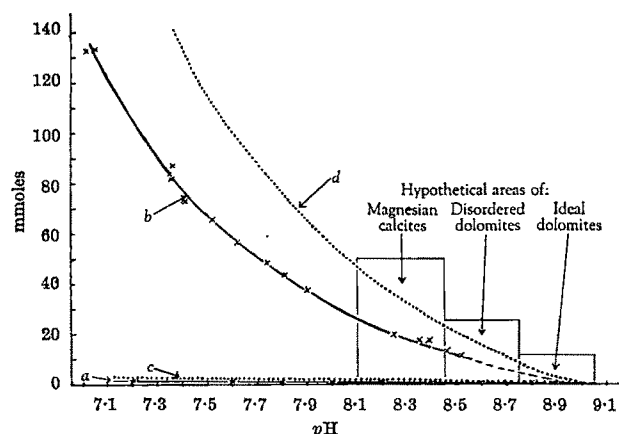


Fig. 4. Solubilities of calcium carbonate (a) (ref. 9) and magnesium carbonate (b) (ref. 11) in normal sea water at 25°C and hypothetical solubilities of calcium carbonate (c) and magnesium carbonate (d) in hypersaline sea water of 4-6 times normal salinity as functions of  $pH$ . (Purely schematic as far as hypothetical curves and areas are concerned.)

the salts being dissolved in purified water to make up 1,000 g. For solutions of higher concentration multiples of the salt mixture were dissolved in purified water and made up to 1,000 g. Calcium sulphate was omitted from many solutions to study the effect of an excess of calcium ions; also none was added to the solution in experiment 57, which was six times the original concentration of sea water. Calcium carbonate in the form of fairly fine calcite crystals was added to each solution in such proportions as to satisfy the dolomitization reactions (2) and (3). Finally, in agreement with the fact that sea water has a preponderance of cations over anions (which gives rise to  $pH$  values ranging from 7.8 to 8.2), the  $pH$  of the solutions was adjusted using a concentrated sodium carbonate solution, aiming at  $pH$  values slightly higher than in normal sea water. This proved impracticable, however, with solutions of higher concentration than three times the original concentration, unless unreasonably high proportions of sodium carbonate were added. Also, after completion of the first series of experiments it was noted that the  $pH$  of all solutions had dropped to values ranging from 7.8 to 7.3. Therefore, in the experiments which followed an additional adjustment of  $pH$  was made in the form of a few drops of diluted ammonia, added to each flask regularly after the completion of the first step and after a certain warming up. This procedure resulted in maintaining a  $pH$  of slightly above  $pH$  8.0 in all mixtures at the time they reached the precipitation stage (step two).

Conical or flat-bottomed round flasks of 500 ml. capacity, fitted with doubly perforated rubber bungs and inlets and outlets for carbon dioxide gas, were filled with 350 g of the experimental solution plus the calculated amount of calcium carbonate. The weight of each flask was then recorded. Each series of experiments consisted of eight flasks linked in series to a carbon dioxide steel cylinder, from which a slow stream of gas was bubbled through the mixtures at a rate of 20-30 bubbles per min. A reaction temperature of 5°-10° C was maintained by cooling with ice. The duration of this first step was 6 h in initial experiments and 12 h in later experiments. The dissolution of calcium carbonate was also helped by occasional shaking of the flasks. After completion of the first step, the flasks were transferred to an electrically heated drying oven, the temperature of which was thermostatically controlled at 43° C  $\pm$  2° C. The flasks were left at this elevated temperature for approximately 60 h, then taken out and the small evaporation losses



made good by the addition of purified water; *pH* measurements in later experiments were carried out at all stages of the process, but only at the start and at the end of the first series.

The temperatures used in these experiments may correspond to actual water temperatures reached at night and in daytime in shallow pools and basins. Maximum temperatures estimated or recorded by various investigators average 40°–50° C (refs. 1 and 14) and may even rise to 70° C under special circumstances. The gradual concentration of the brine by evaporation in the natural environment was duplicated by the use of a range of solutions of definite salt concentrations. The supply of carbon dioxide gas during the first step was undoubtedly more generous in the experiments than in a natural environment. This exaggeration of natural conditions, however, served only to accelerate the dissolution of solid calcium carbonate without affecting the course of events in a fundamental way. Thanks to these measures, one single cycle of dolomite formation took only three days and the accumulating effect was such that one can estimate a total conversion of the calcium carbonate to dolomite as within a few months.

The experiments were performed in an attempt to obtain information on whether it is possible to reproduce the process of dolomite formation in nature by a relatively short laboratory run, envisaging that the process takes shape in alternating steps of dissolution of calcium carbonate and co-precipitation of calcium carbonate and magnesium carbonate. Furthermore, an answer was sought to the question whether the dolomitization reaction is confined to certain limits of salinity as indicated by geological and other evidence; and if it is, then both the lower and the upper limit of salinity could be defined. Also investigated were the effect of an excess of calcium ions, namely, the presence of dissolved calcium sulphate, on the dolomitization reaction, then the suggestion that the presence of ammonium salts, such as ammonium chloride, was absolutely necessary for dolomitization<sup>15</sup>, and finally the effect of *pH* on the course of dolomitization.

Tables 1 and 2 contain all the relevant data of the laboratory experiments.

Some interesting facts emerge from the available data. It has been proved that magnesian calcites, disordered dolomites and probably also well ordered dolomites may be synthesized by a two-step process as already described. The magnesian calcites (as shown by No. 25, Table 1) include some with extremely high contents of magnesium carbonate. The formation of the magnesian calcites and dolomites is very much dependent on the salinity of the sea water. According to X-ray examination, none was formed at normal salinity, although chemical analysis indicated the presence of more than 1 per cent of magnesium carbonate in sample No. 50 after 17 cycles. Whatever the form of this substance is, it may have some

connexion with the presence of the unidentified line at 4.52 Å; however, dolomite formation definitely starts at twice the normal concentration of sea water: weakly if calcium sulphate is present, but quite vigorously if it is left out of the mixture of salts. Production of disordered dolomites appears to reach a peak at three to four times the original salinity, but here again, only if calcium sulphate is not present. As stated before, this condition corresponds to events taking place in the natural environment as soon as 110–120 parts per thousand salinity has been reached. At higher salinities, at least at a salinity of six times the normal concentration of sea water, dolomite is formed at a much reduced rate, but possibly in a condition approaching that of ideal dolomites. The amount of it produced after fourteen cycles was insufficient to prove this point, but sufficient to show the initiation of the main reflexion (104) at 2.88 Å which is characteristic of ideal dolomites. It is also obvious that an upper limit of salinity for the formation of dolomite has not been found in these experiments because it is quite likely that still higher concentrations than six times the normal salinity of sea water may be suitable for this purpose, even if the rate of formation is further reduced. The inhibiting influence of dissolved calcium sulphate is drastically demonstrated by a comparison of numbers 24 and 29, 20 and 22, 52 and 53, 54 and 55. The excess of calcium ions in solution reduces the solubility of calcium carbonate to such a degree that the solubility product remains far below that of magnesium carbonate. The presence of ammonium chloride is not absolutely necessary for the formation of dolomite, although it may be slightly beneficial, as shown by a comparison of numbers 24 and 25, also of 20 and 28.

While the last eight experiments were progressing it was realized that the *pH* at the moment of the start of co-precipitation of calcium carbonate and magnesium carbonate should have reached a higher value than just above *pH* = 8 attained in most samples. Such an adjustment, however, proved a difficult matter because after completion of the first step the *pH* values had dropped in most samples to below *pH* = 6.0. The addition of more ammonia than of a few drops at this stage seemed undesirable because of the possibility of precipitation of basic magnesium carbonates or even magnesium hydroxide. A satisfactory solution of this buffering problem has yet to be found for most salinities, possibly with the exception of six times the normal salinity of sea water. In this latter case the combined effects of increased salinity and slightly increased alkalinity, separately shown in Figs. 3 and 4, may have already created reasonably favourable conditions for the formation of ideal dolomites. The lack of continued *pH* adjustment in the course of the initial experiments has not only adversely affected the quantity but also the quality of the magnesian mineral, as can be seen from a comparison of numbers 24 or 25 with 55. Even in the last experiment

Table 1. EXPERIMENTS CARRIED OUT TO SYNTHESIZE DOLOMITE UNDER CONDITIONS PRESUMED TO CORRESPOND TO A SEDIMENTARY ENVIRONMENT

Experiment No.	Concentrations (× normal salinity)	Solution contains:			<i>pH</i> after adjustment with Na <sub>2</sub> CO <sub>3</sub>	After completion of first step	Measurements:		Duration of steps: 1st (h) 2nd (h)	Number of completed cycles	MgCO <sub>3</sub> * in solid phase (per cent weight)
		CaSO <sub>4</sub>	NH <sub>4</sub> Cl	NH <sub>4</sub> OH			After completion of second step	After completion of experiments			
31	2	—	—	—	8.3			7.3	6	8	0.23
30	2.5	+	—	—	8.2			7.7	6	8	none
29	3	+	—	—	8.6			7.6	6	8	0.40
24	3	—	—	—	8.5			7.5	6	8	1.93
25	3	—	+	—	8.0			7.8	6	8	2.17
22	4	+	—	—	8.6			7.4	6	8	0.40
20	4	—	—	—	7.8			7.4	6	8	1.14
28	4	—	+	—	7.8			7.6	6	8	1.55
50	1	+	—	+	8.3	5.9–6.1	8.1–8.4		12 60	17	1.12
51	1	—	—	+	8.2	5.9–6.1	8.0–8.7		12 60	17	0.37
52	2	+	—	+	8.5	5.7–5.8	8.0–8.1		12 60	16	1.73
53	2	—	—	+	8.5	5.8–6.0	8.2–8.4		12 60	16	5.08
54	3	+	—	+	7.8	5.5–5.7	7.8–8.3		12 60	15	0.74
55	3	—	—	+	8.0	5.6–5.8	8.0–8.2		12 60	15	6.49
56	4	—	—	+	7.7	5.7–6.0	8.0–8.3		12 60	14	6.67
57	6	—	—	+	7.5	5.3–5.7	7.9–8.2		12 60	14	3.12

\*Analytical method. Titration with 1/20 normal EDTA solution using HSN indicator for the determination of calcium (II) and Eriochrome Black T indicator for calcium (II) plus magnesium (II). Because of intensive washing to remove all soluble salts, calcium sulphate was quantitatively removed from the samples and the balance consisted entirely of calcium carbonate.

Table 2a. X-RAY DIFFRACTION DATA\*

		Interplanar spacings in Å of lines referring to magnesian calcites or to dolomites, with hkl indices quoted above.							
No.	?	102	104	110	111 ?	113	?	202	203
31									
30									
29									
24			( )						
25		(3.75)	2.94	((2.74))	((2.43))	((2.23))	((2.04))		((1.32))
22									
20			(( ))						
28			(( ))						
50	(4.52)								
51			( )						
52		((2.96))	((2.92))						
53		(3.74)	2.92†	(2.44)	(2.41)	(2.22)	(2.19)	(2.03)	
54									
55		3.71	2.91 ‡		2.41	2.40	2.20	2.04	2.03
56				((2.79))†					1.97
57			((2.88))						

Key: ( ) = weak, (( )) = very weak, ((( )) = extremely weak.  
 † = Broad peak. ‡ = Peak with improved resolution.

Table 2b. X-RAY DIFFRACTION DATA\*

Estimations from peak heights of:

No.	Magnesian precipitate c/a ratio	Calcite/aragonite ratio	Per cent weight of magnesian calcite or dolomite	Definition of magnesian precipitate	Remarks
31		A little aragonite 19:1			Five weak unidentified peaks
30		Dominant calcite			Five very weak unidentified peaks
29		"			
24		"	Little	Magnesian calcite	
25	3.32 ± 0.02	"		" "	MgCO <sub>3</sub> content possibly 30 mole per cent
22		"			
20		"	Very little	" "	
28		"	A little	" "	
50		1:4			Unidentified line at 4.52 Å
51		1:19	A little	" "	Suggested by slight spread at base of calcite (104)
52		4:1	Traces	Calcite-dolomite intermediates	Suggested by two weak lines at 2.96 Å and 2.92 Å
53	3.30 ± 0.02	13:1	5	Disordered dolomite	
54		9:1			
55	3.33 ± 0.02	Dominant calcite	5	Disordered dolomite	Better resolved main line at 2.91 Å than in No. 53
56		"			
57		"	Trace	Ordered dolomite	Indicated by extremely weak line at 2.88 Å

\* Based on analyses by R. Curtis from smear-mounted samples run at 2°/min on a Philips diffractometer, using copper K $\alpha$  radiation and pulse height discrimination. Additional runs were made of No. 25 at 0.25°/min with and without internal standard.

the results are far from perfect because, owing to the changing laboratory procedure, the dolomite-like products are non-uniform and tend to form broad peaks on X-ray examination. The estimates of dolomite-like products in the solid phase from peak heights do not agree with estimates from chemical analyses. The former are consistently lower and it is suggested that the unusual breadth of the peak may be the cause.

As early as 1917 (ref. 16), N. Kurnakov has found that in the Perekop lakes of the Crimea dolomite was formed by the reaction of calcium bicarbonate carried by rivers with the magnesium salts of the lake. In this special case the dilution of the brine by river water is not critical because the brine represents a highly concentrated solution of magnesium chloride. The difference between dolomite formation in the Perekop lakes and by the two-step process is that in the former case calcium carbonate is being supplied to the brine in a dissolved state, whereas in the second case the brine effects the dissolution of solid calcium carbonate. Even in the Perekop lakes dolomite would be formed by a modified two-step process, probably caused by differences in temperature, corresponding to night—and day—conditions. It may be assumed that this process universally applies to coastal areas with restricted water circulation, as well as to inland lakes which represent remnants of ancient seas.

In connexion with the two-step concept of dolomite formation certain geological problems appear in a new light, among them the deposition of calcium carbonate of organic and inorganic origin, porosity and texture of

dolomites, their role as reservoir rocks for petroleum, the composition of oil-field waters and other problems.

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# Mylonite Zones and Mylonite Banding

by

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Mylonite layers show evidence of thinning and flattening. They are thought to develop approximately perpendicular to the direction of principal stress much the same way as in the development of cleavage in slate

MYLONITE zones have been recorded from many parts of the world. They occur in association with both thrust and wrench faults, and more rarely, as narrow zones which are quite independent of fractures. They exist in both low-grade (for example, the Moine thrust) and high-grade<sup>2</sup> metamorphic environments.

The common misuse of the term mylonite has been noted by Christie<sup>3</sup>; many cataclastic rocks are incorrectly referred to as mylonites (for example, the zone at the base of the Morcles nappe in the Swiss Alps, first recognized by Lugeon, is not composed of mylonite). In Lapworth's<sup>4</sup> definition, mylonite is a banded micro-breccia—that is, it possesses “fluxion structure”. I accept that the presence of banding is crucial but question whether its significance has been properly understood. Lapworth did not explicitly assign a dynamic significance to the banding; he noted that “mylonites formed along thrust planes when two superposed rock systems moved over each other as solid masses”. Later writers have stressed the “rolling out”, “milling”, “differential movements” that were supposed to have operated along the mylonite banding. Turner and Verhoogen<sup>5</sup> define mylonite fabrics as “rock fabrics of *S*-tectonite class that have evolved under the influence of penetrative movement on one set of parallel or sub-parallel *s*-surfaces”; of the banding they say that the “fluxional lamination . . . reflects a sliding movement in a single set of parallel *s*-planes”, also the bands are “surfaces of strong differential movement”. As a general comment on the origin of mylonite zones they state that “notable relative displacements with which they (mylonites) commonly are associated have been distributed through but small thicknesses of rocks”. The latter notion on the origin of the zones is echoed in the use of the term “movement-zone” for the mylonites along the Moine thrust<sup>1,3,6</sup>. Obviously a considerable vagueness persists on the topic of mylonites. But, although nowhere stated explicitly, it seems reasonable to suppose that the common presumption is that mylonites have developed along surfaces of high resolved shear stress<sup>7</sup>, that is parallel to thrust faults.

A simple application of Coulomb theory<sup>7</sup> to mylonite zones is open to general objections, in the same way that the application of the theory to geological fractures is far from satisfactory. It is generally supposed that faults and mylonite zones are alternative expressions of planes of high resolved shear stress in a stress field; the mylonites reflect creep fracture, well known in experimental investigations and characteristic of shear under higher confining pressure and temperature and slower strain rates than the brittle fractures. Alternative theories of brittle fracture in solids, for example, the Griffith theory of fractures<sup>8</sup> or the principle of least work (compare with Kehle<sup>9</sup>), considerably complicate the simple Andersonian concept of faulting. These doubts make it important to reconsider the orthodox interpretation of mylonite zones and mylonite banding.

There are two possible fallacies in the “simple shear” hypothesis for the origin of mylonite zones. The milling and grinding of pre-existing crystals involved in mylonit-

ization do not necessarily involve the operation of a component of high shear stress parallel to the banding, and the streaking out of comminuted rock flour along the banding is more consistent with the banding being a surface of low resolved shear stress, that is, a surface of elongation. Second, it is well known that resistant particles and minerals in mylonites possess an ovoidal shape, for example, porphyroclasts of feldspar in mylonitized granites. The ovoidal porphyroclasts are ellipsoidal, with the longer dimensions parallel to the banding and the shortest dimension roughly perpendicular to it. This pattern of dimensional orientation is also seen in smaller porphyroclasts and in the matrix aggregates of quartz. The banding looks like a coarse slaty cleavage and if this analogy is accurate then the following question arises. Does mylonite banding have the same origin and kinematic significance as slaty cleavage? The evidence in favour of the positive answer to this question may be reviewed by discussing the mylonites from the Moine thrust zone, which have been extensively investigated. Since the nineteenth century it has been apparent that slaty cleavage develops normal to the direction of shortening or in planes of low resolved shear stress<sup>10,11</sup> (along cleavage surfaces rock particles have undergone elongation; compare Cloos's oolites<sup>12</sup>). The relevant evidence as to whether mylonite banding also develops along a plane of low resolved shear stress is summarized here.

The precise geometrical relationship of mylonite banding to fold axial planes is generally unknown. Christie<sup>13</sup> describes the development of “schistosity” in intrafolial folds in the mylonites at the Stack of Glencoul, Assynt, as follows. “During slip on the slip surface, . . . , the surfaces become unstable and bend into flexural slip folds (Biegefallen). . . . A new schistosity develops by shearing of the limbs of the smallest-scale folds. . . . This schistosity is more stable than the original, but it is rapidly rotated toward the plane of the latter”. This description should be clarified as follows. First, the “new schistosity” is the mylonite banding. Second, the “slip surfaces” are bedding planes (which, contrary to Christie's view, give the colour banding in the Stack mylonites). Third, the distinction of bedding (“slip surfaces”) and mylonite banding (“new schistosity”) is obvious in fold cores, but, of course, on the fold limbs the two surfaces are subparallel. These observations can be confirmed in mylonitic rocks from many parts of the Moine thrust zone, also in Torridonian, Lewisian<sup>1</sup>, Cambrian and Moine rocks in this zone. Generally mylonite banding follows a pre-existing compositional layering (foliation in gneisses or bedding in sediments), but at fold cores it transects the layering and thus simulates axial plane cleavage. The postulated analogy between mylonite banding and slaty cleavage is supported by the dimensional orientation of particles in mylonites and mylonite gneisses, a point noted above. Commonly ovoidal porphyroclasts have tails which are normally made up of a recrystallized aggregate of quartz and feldspar which extend out along the banding from the porphyroclast,

tapering-off gradually<sup>1</sup>. The banding curves around the ovoid and the tails, which are sheltered areas, or "pressure shadows", in which macro-crystals have grown. Such a structure is more in accord with the flattening which operates normal to the banding than with the notion of "shear planes" flowing around the porphyroclasts. In fact the ovoids are akin to boudins and they may, indeed, represent relics of once continuous layers of relatively competent material<sup>1</sup>. The "boudins" have become widely separated. Pinched-out forms are also exhibited by small porphyroclasts; and the recrystallized quartz grains in the matrix sometimes show elongation in the direction of the banding. If the banding was a surface of simple shear one could predict that the longest dimensions of particles would lie at an angle to the banding (again, compare the oolites in the South Mountain Fold<sup>12</sup>). Additional evidence that the banding is a surface of tensile strain is that the larger feldspar ovoids in mylonites are commonly split open by tension cracks, which are infilled by notably large quartz grains and lie at a high angle to the banding. Also there are (?) shear fractures in the porphyroclasts: the banding tends to bisect the obtuse angle between intersecting fractures.

The conclusion is that considerable elongation has taken place along the banding during mylonitization; the so-called "streaking out" of rock-particles on the banding shows that the latter was a direction of extension in the strain-field. In terms of the deformation ellipsoid, the longest axis was parallel to the banding. It should be possible to define the deformation ellipsoid fairly accurately using the shapes and orientations of porphyroclasts. In some mylonites there is no obvious preferred orientation of the longest axes of feldspar ovoids. This suggests that extension was permitted in various directions in the banding, which therefore contained the *zy*-plane of an oblate deformation ellipsoid (*S*-tectonites in Flinn's terminology<sup>14</sup>). Other mylonites exhibit a marked linear tendency in the longest dimensions of ovoids (*L*-tectonites). Thus, for the Moine thrust belt, at least, the shapes of the deformation ellipsoids vary from  $K = \infty$  to  $K = 0$  (ref. 15). Possibly the constriction-type deformation ( $1 < K \leq \infty$ ) has been superimposed on a primary *S*-tectonite fabric.

The quartz microfabric in the mylonites from the Moine thrust belt exhibits orthorhombic symmetry. Christie<sup>13</sup> interprets this microfabric as implying non-translative movements following an episode of translative movement expressed by folding. If the orthorhombic quartz microfabric reflects the presence of a flattening component in the folding process, however, there is no need to postulate two episodes for deformation<sup>14</sup>. The orthorhombic microfabric, in which one symmetry plane coincides with banding, accords with the interpretation of mylonite banding as being a plane of flattening.

Further evidence on the kinematic significance of the main foliation in the Moine thrust zone (that is, the foliation—including mylonite banding—and the synchronous schistosity developed outside the actual mylonite zones) is forthcoming from study of the deformation of "pipes" in the Cambrian quartzites. These pipes have previously been neglected although they are useful strain markers.

The pipes have been rotated nearly parallel with the bedding, involving a rotation through 90°. During rotation the pipes have been distorted from an originally cylindrical shape into flattened sheets, some so thin that they resemble leaves of tobacco. The plane containing the pipes is a cleavage, and is obviously a plane of flattening. In some localities the cleavage is roughly parallel to the bedding (for example, above the Moine thrust on the Stack of Glencoul); elsewhere it is oblique to bedding. The progressive deformation suggested by the pipe deformation is that of rotation (and related flattening) of pipes towards an equilibrium position sub-parallel to the bedding, that is, into a sub-horizontal attitude. This

can be depicted as a path of structural movement for a line (pipe) on Flinn's diagrams—the line rotates towards the *zy*-plane of the deformation ellipsoid ( $1 \geq K \geq 0$ ). It may be mentioned that the path of structural movement for the pipes lies in a vertical plane running W.N.W.–E.S.E. and this is perpendicular to the "deformation plane" deduced for the folds (compare Christie<sup>13</sup>).

There is no doubt that the interpretation of the banding in mylonites as a surface of flattening strain applies to mylonites from other places.

In conclusion, a few comments on the general setting of mylonites in the Moine thrust zone are useful in this context. The mylonite zones are zones of intensive flattening ( $0 \leq K < 1$  modified to  $1 < K \leq \infty$  in certain domains) and are not necessarily related to any large scale translative movements. It must be realized, however, that unless mylonitization is accompanied by volume reduction the development of the mylonite zones results in "translative" movement, that is, the flattened mylonitized zone must be displaced relative to the undeformed surrounding rocks.

The development of mylonite zones is correlated with the progressive increase in flattening strain encountered in a traverse from west to east across the Moine thrust zone, that is, structurally upwards. For example, in the Loch Alsh area<sup>6</sup> it is possible to recognize the following changes: (a) in the west around the core of the large-scale Loch Alsh fold there is only weak flattening, clastic grains are undeformed and cleavage is not strongly developed; (b) eastwards across the inverted Torridonian in the Loch Alsh fold flattening becomes more intense, clastic grains are deformed, a strong cleavage is found and various quartz vein systems have rotated towards the plane of cleavage while in some localities (for example, Ard Hill, Balmacara) extensive variations in the axial trends of early folds have resulted from passive rotations of the axes during elongation of the rocks along cleavages; (c) in the overlying Lewisian rocks flattening varies in intensity and is most marked in thin mylonite bands which are oriented roughly parallel to the axial plane of the Loch Alsh fold.

If the discussion is extended to take account of displacement in orogenic belts, then the inferred picture of movement (involving pure compression and extension) for mylonites suffers from the same defects as the generally favoured "flattening hypothesis" for slaty cleavage development. Thus it fails to distinguish between rotational and non-rotational strains. For example, in both pure strain and simple shear it is predicted that composite surfaces with complex kinematic histories can be generated. For example, in progressive simple shear, planes of "flattening" may be rotated into near-parallelism with shear planes. Although such factors complicate the dynamic analysis of geological structures, the deduced picture of movement for simple, regionally developed surfaces like slaty cleavage and mylonite banding probably reflects a uniform strain history for these surfaces.

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# Causes of Birefringence in Diamond

by

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By optical and X-ray techniques many different causes of strain birefringence in diamond can be distinguished. The perfect diamond, free from strain, is an elusive ideal

In a recent paper, Tolansky<sup>1</sup> reports that of 5,000 good quality diamonds he examined, not one was found free from birefringence. Any strain except uniform dilatation will produce birefringence in diamond. Tolansky's observations become less surprising when viewed against the evidence accumulated in the last couple of decades both for the diversity of strain-producing defects in diamond and for their inhomogeneity of distribution in any stone. The "perfect" diamond becomes ever more elusive as it is pursued with increasingly sensitive tests for imperfection. This article attempts to describe some characteristic patterns of birefringence in diamond and to explain them in terms of the distribution of the particular strain-producing defects responsible for them. The types of defect causing strain have been classified, for convenience, into five groups: (i) dislocations, (ii) lattice parameter variations, (iii) precipitates and inclusions, (iv) fractures and (v) plastic deformation.

(i) *Dislocations*. Many diamonds show a concentration of strain birefringence near their geometric centre. Birefringent regions sometimes extend outwards from this centre, like rays. An association of such patterns with dislocations radiating from the crystal nucleus was first suggested by Frank, Puttick and Wilks<sup>2</sup>. Correctly interpreting as etch pits the pyramidal pits which form one class of the equiangular-triangle-bounded pits known as "trigons" commonly observed on natural octahedral faces, they noted that in a specimen possessing a cluster of trigons on every face, each cluster lay roughly at the foot of the perpendicular dropped from the central strain nodulus to the face concerned. Such a disposition of pits would be expected if they were the outcrops of "grown-in" dislocations generated at the imperfect nucleus and subsequently entrained in the crystal along lines radiating outwards to the growing faces. The association suggested by Frank *et al.* was confirmed by X-ray topography<sup>3</sup>. Further X-ray topographic studies, in which the correspondence of dislocation outcrops with apices of pyramidal trigons was demonstrated<sup>4</sup>, and also more recent investigations on both whole stones and sections, have shown that there are many specimens in which radiating bundles of dislocations were obviously the agencies responsible for the major manifestations of birefringence in the specimen.

Fig. 1a shows a birefringence topograph of a polished slice of clear white diamond cut from the girdle region of an octahedron. The print is a positive: black means extinction between crossed polarizers; light is failure to extinguish, that is birefringence. The contrast of the print has been enhanced so as to display better the set of rectangular "frames" of relatively weakly birefringent lamellae which cover most of the section, and which arise from variations in lattice parameter (ii). Visual observation shows that the birefringence is strongest at the centre of the "star" and decreases somewhat outwards along its rays. From the X-ray topograph (Fig. 1b) it appears that this slice has included the nucleus of the crystal, and that it is dense bundles of dislocations radiating from the nucleus that produce the birefringence star. The greater sensitivity

and topographic resolution of the X-ray technique enable the bundles to be resolved into individual dislocations where they are less dense, and disclose in various parts of the slice many individuals which escape detection by birefringence. A uniaxial strain of only one part in  $10^6$  should produce measurable birefringence in a diamond plate 1 mm thick. Such high sensitivity to strain can be realized with simple optical apparatus when the field observed contains strains differing by this amount or more; for then, with strong illumination and careful exclusion of scattered light, the small variations in extinction over the area of the specimen can be recorded directly by high contrast photography. The X-ray diffraction method requires strain gradients for the production of contrast:

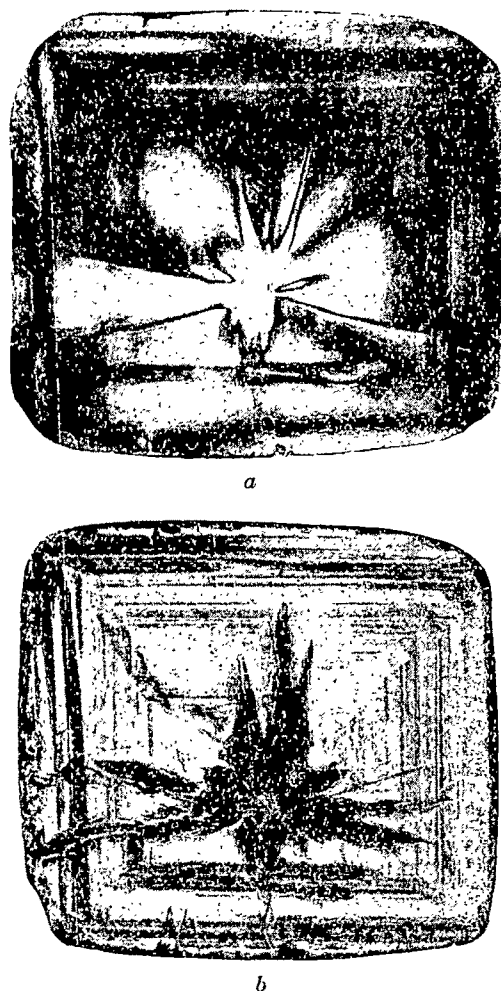


Fig. 1. Diamond slice cut parallel to (001), edge length 6 mm, thickness 1 mm. a, Birefringence topograph. b, X-ray topograph, 400 reflexion, molybdenum K $\alpha$  radiation.



only inhomogeneous strains can be detected, but with a sensitivity that surpasses that of the optical method. An earlier report<sup>4</sup>, dealing with observations on complete natural octahedra, stated that "possibly some hundreds of dislocations are required to produce noticeable birefringence". With polished slices, which are free from strains due to surface damage, it appears possible to detect by birefringence small bundles of dislocations the resultant Burgers vector of which is perhaps only 3 or 4 unit Burgers vectors.

(ii) *Lattice parameter variations.* The concentric rectangular "frames" which appear in Figs. 1a and, better resolved, in Fig. 1b are traces of octahedral planes. Such patterns, together with those produced by etching polished slices<sup>5,6</sup>, give a valuable stratigraphic record of the growth history of the stone. They testify to non-uniform conditions of growth, with consequent non-uniform concentrations of incorporated impurity. The record in Figs. 1a and b is unusually simple for diamond: it suggests growth with unchanging regular octahedral habit. A more complicated history, involving a change from rounded to octahedral habit, is indicated by the birefringence, X-ray diffraction and ultra-violet absorption topographs of one of the specimens investigated by Takagi and Lang<sup>7</sup>, and is displayed in striking form in the etch patterns of Tolansky<sup>1,6</sup> and some of those of Seal<sup>8</sup>. (A straightforward explanation of the geometry of Seal's and Tolansky's etch patterns has recently been put forward by Frank<sup>9</sup>.) The chemical nature of the impurities whose non-uniform incorporation produces strain in Fig. 1 cannot be discovered from birefringence and diffraction topographs. Neither can it be stated whether they are incorporated on an atomic or coarser scale, except that the state of division must be finer than about  $1\mu$ . Larger aggregates would probably produce strain fields which could be individually resolved by both the X-ray and optical techniques. It is pertinent to recall the pioneer birefringence topographs of Raman and Rendall<sup>9</sup> and the X-ray topographs of Ramachandran<sup>10</sup>. Raman and Rendall's specimen D179 (shown in their Fig. 1) contains a central concentration of birefringence like that shown in Fig. 1a here. Ramachandran's X-ray topograph of D179 shows a corresponding central region of strong diffraction contrast, but does not resolve dislocations. Raman and Rendall's specimens D174, 178 and 179 show growth stratifications parallel to octahedral faces, forming concentric octahedral shells. Ramachandran's careful determination of the sign of the uniaxial birefringence of individual lamellae interspersed in diamonds (Type I) opaque to ultra-violet showed that some lamellae are in a state of tension and some in compression<sup>11</sup>. This certainly suggests that more than one type of impurity is responsible for the differences in mean lattice parameter of adjacent layers of diamond, from which differences the coherency strains arise. Mismatch of this sort can be accommodated by arrays of dislocations lying in the surface separating regions of different lattice parameter. An array probably performing this function is seen in plan in Fig. 7 of the article by Takagi and Lang<sup>7</sup>. Others, seen edge-on, appear in Fig 2c. The slice of which the birefringence, ultra-violet transmission and X-ray diffraction topographs appear in Figs. 2a, b and c, respectively, had the same thickness and orientation as the slice shown in Fig. 1, but was somewhat larger. It was also much more imperfect, containing cracks and large inclusions whose strainfields dominate the birefringence pattern Fig. 2a and which, as seen in the X-ray topograph Fig. 2c, cause either strong diffraction contrast or, in some regions, a misorientation sufficient to tilt the region away from the Bragg reflecting position altogether. The one impurity known to be present in variable concentration is nitrogen, since its presence, when precipitated in platelet form<sup>7,12</sup>, is associated with ultra-violet absorption<sup>13</sup>. The ultra-violet transmission topograph Fig. 2b (kindly provided by Dr. W. Kaiser) shows a roughly square band of relatively highly transmitting material. This appears because the slice cuts through an octahedral



Fig. 2. Diamond slice roughly parallel to (001), edge length 9 mm, thickness 1 mm. a, Birefringence topograph. b, Ultra-violet transmission topograph. (Blackening on image corresponds to high transmission.) c, X-ray topograph, 220 reflexion, silver  $K\alpha$  radiation.

shell, roughly 1 mm thick, in which the nitrogen concentration is about  $2 \times 10^{19}$  atoms/c.c., which is about a fifth of that ( $\sim 10^{20}$  atoms/c.c.) in the more absorbing central and outer parts of the slice. (Diagonal striae on Fig. 2b are to be ignored: they arise from refraction at saw-cut grooves on the specimen faces.) A relatively steep nitrogen concentration gradient appears to be present at the lower, horizontal outer boundary of the band. This boundary correlates with the innermost of three horizontal bands of blackening on the X-ray topograph. From this and other topographs it appears that these bands contain arrays of dislocations; some of the dislocations in each band become entrained in the growing crystal and form the "cascade" of dislocations seen in the lower part of Fig. 2c. The regions of high gradient of nitrogen concentration, that is the inner and outer boundaries of the transmitting octahedral shell cut in Fig. 2b, have no manifest correlation with any feature on the birefringence topograph Fig. 2a, though the strains due to cracks and inclusions may be masking small birefringence variations. It is clear, on the other hand, that the bands of strongest diffraction contrast (note especially the bands running vertically near the left margin of Fig. 2c) and which correlate well with bands of birefringence on Fig. 2a, have no corresponding feature on Fig. 2b. If they are associated with abrupt changes in nitrogen concentration, then such changes must be confined within layers not more than about  $10\mu$  thick in order to escape detection in the ultra-violet transmission topograph (Fig. 2b) assuming that the layers are parallel to octahedral planes and hence make  $35^\circ$  with the specimen normal.

(iii) *Precipitates and inclusions.* The strains arising from incorporation of inclusions large enough to be easily visible optically are an obvious cause of birefringence. Such inclusions may or may not be the source of dislocations generated by lattice closure errors arising as the foreign body is enveloped by growing crystal: this question will be answered by X-ray rather than birefringence topography. Generation of dislocations at inclusions is very common. Small bodies in the micron range of size are a cause of birefringence less readily identified optically. These small bodies may be inclusions mechanically incorporated at the growing crystal surface, or precipitates formed after growth: the distinction can probably be made if dislocations are present. Inclusions often are a source of grown-in dislocations, as already mentioned, whereas precipitates are observed strung along previously grown-in dislocations, like beads on a thread<sup>14</sup>. In general only the larger strain centres visible on X-ray topographs can be correlated individually with those detected by birefringence<sup>7,14</sup>.

(iv) *Fractures.* Strains associated with internal cracks produce both X-ray diffraction contrast and visible birefringence. Cracking is frequently found in the otherwise rather perfect cores of coated diamonds<sup>15</sup>. Natural diamonds have suffered much surface damage: edges are burred and faces bear many percussion marks in the form of partial or complete ring cracks. On X-ray topographs of such surfaces, it is only too evident that all faces are peppered with images of ring cracks. It is found that ring cracks the diffraction images of which spread  $25\mu$  or more wide on topographs taken with molybdenum  $K\alpha$  or silver  $K\alpha$  radiation produce, individually, clearly visible birefringence figures. Cracks of such magnitude occur with a density around  $10/\text{mm}^2$  of surface. To the extent, then, that all natural diamonds have damaged surfaces, all are birefringent. (It may be noted that the birefringence generated by deliberately produced ring cracks with diameters of  $100\text{--}200\mu$  is extremely intense<sup>16</sup>; but the birefringence produced by the strainfields of micro-abrasions<sup>17</sup> is quite slight.) If cleavage occurs in an irregular fashion, as it does when diamond plates fail during bending experiments<sup>18</sup>, strong birefringence and diffraction contrast are generated by strains associated with subsidiary cracks accompanying the fracture<sup>19</sup>. It is

thus not unexpected that a high incidence of birefringence is found among diamond chips (that is, cleavage fragments)<sup>1</sup>.

(v) *Plastic deformation.* A type of birefringence found in many Type II (nitrogen-poor) stones<sup>9,11,20</sup> is exemplified in Fig. 3. Such specimens were called "laminated diamonds" by Ramachandran. A descriptive term proposed<sup>7</sup> for this pattern which avoids any suggestion of connexion with growth layers is "tatami"—after the Japanese rice straw mat. The birefringence is very strong. In Fig. 3 it occurs in lamellae dominantly parallel to one octahedral plane. In other specimens equally well developed intersecting birefringent lamellae parallel to more than one octahedral plane can occur. Ramachandran reported that lamellae occasionally occur parallel to dodecahedral planes. Significant features of the tatami pattern are that lamellae parallel to different crystallographic planes intersect each other and also that they cut right through growth stratifications. The latter occurrence could be demonstrated with the specimen of Fig. 3 which was found by M. Takagi to contain some growth layers with a sufficient nitrogen platelet content to show up in ultra-violet transmission topographs. They formed a series of roughly concentric shells centred about a point slightly to the right of the centre of Fig. 3. The similarity of the tatami birefringence patterns to those produced by slip-bands in plastically deformed transparent crystals<sup>21</sup> is so close that its explanation, proposed by Frank, as due to varying amounts of slip, on one or more slip planes, at some stage after growth, appears irresistible. The lower plastic yield stress of nitrogen-poor diamonds (which often show tatami patterns) compared with nitrogen-rich diamonds<sup>18</sup> could account for the greater probability of finding plastic deformation in the former.

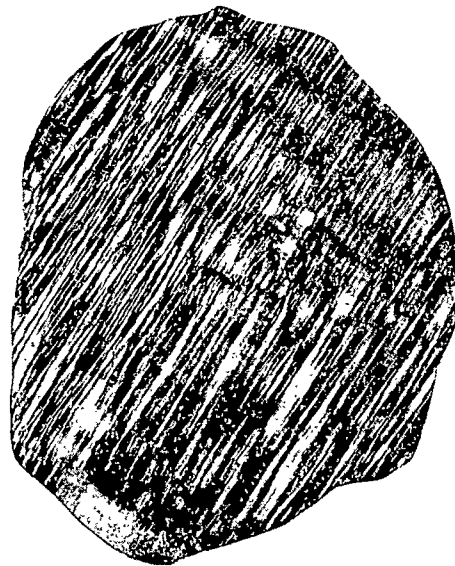


Fig. 3. Birefringence topograph: "tatami" structure. Specimen width 8 mm, thickness 1 mm.

Another type of birefringence pattern found in Type II diamonds is shown in Fig. 4. This is very weak: an exposure forty times that of Fig. 3 was needed to record the pattern, and it could easily escape notice. It was the highly imperfect crystal lattice, found by X-ray topography to approach the ideal "mosaic", that first attracted attention<sup>19</sup>. The X-ray and birefringence topographs look rather alike. There is some overall long range warping, but the characteristic feature is the division of the whole specimen into cells ( $\sim 10\mu$  diameter) in the walls



Fig. 4. Birefringence topograph: "annealed" structure. Specimen width 4 mm, thickness 0.5 mm.

of which reside strains, and, presumably, dense, unresolvable dislocation networks. It is believed that this pattern represents an annealed plastically deformed crystal: strains have been greatly reduced compared with those seen in Fig. 3, and the dislocations have been largely re-arranged to lie in cell walls which bear no obvious relation to the original slip planes.

The foregoing account has indicated the variety of causes of birefringence of diamond. Tolansky's<sup>1</sup> invocation of an ever present intermixing of Type I and Type II layers is unnecessary. Indeed there is evidence that it is not generally present. Growth in layers alternately poor and rich in nitrogen platelets, repeating with a period of 5 $\mu$  or less, would be detected by electron microscopy<sup>12</sup>; and if such occurred with a larger period, and was accompanied by strains sufficient to produce birefringence, it would be detected by X-ray topography. Sharp gradients of platelet concentration do, however, exist at particular

growth horizons<sup>7,12</sup> and a single such gradient in the specimen should produce observable birefringence unless dislocation arrays exactly compensate the dimensional mismatch across the boundary where a given number of unit cells of pure diamond meets the same number of cells of a mixture including some cells of platelet<sup>22</sup> structure with those of pure diamond. But birefringence demonstrably arising from other causes is usually observed.

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## Flavins in a Solid Matrix

by

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Organized aggregates of flavins and other chromophores play a part in the functioning of biological photoreceptors; this has prompted a study of photochemistry and energy transfer of flavin molecules in methyl cellulose films

THE increasing awareness that chromophores in biological photoreceptors form part of an organized or semi-organized system led us to examine the photochemistry of flavins in transparent films of methyl cellulose. The solution photochemistry of these compounds is quite well understood<sup>1</sup>.

**Properties of the films.** Thin (0.0025 cm) transparent films of methyl cellulose can be prepared by spreading a layer of aqueous methyl cellulose solution (2.25 g/100 ml.) on a glass plate, and allowing it to dry. If the solution contains other compounds, then they are included in the film. Methyl cellulose is hygroscopic, and after atmospheric drying the films retain 5 per cent water by weight (measured by heating to constant weight) which shows as an absorption at 1,640  $\text{cm}^{-1}$  in the infra-red spectrum of the film<sup>2</sup>. Flavin mononucleotide (FMN) does not alter the infra-red spectrum of an empty film except to superimpose the flavin absorptions which are at 1,545, 1,580, 1,640 and 1,710  $\text{cm}^{-1}$  (ref. 3). Compared with the spectra in water the visible absorption band of FMN is unchanged in methyl cellulose ( $\lambda_{\text{max}}$  447  $\text{m}\mu$ ), but there are small shifts in the near ultra-violet band (375–368  $\text{m}\mu$ ) and in the fluorescence emission (530–535  $\text{m}\mu$ ). These shifts are compatible with the film providing a less polar environment than an aqueous solution<sup>1</sup>.

It might be expected that trapping a chromophore in a solid film would considerably restrain, if not completely prevent, its molecular motion (translation and rotation). The fluorescence polarization of FMN and flavin-adenine dinucleotide (FAD) in our films showed that rotational

restraint is incomplete. The period of rotation of FMN calculated from its fluorescence polarization in water (0.01) by Weber's method<sup>4</sup> is  $10^{-8}$ – $10^{-9}$  sec. Low polarizations were also measured when FMN, FAD, rose bengal and eosin were dissolved in concentrated aqueous solutions of methyl cellulose which had macroscopic viscosities comparable with glycerol (Table 1). Even in films dried over calcium chloride for 12 h complete rigidity was not observed, the polarization of FMN rising to 0.31 (Table 2). This is well below the value of 0.43 which is measured for a solution of FMN in glycerol.

Table 1

(a) FLUORESCENCE POLARIZATIONS IN AQUEOUS METHYL CELLULOSE SOLUTIONS

Methyl cellulose concentration (g/l.)	FMN	Fluorescence polarization FAD	Eosin	Rose bengal
0	0.014	0.024	0.075	0.142
3.26	0.017	0.029	0.114	
6.41	0.015		0.145	
9.62	0.020	0.034	0.162	
12.83	0.022		0.177	
17.66	0.025	0.043	0.189	
22.50	0.029	0.043	0.195	0.204

(viscosity = 5.5 poise)

(b) FLUORESCENCE POLARIZATIONS OF FMN IN WATER/GLYCEROL MIXTURES

Viscosity of water/glycerol mixture (in poise)	Fluorescence polarization
10	0.430
6.6	0.392
4.6	0.384

Table 2. FLUORESCENCE POLARIZATIONS OF FMN IN METHYL CELLULOSE FILMS

Optical density of FMN	Fluorescence polarization
0.2	0.28
0.2*	0.31
0.2†	0.25
0.2*†	0.28
0.5	0.29
~5.0	0.25
~10.0	0.20

\* These films were dried for 12 h over calcium chloride.

† These films were made from solutions of methyl cellulose concentration half that normally used.

The low polarizations in methyl cellulose solutions and films might have been caused by light scattering from the methyl cellulose or energy transfer between chromophores. The emitted light is observed at 90° to the exciting beam which necessitates the use of an angled sample position for solids. This does not affect polarization values by more than 15 per cent as was found by studying a solution in the angled and normal positions. Depolarization by light scattering was shown to be unimportant in two sets of experiments: (a) FMN polarization in glycerol was only lowered by 10 per cent in the presence of solid 'Sephadex' which scattered far more light than a methyl cellulose film, and (b) the fluorescence polarization of the flavo-protein lipoyl dehydrogenase (molecular weight 10<sup>6</sup>) is 0.43 in both water and a concentrated aqueous solution of methyl cellulose. The result of (b) also means that depolarization by energy transfer is unlikely. For a given concentration of FMN the fluorescence in films made from solutions of different concentrations of methyl cellulose differs (Table 2). The more concentrated solutions give films with higher polarizations, which is compatible with depolarization by molecular rotation of the chromophore but not with energy transfer, although this does occur at high FMN concentration (see later).

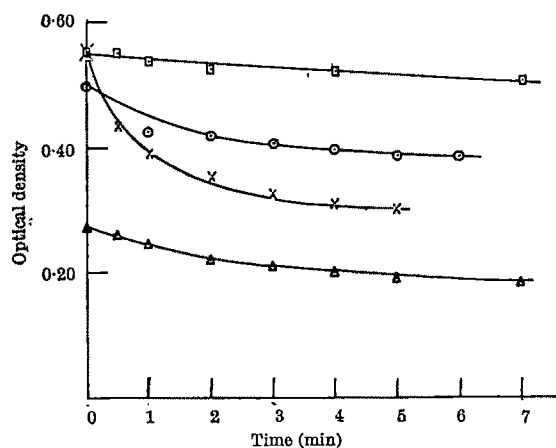


Fig. 1. Photoreductions of flavins in methyl cellulose films. ○, FMN alone; ×, FMN + EDTA; □, FMN + EDTA + serotonin; △, lumi-flavin alone.

These results suggest that the microscopic viscosities of concentrated methyl cellulose solutions and films are considerably lower than the macroscopic viscosities (compare Tables 1 and 2). Thus we picture both as having channels or cavities in which chromophores (and probably water too) are constrained, but within which they have certain freedom of movement. The observations that oxygen has no effect on the photoreductions (see later) and that when FMN is inhomogeneously distributed in concentrated aqueous methyl cellulose homogeneity is not achieved after several hours support the assumption that diffusion processes in a film are slow. The small changes in the absorption spectrum of FMN in a film, and the fact

that the fluorescence intensity of FAD is unchanged in the presence of methyl cellulose, are evidence that the chromophore is in a largely aqueous environment<sup>5</sup>.

**Photochemistry.** In anaerobic aqueous solutions FMN is photobleached in the absence of external reducing agents, and photoreduced in the presence of ethylenediamine tetraacetic acid (EDTA) and methionine<sup>1</sup>. These reactions also occur in methyl cellulose films (Fig. 1) though no precautions to exclude oxygen are necessary. The films were not inert during these reactions as lumi-flavin (6,7,9-trimethylisoalloxazine), which is not photobleached in solution by the light intensity used, reacted in methyl cellulose. In the photobleaching of FMN hydrogen is abstracted from the ribitol side chain<sup>6,7</sup>, so methyl cellulose can probably donate hydrogen in a similar fashion. The photoreductions in films resemble those in solution in susceptibility to the action of inhibitors<sup>8</sup> (Fig. 1).

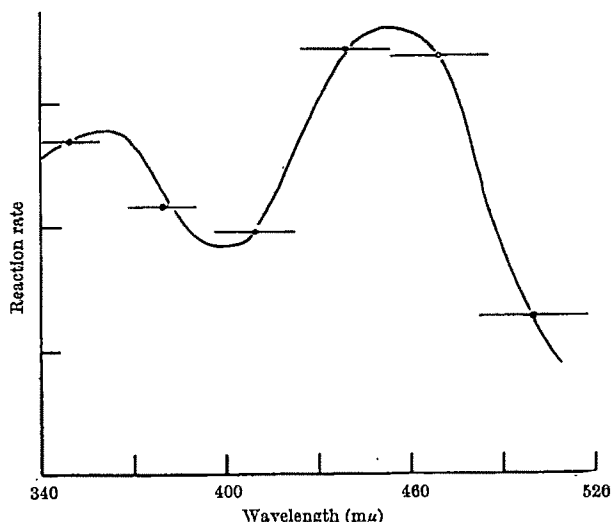


Fig. 2. Action spectrum for the photoreduction of FMN in a methyl cellulose film.

Because of the ready reactivity of flavins and the reduction of diffusion problems in films, we have been able to study action spectra for photoreduction. Using the monochromatic exciting light of the spectrofluorimeter to induce reaction, progress was followed by the fall in intensity of flavin fluorescence. The action spectrum has the same shape as the absorption spectrum (Fig. 2),

Table 3. SPECTRAL PROPERTIES OF THE DERIVATIVES CHOSEN FOR MIXED FILM EXPERIMENTS

Derivative	D	A
Visible absorption maximum		
(a) In solution	445 mμ	470 mμ
(b) In a methyl cellulose film	445 mμ	475 mμ
Fluorescence maximum		
(a) In solution	505 mμ	540 mμ
(b) In a methyl cellulose film	530 mμ	570 mμ

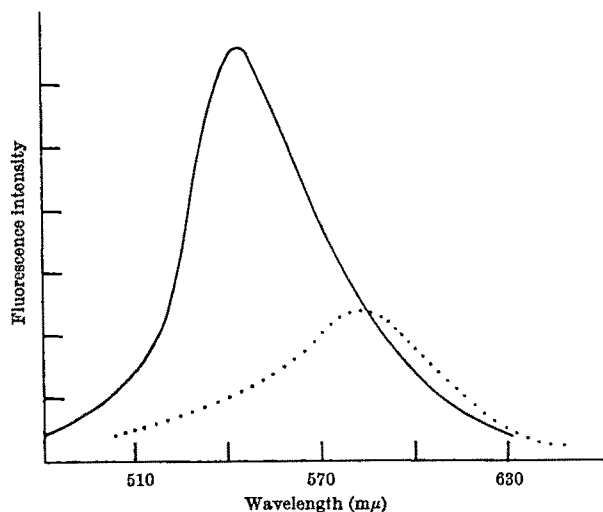


Fig. 3. Fluorescence emission spectra of pure and mixed flavin derivatives in a methyl cellulose film. —, *D* alone; ····, *A* alone and 1:1 *A*:*D*.

confirming that the quantum yield of reaction is independent of the wavelength responsible for the excitation.

**Energy transfer.** Energy transfer is most likely when the emission spectrum of the donor overlaps the absorption spectrum of the acceptor<sup>9</sup>. This situation can be realized by using the two flavin derivatives the formulae and spectral properties of which are shown in Table 3. No energy transfer can be observed between the flavins in dilute solutions. In mixed films the absorption spectra are additive, but the fluorescence emissions are not. A little *A* (20 per cent or more total flavin) completely quenches the fluorescence emission of *D* (Fig. 3). In a 4:1 (*D*:*A*) film the reductions of exciting and emitted light (at 530 mμ) as a result of absorption by *A* are about 15 per cent and 10 per cent, respectively, for excitation at 350 mμ. This alone cannot explain the fluorescence quenching of *D*, which suggests that a singlet-singlet energy transfer from *D* to *A* occurs.

Using Förster's formula<sup>9</sup> the mean intermolecular distance at which energy transfer from an excited singlet is as likely as deactivation by other processes ( $R_0$ ) can be calculated. Assuming that the quantum yields of fluorescence in films are the same as those measured in solution (Wentworth, S., and Radda, G. K., unpublished results), critical transfer distances for FMN alone and for a mixture of *A* and *D* are 14 and 21 Å, respectively. The average intermolecular distance in a mixed film of optical density 1.5 is 30 Å, and for FMN alone with an optical density of 0.5 the distance is 45 Å. At very high concentrations of FMN depolarizations greater than 15 per cent have been measured (Table 2). Under these conditions the intermolecular distance is 20–25 Å. The fluorescence polarization of *A* in a film (optical density 0.55 at 475 mμ) is also reduced in the presence of *D* by 15 per cent for excitation at 450 mμ. Thus it appears that energy transfer can be detected for mean intermolecular distances about 50 per cent greater than the calculated  $R_0$  values. This divergence could be caused by our assumption that the refractive index of a methyl cellulose film is 1.33. Nonetheless, energy transfer occurs in far more dilute mixtures of *A* and *D* than are required to observe it for FMN alone, which is in qualitative agreement with calculations. The observation that polarization is constant for low concentrations of FMN in films shows that energy transfer depolarization is not occurring (Table 2).

It is clear that methyl cellulose films not only provide a simple system in which the photochemical properties of flavins and other dyes can be studied, but also offer environments which may be closer than homogeneous aqueous solutions to the natural ones.

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## Antibodies of the ABO System and the Metabolism of Human Spermatozoa

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Antibodies of the ABO system can affect the metabolism of human spermatozoa. The main effect is that the anaerobic glycolysis of sperm from men with A or AB secretor phenotypes is lowered in anti-A or anti-B antisera. Respiration, on the other hand, is increased

HUMAN spermatozoa can respire in the aerobic condition, and are also capable of anaerobic glycolysis. It is agreed that glycolysis, aerobic and anaerobic, is quantitatively more important than oxidative function. It was questioned for some time whether human spermatozoa were indeed capable of oxidative reactions or respiration<sup>1-3</sup>. Although at least one earlier worker<sup>4</sup> reported measurable respiration (oxygen uptake), the demonstration was not entirely convincing until the work of Turner<sup>5,6</sup>. Despite the predominance of glycolytic metabolism, it is possible that respiratory function is important *in vivo*, where ejaculated spermatozoa might most efficiently utilize oxidizable intermediates such as lactate<sup>5</sup>.

Many students of mammalian semen consider that the metabolic activity of spermatozoa is correlated with their

fertilizing capacity. In particular, anaerobic fructolysis—production of lactic acid with fructose as the reducing sugar—has been related to number and motility of human spermatozoa<sup>7,8</sup>. Sakakura has found no relation between the number of motile spermatozoa and the production of lactic acid, at concentrations greater than 10 million spermatozoa per c.c. (ref. 9). In many species, respiratory activity is related to spermatozoan motility and concentration<sup>10</sup>.

The subject of this study is the effect of incubation with antibodies of the ABO system on the metabolism of human spermatozoa. It is clear that spermatozoa of secretor-type individuals will absorb specific antibody<sup>11,12</sup>, and there is evidence to suggest that the spermatozoa of non-secretor individuals may also do so<sup>13,14</sup>. Fertility



differentials for matings which are incompatible in the ABO system<sup>16-18</sup> and distorted genotypic ratios in the offspring of such matings<sup>19</sup> may be partly the result of prezygotic selection exercised by antibodies in the reproductive tract. On this assumption, the adsorption of antibody may in some way impair the physiological function of the affected spermatozoa.

Since there is reason to believe that there is a relationship between the fertilizing potential of spermatozoa and their metabolic activity, it was supposed that the influence of ABO antibodies on gametes may be revealed by gross metabolic studies. The metabolic apparatus of mammalian spermatozoa is located in the midpiece and tail; in human spermatozoa, succinic dehydrogenase activity has been found in the midpiece and, to a much lesser extent, in the head<sup>20</sup>. The localization of the antigens of the ABO system on spermatozoa is not known, but antigen should be ubiquitous for secretor donors<sup>21</sup>. For non-secretors there is nothing to be said; it is possible that antigen, if it does exist, may be generally distributed and present at localities on the spermatozoa concerned with metabolic activity. Spermatozoa of non-secretor donors, when incubated with specific ABO antibody conjugated with isothiocyanate, show fluorescence on head and on midpiece and tail<sup>22</sup>.

### Materials and Methods

(1) *Apparatus*. The metabolic studies were carried out with a Warburg rotary apparatus, using micro-Warburg vessels with centre well and single sidearm. Vessels and manometers were calibrated and vessel constants computed by standard methods<sup>21,22</sup>. A thermobarometer was used in each experiment to compensate for local variations of atmospheric pressure.

(2) The concentration of sperm in all specimens of semen was normal or greater than normal. Volume and morphology were normal. The fertility of most donors was established. No attempt was made to use the same concentration of spermatozoa in the different experiments, but variations did not affect experimental results.

(3) *Antisera*. In most cases, commercial immune antisera, diluted with bovine albumin, were used. These were group A, anti-B, and group B, anti-A. Alternatively, undiluted samples of serum from the blood bank of the University of Michigan Medical Center were used, in which cases it was not determined whether the sera contained immune or naturally occurring antibodies; these sera contained the aforementioned types as well as group O, anti-AB.

(4) *Diluents*. Two types of diluent were used. In measurements of respiratory activity, spermatozoa were suspended in Mann-Ringer phosphate (pH 7.8) and in measurements of anaerobic fructolysis the suspending medium was Mann-Ringer fructose phosphate (pH 7.6) (ref. 10). The diluents were not sterile but were replaced periodically to avoid contamination.

(5) *Procedure*. Only one semen specimen was studied at a time. Immediately after liquefaction, the specimen was divided into two parts in proportions determined by the numbers of vessels to be used for respiration measurements and for fructolysis. Each part was washed once in the appropriate diluent for 10 min at 2,500 r.p.m., so as to separate the spermatozoa from the seminal plasma. The spermatozoa were then resuspended in the appropriate diluent.

Six reaction vessels were usually used: two were used for measuring respiration, the sidearm of one containing anti-A and that of the other anti-B. The centre well of each contained 0.5 ml. 10 per cent potassium hydroxide. Two sets of two were used to measure fructolysis anaerobically. In each set, 1.5 ml. of the same antiserum was pipetted into each sidearm, but the centre well of one contained 0.5 ml. 10 per cent potassium hydroxide and that of the other did not.

The appropriate suspension of spermatozoa was pipetted into each vessel to a volume of 1.5 ml. The volume of antiserum in each sidearm was 1.5 ml. The flasks were calibrated for a liquid phase of 3.0 ml. Filter paper folded accordion-wise was inserted into each centre well containing potassium hydroxide to provide sufficient surface for the absorption of carbon dioxide. The vessels were connected to manometers placed in the Warburg water bath, which was maintained at 37° C. The vessels for anaerobic measurements were then gassed for 10 min with pure nitrogen. After the vessels had equilibrated for 15 min, the antisera in the sidearms were tipped into the flasks. Measurements then were recorded every 15 min for 4 h.

### Results

The consumption of oxygen, or the production of lactic acid as measured by displacement of carbon dioxide from the diluent<sup>1</sup>, was computed for each hour<sup>23</sup>, and the results were expressed in  $\mu$ l. per  $10^6$  spermatozoa per hour.

These results were analysed by the covariance method and the number of  $\mu$ l. of gas produced or consumed was taken as the dependent variable. Covariance analysis allows (a) determination of the significance of each regression, (b) comparison of the slopes of the regressions, and (c) testing of the adjusted means of the regressions.

The respiration of sperm from group A secretors is increased in both antisera, compared with that in normal serum. There is significant heterogeneity among the adjusted means ( $F = 4.22^*$ ,  $df = 2,36$ ); the less sensitive method of planned comparisons between means did not, however, allow us to determine the source of this heterogeneity. Glycolysis in these sperm is significantly inhibited by both antisera ( $F = 7.15^{**}$ ,  $df = 2,48$ ).

Neither anti-A nor anti-B had any effect on either respiration ( $F = 1.57$ ,  $df = 2,32$ ) or glycolysis ( $F = 3.02$ ,  $df = 2,24$ ) in sperm from group B non-secretors. Covariance analysis shows significant heterogeneity among the adjusted means of respiration in sperm from group O secretors ( $F = 23.31^{***}$ ,  $df = 2,20$ ). The source of this heterogeneity is the influence of anti-A—the difference between anti-A and normal serum means being  $0.56 \pm 0.19 \mu$ l.\*; anti-B has no effect. There was no effect on glycolysis in these sperm.

In the case of sperm from group O non-secretors, neither respiration ( $F = 2.27$ ,  $df = 2,12$ ) nor glycolysis ( $F = 2.26$ ,  $df = 2,16$ ) was affected by either antiserum.

Comparison of within and regression mean squares for the results on the respiration of sperm from group AB secretors shows that the regressions of volume on time are not parallel: the covariance analysis is therefore not appropriate. In this case, adjusted means were computed for each dependent variable,  $y$ . A value of  $y$ , extrapolated to zero time,  $E(y_0)$ , was taken in order to allow comparisons between means free from the effects of the independent variable:  $E(y_0) = \bar{y} + b(x_0 - \bar{x})$ . The normal deviate for each combination of pairs was computed in order to compare these adjusted means

$$\text{Normal deviate} = \frac{E(y_{0A}) - E(y_{0B})}{\sqrt{(\sigma_A^2 + \sigma_B^2)}}$$

Neither antiserum had any effect on the respiration of these cells.

Covariance analysis showed no heterogeneity among means for glycolysis in these sperm. The adjusted means for glycolysis in both antisera were, however, remarkably depressed compared with normal serum values. As  $N$  was small in this case, the values for anti-A and anti-B were pooled and compared with those for normal serum ( $t = 3.00^{**}$ ,  $df = 18$ ). On this basis, glycolytic inhibition in anti-A and anti-B is taken to be significant.

The respiration of sperm from group AB non-secretors was not affected by anti-A, but was significantly enhanced in the presence of anti-B ( $F = 4.20^*$ ,  $df = 2,20$ ). Differ-

ences between anti-B and normal serum were  $0.08 \pm 0.06 \mu\text{l.}^*$ . Incubation with either antiserum had no effect on glycolysis in these cells.

Before proceeding to a discussion of these findings, the values for oxygen consumption and carbon dioxide production can be compared with those achieved by other workers using manometric techniques. Shettles<sup>4</sup> measured the oxygen consumption of spermatozoa in undiluted whole semen in the Warburg apparatus. He recorded values on fresh specimens of approximately  $0.14 \mu\text{l./h/10}^6$  cells. Only one experiment in the present series is comparable. Type AB spermatozoa in seminal plasma with 0.6 ml. Mann-Ringer-phosphate, with an equal volume of anti-A and anti-B, give an average oxygen consumption of  $0.42 \mu\text{l./h/10}^6$  cells. Most measurements were taken on spermatozoa free from seminal plasma, suspended in Mann-Ringer-phosphate, with an equal volume of antiserum. Under these conditions values were around  $0.125 \mu\text{l./h/10}^6$  cells. Type AB spermatozoa free from plasma in Mann-Ringer-phosphate gave a value of  $0.075 \mu\text{l./h/10}^6$  cells. One could conclude from these results that the contribution to respiration by serum is about 7 per cent of the total, and that of seminal plasma about 83 per cent, while the basic respiration of isolated cells is about 10 per cent of the total. There is internal consistency in these results as it is known<sup>10</sup> that absorption of oxygen by human semen is predominantly associated with the seminal plasma. The discrepancy between the figures of  $0.42 \mu\text{l./h/10}^6$  cells and  $0.14 \mu\text{l./h/10}^6$  cells<sup>4</sup> can be accounted for at least in part by the dilution of the former specimens with serum and phosphate buffer. Turner<sup>5</sup> attempted to measure respiration in human spermatozoa washed and suspended in Krebs saline, by manometric means, and obtained no readings.

Carbon dioxide production in the anaerobic condition (95 per cent nitrogen; 5 per cent carbon dioxide) has been measured on two occasions by the Warburg technique<sup>1,3</sup>. The spermatozoa in each case were free of seminal plasma and resuspended in Ringer solution. The average values were 0.1 and  $0.25 \mu\text{l./h/10}^6$  cells. In the present series, the following approximate average values were obtained: washed spermatozoa in Mann-Ringer-

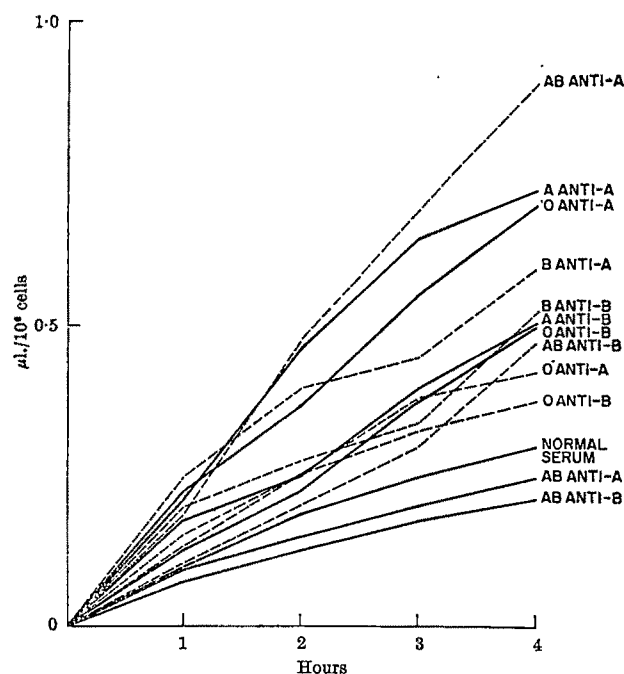


Fig. 1. Oxygen consumption. Solid lines indicate oxygen consumption of sperm from secretor donors; broken lines indicate that of sperm from non-secretor donors. In the description of each curve, the blood group of the donors is given first, followed by the designation of the antiserum in which the sperm were incubated.

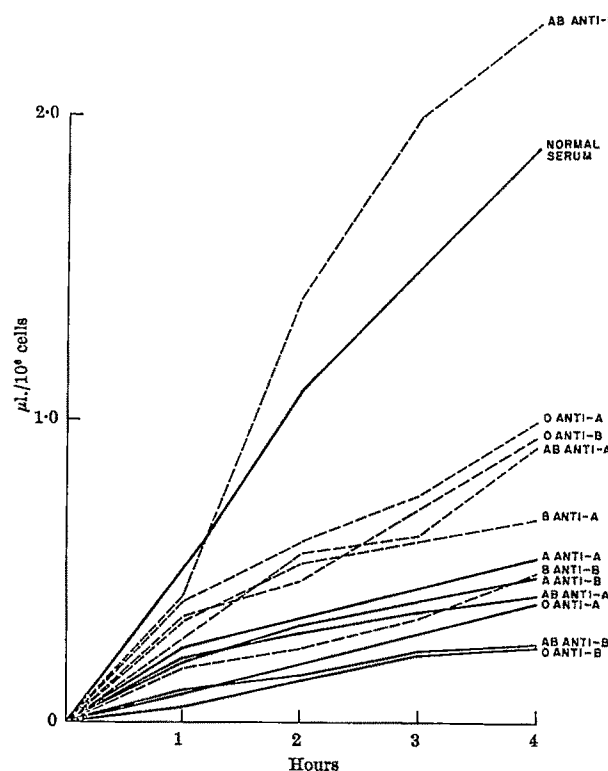


Fig. 2. Carbon dioxide production. Solid lines indicate carbon dioxide production of sperm from secretor donors; broken lines indicate that of sperm from non-secretor donors. In the description of each curve, the blood group of the donors is given first, followed by the designation of the antiserum in which the sperm were incubated.

fructose-phosphate,  $0.03 \mu\text{l./h/10}^6$  cells; in normal serum,  $0.45 \mu\text{l./h/10}^6$  cells; in antiserum,  $0.15$ – $0.25 \mu\text{l./h/10}^6$  cells; spermatozoa in antiserum, seminal plasma and 0.6 ml. fructose-phosphate,  $0.34 \mu\text{l./h/10}^6$  cells. With the exception of the first, these results are comparable with those of MacLeod. The value of  $0.03 \mu\text{l.}$  for spermatozoa in fructose-phosphate is lower by several orders of magnitude than that of MacLeod for roughly similar conditions. This may, in part, be accounted for by three factors: (a) MacLeod's use of 200 mg per cent glucose in his suspensions, as opposed to the use of 5 mg per cent fructose in the present experiment; (b) an initial deficiency of carbon dioxide in both gas and liquid phases in the present experiment compared with those of MacLeod; (c) individual variability; that is, MacLeod's result is an average of more than twenty-five experiments, while we have only carried out a single experiment of this type. In addition, MacLeod<sup>3</sup> apparently pooled the spermatozoa of several donors to minimize variability between specimens.

Four conclusions can be drawn from the observations reported here. (a) Specificity of antiserum in the ABO system has no effect on respiration or anaerobic glycolysis of human spermatozoa from donors of either secretor status. (b) There is a tendency for respiration in sperm of all types to be enhanced in any antiserum, but oxygen consumption is significantly elevated in only four of the twelve combinations tested. In three of these four cases, spermatozoa were those of secretor donors. (c) Anaerobic glycolysis is significantly lower in either antiserum than in normal serum for spermatozoa from secretors of A or AB phenotypes. (d) Anaerobic glycolysis in spermatozoa from non-secretors does not differ from that of the same cells in normal serum.

Thus the only consistent determinant of differential metabolic performance of cells under these experimental conditions is the secretor status of the semen donor. Secreted red blood cell antigen and the presence of any

antibody interact to reduce glycolytic function. Concomitant with the glycolytic depression, there is apparently some tendency to increased respiratory activity. This suggests that the interaction of antigen and antibody tends to force a change-over, in the affected cells, from the primary glycolytic metabolic pathway, to the secondary, or oxidative, pathway. Such a change-over might reasonably be expected to decrease the viability of the affected cells, and consequently their fertilizing capacity.

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## Haptotaxis and the Mechanism of Cell Motility

by

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Dr. Carter puts forward a hypothesis for the basis of tissue cell motility involving considerations of thermodynamics. He uses it to explain experimental haptotaxis and other features of cell motility such as ruffling and circus movement. Dr. Moilliet shows that the hypothesis is thermodynamically feasible

In a previous article<sup>1</sup> I described the motility of cultured tissue cells on specially prepared surfaces. The cells used in these experiments did not adhere to surfaces of pure cellulose acetate, but adhered readily if the acetate surface was previously coated with evaporated palladium.

By controlling the amount of palladium deposited, it was possible to make a range of surfaces which gave different degrees of cell adhesion. Experiments with these surfaces emphasized the importance of the underlying substrate in determining cell behaviour. For example, "L" cells showed mutual contact inhibition<sup>2</sup> on a surface to which they adhered strongly, but not on one to which their adhesion was relatively weak. Other experiments involved a gradient of progressively increasing metal deposition on a surface of cellulose acetate. Such a surface presents a corresponding gradient of cell to substrate adhesion, and cells were found to move up this adhesion gradient in a highly directional manner. I called this phenomenon "haptotaxis", and suggested that cell movement from a less adherent to a more adherent surface is the dominant principle in tissue cell motility. I indicated how all movements of tissue cells could be interpreted on this basis, and considered particularly how the principle of haptotactic movement could be applied to chemotaxis and to cancer invasion.

The present paper attempts to relate the phenomenon of haptotaxis to the mechanism of cell movement. This is necessary if it is to be established as a general and overriding principle of tissue cell motility.

As in previous experiments, the cells used were mouse fibroblasts (Earle's "L" strain). These were cultured in Eagle's medium<sup>3</sup> (with 8 per cent heat inactivated calf serum), using oil sealed culture chambers<sup>4</sup>. The technique of preparing cellulose acetate films on cover glasses was described in the previous paper<sup>1</sup>.

Tissue cells depend for their motility on attachment to a suitable substrate. True motility involves the displacement of the whole cell in relation to this substrate. In the simplest analysis, the movement of a cell on a solid material such

as glass involves two prime requirements: the leading margin of the cell must move forward, and the trailing margin must follow in the same direction. It is convenient to consider these requirements separately.

*Movement of the leading margin.* A thin film of cellulose acetate on a cover glass can be scraped to expose areas of underlying glass. When the leading margin of a cell moving on the exposed glass meets the edge of the acetate film, forward movement ceases immediately.

This behaviour resembles the "contact inhibition" which occurs when a cell meets another cell moving on glass, and the operative mechanism appears to be the same.

Fig. 1 shows a cell approaching the edge of a film of cellulose acetate, and the same cell shortly after it has made contact with the film. It will be seen that the film completely blocks the forward movement of the cell. If the acetate surface is sufficiently clean, no visible extension takes place over it. The simplest and most satisfying explanation for this effect is that the leading

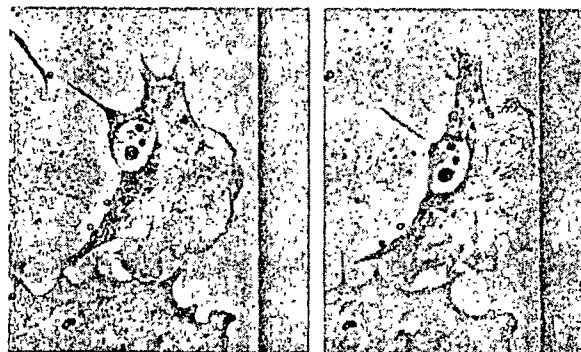


Fig. 1. A cell on glass approaching the edge of a cellulose acetate film, and the same cell after making contact with this edge. Forward movement of the leading margin of the cell ceases as soon as contact is made. (Phase contrast  $\times 375$ .)



Fig. 2. The leading margin of a cell moving on a surface of alternating strips of cellulose acetate and glass. The margin bulges forward on the lighter (glass) strips, and is held back on the darker (acetate) strips. (Phase contrast  $\times 1,000$ .)

edge of a cell moving on glass extends by passive spread, and not by forces arising inside the cell. The glass provides a wettable surface on which spreading can occur, but the acetate presents a surface which cannot be adequately wetted by the cell. In the same way a drop of water spreading on a glass plate will stop spreading exactly at the edge of a film of grease.

Further evidence for the passive nature of cell extension is provided by the appearance of a cell moving on a substrate consisting of alternate strips of cellulose acetate and glass (Fig. 2). The leading edge of this cell is seen to bulge forward on the lighter glass strips, and to be held back on the darker acetate strips. The same pattern is evident when a drop of gamma-globulin solution (for example) spreads passively on the same type of surface (Fig. 3).

Weiss<sup>5</sup> argues that the expansion of the cell surface at an interface cannot be regarded as a basically passive process. In support of this he cites the finding of Lettré<sup>6</sup> that cells dosed with ATP tend to remain extended instead of rounding up when undergoing mitosis. Even if it were shown that the presence of ATP at or near the cell surface is a necessary requirement for cell extension, this would not exclude the possibility that such extension depends on passive spread. All that is implied by "passive" in this context is that the final process in the movement of the leading edge of the cell involves intermolecular forces acting between the surface of the cell and the surface of the substrate. Metabolically derived energy can be represented in the form of surface free energy as well as any other, and there is no reason why it should not act in this form to bring about cell movement. In fact the terms "active" and "passive" applied to particular processes become meaningless when considered at the molecular level. Nevertheless I think the concept of passive spread (as defined) is valuable in attempting to understand the mechanism of cell movement, provided it is not taken to mean that the cell does not contribute any energy to the process.

The nature of the forces concerned with cell adhesion is poorly understood and largely disputed. Curtis<sup>7</sup> has used interference reflexion microscopy to investigate this problem. He has calculated that the closest approach of the cell surface to a glass substrate is about 100 Å. This type of calculation necessarily involves making a large number of assumptions, but there is also the tacit assumption that this gap is constant in width over an unspecified area of surface. The size of this area depends on the resolving power of the method as a whole, but this cannot be better than the optical resolving power of the microscope. Even at its theoretical minimum, therefore, this area will be very large in relation to a gap of 100 Å. It seems highly unlikely that a macromolecular surface would present a regular contour on this scale.

If a gap of this order does exist, then it cannot strictly be said that the glass is wetted by the cell surface. Nor can the relationship of the two surfaces be considered by reference to a single interfacial or boundary tension. Until these problems are settled, however, it seems preferable to use these terms provisionally, as there is no virtue in introducing new terminology before it can satisfactorily be defined.

The idea that surface tension forces are involved in cell motility is a very old one. In 1886 Berthold<sup>8</sup> interpreted amoeboid movement in terms of local variations in surface tension, and Rhumbler<sup>9</sup> extended these ideas further. Many other workers adopted a similar approach to problems of cell movement. Harvey<sup>10</sup> gives a useful historical summary of this early work.

Theories of cell movement which involve surface tension forces have now become unfashionable. When they are mentioned at all it is usually to exclude them from serious consideration. The older ideas regarded the cell as a drop of protoplasm of which the surface simply represented the outermost exposed layer of this material. The realization that the cell was surrounded by a distinct membrane completely invalidated this earlier concept, but the presence of a membrane does not in any way exempt the cell from the influence of forces due to boundary tensions. Naturally, the membrane will modify the effect of these forces on the cell as a whole, and the properties of the membrane as a physical structure must be taken into account.

Cells can, of course, extend into a liquid medium in the total absence of an extraneous surface. Such extensions are usually small and occur during anaphase "boiling", for example. Free extensions of this type are not in any way incompatible with the idea of passive spread on a surface. Both types of extension can be interpreted as a local reduction in the boundary tension at the cell surface. In the case of a free extension this could be due to local differences in the distribution of a surface active material. In the case of passive spread the wettable surface itself may lower the boundary tension at the point of contact.

*Movement of the trailing edge.* When considering the movement of the trailing edge of the cell, it is helpful to distinguish the situation in which a cell is moving on a gradient of adhesion from that in which it is moving on a uniform surface.

In the case of an adhesion gradient (such as that produced by a graded deposition of palladium on cellulose acetate), the margin of the cell which lies highest on the gradient will be subject to the greatest spreading tension. Correspondingly the opposite margin, on a lower part of the gradient, will be in contact with a less wettable surface and subject to a lower spreading tension. If the gradient

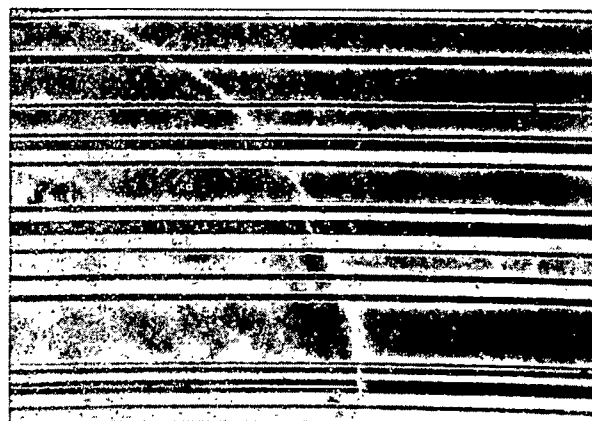


Fig. 3. The margin of a drop of gamma-globulin solution spreading on the same surface as that shown in Fig. 2. The margin of the drop presents the same pattern as the margin of the cell, bulging forward on the glass strips and held back on the strips of cellulose acetate. (Phase contrast  $\times 480$ .)

is sufficiently "steep" and the cohesive forces of the cell sufficiently strong, then the stronger pull on the side of the cell farthest up the gradient might be sufficient to draw the cell bodily up the gradient. In theory at least, the cell could move up such a gradient without expending any metabolic energy, since it will have moved to a position of lower total free energy. In practice, however, with artificial gradients of this type, I think that the energy contributed by the gradient itself represents only part of the energy required to move the cell. This contribution is nevertheless sufficient to determine its direction of movement. For the rest it is necessary to invoke the kind of mechanism which I shall propose for movement on a uniform surface.

Before discussing this mechanism, it may be useful to consider certain aspects of the nature of adhesion. For present purposes adhesion will be considered in terms of relative wettability. When a cell adheres to a glass surface it is commonly described as though the only surfaces involved were those of the cell and the glass. But this is fundamentally a system of three phases in which the cell, the glass, and the fluid medium in which they are immersed all need to be considered equally. This means that the medium/glass interface is not only relevant to the adhesion of the cell: it should be accorded exactly the same importance as the cell/glass interface. Indeed, the adhesion of a cell to a solid surface represents a three way competition, each phase being competed for by the other two. In the argument which follows, however, the consequences of changes at the cell/medium interface will not be considered, and for descriptive purposes the adhesion of the cell will be discussed in an over-simplified form in terms of a competition for the solid surface between the cell and the medium. The concept of a cell failing to adhere because the adhesion forces between it and a particular surface are too weak is incomplete. Adhesion may also fail because the adhesion forces between the medium and this surface are particularly strong. This would enable the medium to displace the cell from contact with it. A drop of oil may spread on a glass surface in air, but the same oil may fail to spread on glass under water because the water can successfully compete with it for the glass surface.

To return to the subject of cell movement on a reasonably uniform surface such as glass, the problem is to account for the fact that the trailing margin of the cell moves in the same direction as its advancing margin. If the movement of the advancing margin is due to passive spread, this spread would be expected to be equal in all directions. In the case of a cell showing directional movement on such a surface, this is clearly not the case, and it is necessary to find a reason for this loss of symmetry.

Using the technique of ellipsometry, Rosenberg<sup>11</sup> has investigated the submicroscopic layer of material which appears to be deposited by cells when in contact with certain substrates such as chromium coated glass. He called this material a "microexudate" and considered that it was transferred directly to the substrate by contact with the cell, since none could be demonstrated in the supernatant from cell suspensions. Chemically it appeared to be a complex of materials including protein. Rosenberg also showed that culture media containing serum very rapidly produced an adsorbed layer of protein on chromed glass. Microexudate also appeared to be deposited on such a protein film when cells were cultured on it, though the rate of deposition was reduced. The probable existence of an adsorbed layer of protein should clearly be borne in mind when considering surfaces in contact with media containing serum, and all the experiments described here were carried out in the presence of serum.

Taylor<sup>12</sup> has investigated the adhesion of cells to a variety of different substrates. He found that the adhesion of cells to glass is greatly reduced if the glass is covered by a layer of microexudate produced by previous contact with cells. The loss of cell adhesion on glass surfaces

"contaminated" by microexudate could be due to a reduction in the affinity of the altered glass for cell surfaces corresponding to an increase in the cell/glass boundary tension. As we have seen, however, an equally possible explanation is that the affinity of the glass for medium has been increased, corresponding to a reduction in the medium/glass boundary tension. In fact this is the more likely explanation, since if the substance responsible for this effect is transferred by direct contact, it is highly improbable that such a transfer could result in an actual increase in the boundary tension between the contacting surfaces.

If a cell margin which is spreading passively outwards on a wettable surface such as glass should be momentarily reversed at a particular point, then a small area of glass which has been contaminated by microexudate (by contact with the cell) would be exposed. If the wettability of the contaminated glass for medium is increased as suggested, this could enable the medium to compete successfully with the cell and displace it from the glass at this point. The reversal of the cell margin which is necessary to initiate this process has only to expose a very small area of altered glass surface. A random Brownian event may well be sufficient for this purpose.

The situation is now no longer symmetrical, and a potential direction of movement has been determined. The medium would be expected to continue to displace the cell from the glass where the contaminated area is exposed, so that this becomes the trailing margin of the cell. The leading margin is now able to advance on uncontaminated glass with improved efficiency. This is because it is relieved of the opposing tension of the opposite cell margin transmitted by the cohesion of the cell. These factors would tend to reinforce each other, so that once the direction of the cell has been randomly determined this direction would tend to be preserved, provided the newly encountered surface is homogeneous and free of the contaminating influence of other cells.

In practice, when a cell is newly seeded on glass, it may be a considerable time before one margin of the cell gains a sufficiently strong advantage over the others to result in directional movement. Many "random" reversals of outward spread may occur before a sufficiently asymmetrical position is established to ensure continuity of movement in a particular direction. Until this position is reached, the cell may undergo many changes of shape and minor shifts of position. In marked contrast to this, directional movement on an adhesion gradient is immediate.

The suggestion that the trailing margin of a cell moves forward because it is displaced by medium may seem an even more "passive" movement than that proposed for the leading edge. Again there is no intention of suggesting that the cell does not provide energy for its own movement. Although no attempt is made here to identify the energy conversions involved, it should be borne in mind that the driving force of the medium would be provided on this theory by the surface active material deposited by the cell. This material would require the expenditure of metabolic energy for its synthesis, and in the process of movement it would need to be continuously replaced.

A basically similar mechanism may be operating to produce the curious phenomenon of circus movement which Holtfreter and others have described for certain embryonic cells<sup>13</sup>. In contact with glass these cells may produce a bleb of cytoplasm which protrudes at one side and proceeds to move round the periphery of the cell. When speeded up by time lapse photography each cell appears to be spinning in a clockwise or anti-clockwise direction. Again the direction of movement of the cytoplasmic bleb may be the consequence of a random event which tips the balance in a particular direction. For reasons already discussed the bleb would be expected to continue round the cell, its leading margin being drawn on by passive spread, and its trailing margin giving way



to the medium as a result of a surface active material deposited in its wake. It is only necessary to postulate that this material is desorbed or otherwise inactivated after an interval of time, so that it is reduced or rendered less effective by the time the bleb has gone full circle. The bleb would then continue to move round the cell indefinitely.

If adhesion is considered in terms of the relative success of the cell in its competition with the medium for contact with the glass, then the mechanism of movement proposed for tissue cells on a homogeneous surface such as glass may also be considered as representing a movement from a surface offering relatively poor adhesion to a surface offering better adhesion. Seen in this way, the movement of the cell on a uniform surface is also an example of haptotactic movement. Indeed, the only distinction between this and movement on a surface representing an adhesion gradient is that the cell is considered to be creating its own adhesion differential as it moves. An important consequence of this is that once the cell is in motion and has established its own adhesion gradient, it may continue across a line dividing two dissimilar surfaces in the "wrong" direction, that is towards the surface offering the lower relative adhesion. This is because the differential generated by the moving cell may represent a greater difference of adhesiveness than that between the two dissimilar surfaces.

*The ruffled membrane.* The picture of cell movement so far presented has taken no account of the ruffled membrane. Ruffling movements of the cell membrane are characteristic of many tissue cells moving on glass, and are well shown by the "L" cell. Ruffling can occur at any margin of the cell in relation to its direction of movement, and it is by no means confined to the leading edge. Nevertheless, there is a tendency in rapidly moving cells for the ruffled membrane to be more fully developed at the front of the cell, and the trailing margin may show no ruffling at all. For this reason it has been suggested<sup>14</sup> that the ruffled membrane may be looked on as the "organ of locomotion" of cells which demonstrate this type of behaviour. This view is widely held, and theories of cell movement<sup>15</sup> have been based on the idea that the ruffles are essentially wave-like in character, and that their rearward movement across the surface of the cell is responsible for the forward movement of the cell as a whole. The formation of a ruffled membrane, however, is dependent on a surface (such as glass) which offers a relatively high degree of adhesion. "L" cells, for example, remain essentially spherical on a surface offering little adhesion (such as cellulose acetate), and do not show any ruffling, yet they are able to move as efficiently as on glass. It is probably misleading, therefore, to regard the ruffled membrane as the organ of locomotion of the cell.

In order to interpret the ruffled membrane, it is first of all necessary to have an accurate picture of the physical pattern which it presents. Even with the help of time lapse cinematography, this pattern is complex and difficult to follow. Cells which have been trypsinized and newly seeded on glass are particularly valuable for investigating the pattern of ruffle formation. In many cases these cells attach and spread out without ruffling. The first ruffles to appear tend to be single and isolated, so that their formation can be observed critically, and a clear sequence of events can be followed. Fig. 4 shows such a cell photographed at intervals against a reference grid. The first photograph shows the cell spreading outwards, presenting the regular circular outline which would be expected for passive spread on a uniform surface. In the second photograph the cell margin has extended further. The cell outline is less regular but there is no true ruffling. The first ruffle appears in the third photograph, and is indicated by a long arrow. Reference to the grid lines shows that the formation of the ruffle is accompanied by a retreat of the cell margin at this point. The ruffle itself appears to be formed by the piling up of cytoplasm as the

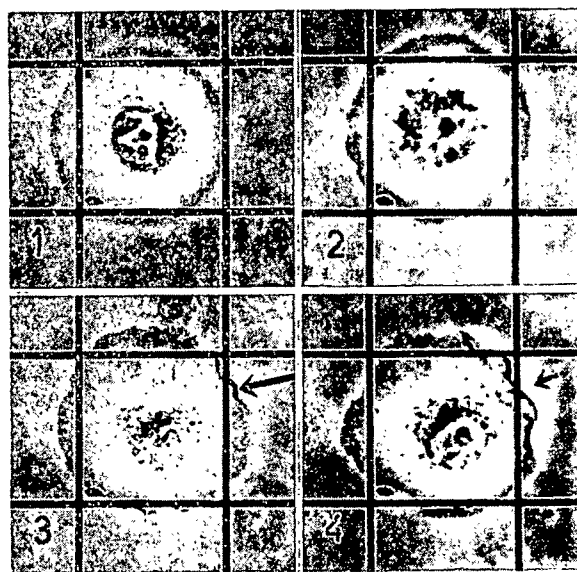


Fig. 4. A trypsinized cell, newly seeded on glass and photographed at intervals against a reference grid to illustrate the development of the ruffled membrane. The arrows indicate features mentioned in the text. (Phase contrast  $\times 800$ .)

cell margin moves back. Apart from this initial movement, there is no tendency for the ruffle to be propagated backwards over the surface of the cell. The fourth photograph shows a rounded extension (indicated by a short arrow), reappearing in the same region. This extension will in turn retreat to form another ruffle. This sequence can also be seen in the fully developed ruffled membrane, but its essential simplicity is obscured by the number of ruffling movements which are then superimposed.

This pattern of movement can be interpreted in precisely the same way as I have attempted to interpret the movement of the cell as a whole. Each ruffle represents a retreat of the cell margin in the face of medium which is successfully competing with the cell at that point by virtue of the microexudate deposited by the cell. The re-extension of this margin can occur when this material has become partly desorbed or inactivated, as discussed in relation to circus movement.

Little is known about the way in which additional cell membrane is made available to accommodate new cell extensions. Whatever the mechanism, there must be a limit to the rate at which new membrane can be made available, and this may be an important controlling factor in the rate of cell extension. Ruffling is seen particularly when the cell is well spread, and the cytoplasm may form an extremely thin layer at the front of the cell, except where it is locally thickened as a ruffle. In this situation the local resources for providing new surface membrane to allow further extension may be particularly limited. If these resources become momentarily exhausted, the advancing margin must stop, and at this point the margin may be especially vulnerable to the random thermal factors already discussed which could initiate the reversal of the cell margin. The reversal itself would be checked by the development of tension in this part of the membrane as a result of the movement of other parts of the margin which are still making forward progress.

If this is a basically correct interpretation of ruffle formation, then, far from representing the organ of locomotion of the cell, the ruffling of the membrane actually impedes the progress of the cell. This is because each ruffle may be regarded as a temporary set-back to the forward movement of the leading cell margin.

In so far as ruffling may reflect the principles which determine cell movement, however, the detailed study of ruffle formation is highly relevant to the investiga-

tion of cell motility. In this context, the behaviour of recently trypsinized cells may be of particular value. Superficially, the failure of such cells to show ruffling movements until they have fully recovered from their exposure to trypsin suggests that this enzyme may destroy some component of microexudate on which ruffling may depend, or that it interferes in some way with microexudate production. Rosenberg<sup>11</sup> has presented evidence that trypsin adsorbed to the cell surface may continue to be active in this way.

It is still necessary to explain the relative or total lack of ruffling at the trailing margin of the cell.

Since the interpretation of ruffle formation previously discussed involves the alternate spreading and retreating of a thin layer of cytoplasm, it depends for its continuation on the continued ability of the cell margin to extend. The surface relationships of the cell membrane at the trailing margin do not favour extension, and the general state of tension in the membrane in this region (as evidenced for example by the tautness of the cytoplasmic strands which are often dragged behind the cell during its progress) also militates against the local expansion of the membrane which extension must involve. It is noticeable that should this tension be generally or locally relieved by a change in cell direction, for example, ruffling at the trailing margin is resumed. When a cell on glass meets the edge of an acetate film, or demonstrates "contact inhibition" on meeting another cell, there is also a tendency for ruffling to be reduced or cease at the contacting margins. This could again be because the initial extension on which the formation of a ruffle depends is now restricted.

Although the mechanism of cell movement here proposed is basically very simple, a more complete interpretation along these lines becomes extremely complex. This is particularly true if the potential influence of the boundary tension between the inner surface of the cell membrane and the cytoplasm is taken into account<sup>16</sup>. Each situation demands the consideration of a number of factors which may sometimes oppose, and sometimes support, each other. In addition it will seldom be clear in a given instance which are the main factors concerned and which make only minor contributions. A local feature of a moving cell may have an essentially local explanation, but it is also liable to be influenced by situations obtaining elsewhere in the cell. In assessing the relative importance of these factors, it would be extremely useful to know, for example, to what extent the forces due to a spreading tension at one margin of the cell may be transmitted to another part of the cell by tension in the cell membrane. This problem would be complex enough if the cell membrane were a stable structure, but the fact that it may be in a dynamic state, involving the continuous interchange of material between the membrane and the cell interior, could make its formal solution virtually impossible.

The theory of cell movement I have put forward does not invoke any internal mechanisms for moving the cell, such as specialized contractile elements. If such mechanisms can be shown to exist in a particular type of cell, they must clearly be taken into account. Apart from this, the theory is intended as a general basis for the motility of all types of tissue cell. On the other hand it is not intended to apply in this form to the mechanism of "true" amoeboid movement such as that shown by *Amoeba proteus*.

The purpose of investigating the movement of tissue cells *in vitro* is to try to identify the principles which govern their motility, in the hope that these principles may also be applied to such cells in their normal environment *in vivo*. Extrapolation of *in vitro* results in this way is a hazardous but necessary step. In attempting to transfer these ideas to their proper context in the living animal, an important question is raised: to what extent may the movement of a particular cell be due to "pre-

existing" gradients of surface adhesion presented to it, and to what extent could it be due to a gradient of the cell's own making? If it is mainly a question of pre-existing adhesion differentials which are presented to the cell, then a rather disturbing possibility has to be entertained—that cell movement on artificial materials such as glass may be dependent on a mechanism which is largely irrelevant to cell movement *in vivo*.

I thank Dr. J. L. Moiliet for valuable discussions on surface thermodynamics, and Miss Janet Way for technical assistance.

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### Elementary Surface Thermodynamics of Carter's Theory of Haptotactic Cell Movement

THE mechanism of haptotactic cell movement proposed by Carter<sup>1,2</sup> can readily be shown to be feasible in terms of the thermodynamics of surfaces, provided that the inner and outer surfaces of the cell membrane can be treated as interfaces with positive boundary tensions. The simplest case is represented in Fig. 1, which shows the cross-section of a cell, represented as a droplet of liquid (2), on the surface of a solid substrate (3), in competition with another liquid (1). Suppose now that  $\gamma_{13}^x$ , the value of the interfacial tension (1)-(3) at  $X$ , is less than the value  $\gamma_{13}^y$  at  $Y$ . As Carter has pointed out, this could be due to a surface free energy gradient on the surface of the solid, produced for example by differential metal shadowing. Alternatively, or perhaps at the same time, a surface-active material might be adsorbed or deposited at the (2)-(3) interface and the droplet slightly displaced by accident to the left, leaving a "contaminated" (1)-(3) interface of lower interfacial tension immediately to the right of  $Y$ .

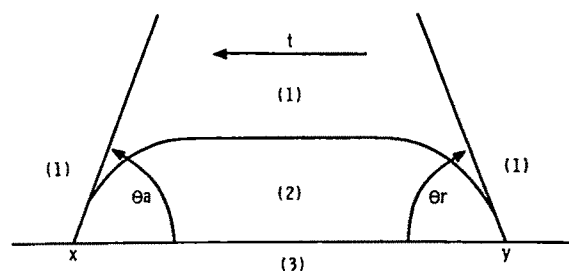


Fig. 1.

The net spreading tensions at  $X$  and  $Y$  are given respectively by

$$t_x = \gamma_{13}^x - \gamma_{23}^x - \gamma_{12}^x \cos \theta_a \quad (\text{to the left})$$

$$\text{and } t_y = \gamma_{13}^y - \gamma_{23}^y - \gamma_{12}^y \cos \theta_r \quad (\text{to the right})$$

the resultant tension (to the left) being

$$t = t_x - t_y = (\gamma_{13}^x - \gamma_{13}^y) - (\gamma_{23}^x - \gamma_{23}^y) - (\gamma_{12}^x \cos \theta_a - \gamma_{12}^y \cos \theta_r) \\ = \Delta\gamma_{13} - \Delta\gamma_{23} - (\gamma_{12}^x \cos \theta_a - \gamma_{12}^y \cos \theta_r) \quad (1)$$

$\theta_a$  and  $\theta_r$  not necessarily being equilibrium contact angles.

It is such a possible condition that the values of the  $\gamma$ 's be such that  $t$  is greater than zero. On a differentially

shadowed surface, for example,  $\gamma_{12}^x$  and  $\gamma_{12}^y$  would probably be equal, but  $\Delta\gamma_{13}$  could be larger than  $\Delta\gamma_{23}$ , especially if the (2)-(3) boundary tensions were in general lower than the (1)-(3) tensions. To take a second case, if  $\Delta\gamma_{13}$  is positive because of contamination of the (1)-(3) interface to the right of  $Y$  by materials from the cell  $\Delta\gamma_{23}$  would be zero and  $\gamma_{12}^x = \gamma_{12}^y$ , except in so far as movement of the cell disturbed the adsorption equilibria at  $X$  and  $Y$ . In either or both of these possible cases,  $t$  would be zero only if  $\cos \theta_a > \cos \theta_r$ , but this would destroy the symmetry of the drop, the radii of curvature of the (1)-(2) interface becoming smaller near  $Y$  than near  $X$ . This dissymmetry would cause a pressure to the left, which would prevent the differences in the two contact angles from becoming large enough to reduce  $t$  to zero. (For the simple case just mentioned, that  $\Delta\gamma_{23} = 0$  and  $\gamma_{12}^x = \gamma_{12}^y$ ,  $t$  would become zero only if  $\cos \theta_a = \cos \theta_r + \Delta\gamma_{13}/\gamma_{12}$ , but the pressure due to the dissymmetry would cause  $\cos \theta_a$  to lie between this value and  $\cos \theta_a = \cos \theta_r$ .)

The kinetics of the proposed mechanism of haptotaxis, which involves the kinetics of adsorption processes at the various interfaces, as well as viscous and frictional effects, are obviously very complicated. These effects are not considered in the present treatment, which suggests only that a mechanism based on boundary tension effects is thermodynamically possible. The energy for the process, on this picture, would of course come from a decrease in the total free energy of the system. On a differentially shadowed surface, for example, a contribution to this decrease would come from a movement of the cell towards a position of lower total surface free energy; on the hypothesis of a lowering of  $\gamma_{13}$  near  $Y$  by adsorbed material, the energy would come from the total free energy of adsorption of this material.

The present treatment can be extended to the true situation, in which the cell is enclosed in a membrane. Suppose that the droplet in Fig. 1 contains a third liquid phase (4), which has exactly the same shape as the droplet, leaving a "membrane" layer of (2), which is so thin as to occupy a negligible fraction of the total volume of the droplet, but is thick enough to have an internal boundary tension  $\gamma_{24}$ . We also assume that this very thin layer is kept in place by a structure of some sort, but that this structure does not interfere significantly with the effects of the boundary tensions  $\gamma_{12}$ ,  $\gamma_{23}$ , and  $\gamma_{24}$ . It can be shown

in such a case that the contact angle (measured inside the composite droplet) is given under equilibrium conditions by

$$\cos \theta' = \frac{\gamma_{13} - \gamma_{23} - \gamma_{24}}{\gamma_{12} + \gamma_{24}} \quad (2)$$

(This expression can be derived in an analogous manner to Young's equation, for example by finding the dimensions of the composite droplet which corresponds to a minimum in the total free energy of the system, at constant temperature, pressure, and composition.) Similarly, the net spreading tension will be given in this case by

$$t' = \Delta\gamma_{13} - \Delta\gamma_{23} - \Delta\gamma_{24} - [(\gamma_{12}^x + \gamma_{24}) \cos \theta'_a - (\gamma_{12}^y + \gamma_{24}) \cos \theta'_r] \quad (3)$$

where  $\theta'_a$  and  $\theta'_r$  are the prevailing contact angles (not necessarily equilibrium ones) obtaining at  $X$  and  $Y$ , respectively. The same general argument applies as before for the case that  $\Delta\gamma_{13} > 0$ , taking account of the appearance of  $\gamma_{24}$  in equation 3, and again leads to the conclusion that it is possible for  $t$  to exceed zero. Several points of special interest are, however, worth noting.

(a) It follows from equation 2 that, since  $\gamma_{24}$  is positive, each  $\cos \theta'$  will be smaller than the corresponding  $\cos \theta$ , other boundary tensions being unaltered; that is, the general effect of the presence of the internal interface is to increase the contact angle.

(b) The kinetics of adsorption will obviously be even more complicated for the case of a composite droplet.

(c) Fluctuations in the shape of the (2)-(4) interface, and local fluctuations in the value of  $\gamma_{24}$ , could contribute to the "ruffling" of the cell membrane which is frequently observed in motile tissue cells. If, for example, the (2)-(4) interface near  $X$  oscillated between the shape shown in the diagram and a rounded-off shape as the drop advanced, a fluctuation would be imposed upon  $\theta'_a$ , and ripples would be generated along the double boundary between phase (1) and the interior of the cell.

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<sup>1</sup> Carter, S. B., *Nature*, **208**, 1183 (1965).

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## Effects of Cytochalasins on Mammalian Cells

by

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The cytochalasins—a group of mould metabolites—inhibit movement and cytoplasmic cleavage in cultured cells. At higher doses they cause nuclear extrusion which may lead to total enucleation

SEVERAL species of moulds have been found to produce a number of chemically related metabolites which show unusual biological activity. The name "cytochalasin" (Greek *cytos*, a cell; and *chalis*, relaxation) is suggested for this new class of compounds. The name is intended as a general description of the effects which are characteristic of these substances. It is not meant to imply a particular mode of action.

Four cytochalasins have been isolated in these laboratories by Dr. W. B. Turner. These have been designated A, B, C, and D. Cytochalasins A and B were obtained

from culture filtrates of *Helminthosporium dematioides*. Cytochalasins C and D were isolated from *Metarrhizium anisopliae*. Cytochalasin-like activity seems to be widely distributed. Filtrates from nine other species (representing four orders of fungi) have been found to produce similar effects. The four cytochalasins so far isolated show essentially similar activity but differ in potency. Cytochalasins C and D are about ten times as active as cytochalasins A and B.

The experiments described here were carried out with cytochalasin B. This compound has a novel macrolide

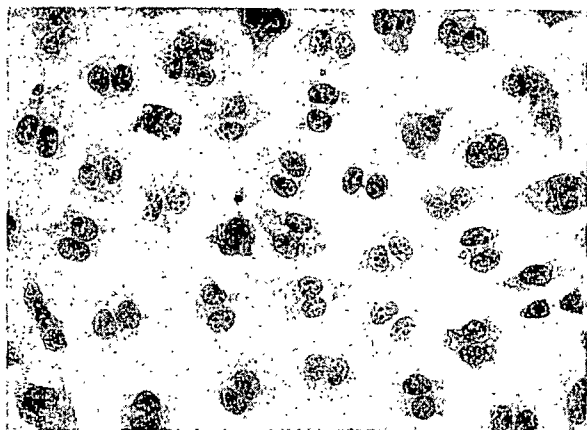


Fig. 1. Binucleated "L" cells produced by exposure to cytochalasin B at 1.0 µg/ml. for 24 h ( $\times 250$ ).

structure in which the lactone ring is joined to a bicyclic lactam system. Its molecular structure is reported elsewhere<sup>1</sup>.

Earle's "L" strain of mouse fibroblast was used in most of these experiments. The cells were cultured on glass cover slips using Eagle's medium<sup>2</sup> with 8 per cent inactivated calf serum. For observations on living cells, oil-sealed culture chambers were used<sup>3</sup>. The compound is poorly soluble in water, and appropriate dilutions in culture medium were made from a stock solution in dimethyl sulphoxide (0.1 per cent).

**Prevention of cytoplasmic cleavage.** Cytochalasin B prevents cytoplasmic cleavage at a concentration of 0.5 µg/ml. After 24 h exposure to this concentration, many cells are found to contain two nuclei. At 1 µg/ml., inhibition of cytoplasmic cleavage is virtually complete (Fig. 1). Nuclear division proceeds normally, and following this a deep cleavage furrow may develop. This furrow fails to separate the cells completely, however, and subsequently retreats. If the compound is removed by perfusion with normal medium, the binucleated cells produced undergo multiple cytoplasmic cleavage following the next nuclear division. No compound has previously been described which specifically and consistently prevents cytoplasmic cleavage without interfering with nuclear division. Wolpert has pointed out the potential value of such a compound for investigating the cleavage mechanism<sup>4</sup>.

Cells remain viable for many days in the continuous presence of cytochalasin B. Although the mitotic rate is reduced, many consecutive nuclear divisions may take place without cleavage. Very large multinucleated cells are produced which are clearly visible to the naked eye. All the nuclei in a particular cell appear to enter mitosis synchronously. Synchrony of nuclear division in multinucleated cells is regarded as the rule in a very wide range of biological material. If all the nuclei divide together, it would be expected that the number in each cell would double after each mitotic cycle. In fact this does not happen. More than a hundred individual cells have been followed over a period of 7 days under continuous treatment with cytochalasin B, to investigate the progressive increase in nuclearity which occurs. The usual pattern is for the nuclear complement of each cell to increase one at a time. This is a surprising finding which seems to conflict with an established cytological principle. It may be that synchronous nuclear division in multinucleated cells cannot safely be inferred from fixed preparations in which all the nuclei appear to be in the same mitotic phase. The simplest explanation for the production of only one nucleus per cycle is that a multinucleated cell may enter mitosis when only one of its nuclei is fully prepared and competent to divide. This implies that the other nuclei may be

induced to go through a "pseudomitotic" cycle which closely imitates mitosis, but involves no overall change in the nuclei taking part. Such a cycle could entail chromatin condensation and "chromosome" formation, followed by complete reconstruction of each nucleus in its original form. It is difficult to obtain direct evidence for this explanation because of the large number of chromosomes involved. If it can be shown to occur, however, pseudomitosis would have important implications for the initiation of nuclear division and the mechanism of chromosome formation.

**Inhibition of cell motility.** Time lapse cinematography reveals that "L" cells stop moving when the culture is perfused with cytochalasin B at 0.5 µg/ml. The effect is immediate and is reversed with equal rapidity when normal medium is restored. Treated cells remain in the same position for many days. This greatly facilitates the long term investigation of nuclear changes by time lapse techniques.

Ruffling of the cell margin is also inhibited. Ruffle formation is resumed as soon as the compound is removed.

No effect has been observed on the motility of a number of fresh water ciliates and flagellates even at doses as high as 50 µg/ml. Similarly the motility of mouse spermatozoa was unimpaired at this concentration.

**Extrusion of nuclei.** Nuclear extrusion is occasionally observed in "L" cells exposed to cytochalasin B at 1 µg/ml. At a dose of 10 µg/ml., however, a high proportion of cells extrude their nuclei (Figs. 2 and 3).

The initial stages of this process are dramatically rapid. Within minutes the nucleus has formed a prominent bulge in the cell membrane. This bulge progresses rapidly



Fig. 2. Living "L" cells before exposure to cytochalasin B. (Phase contrast  $\times 350$ .)

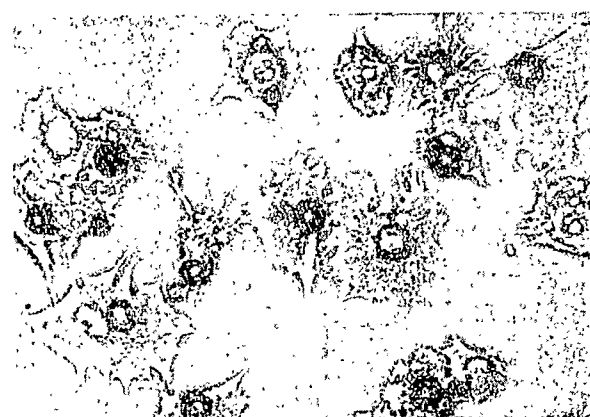


Fig. 3. The same field as Fig. 2 showing nuclear extrusion 1 h after exposure to cytochalasin B at 10 µg/ml. (Phase contrast  $\times 350$ .)

until the nucleus appears to be entirely outside the cell, but still attached by a delicate cytoplasmic thread. At this stage the process can be reversed by washing with fresh medium. The nucleus is drawn back into the cell and is functionally unimpaired. If the nucleus is allowed to remain fully extruded for several hours, irreversible damage may be done and the nucleus becomes pycnotic.

The thin thread connecting nucleus and cytoplasm becomes progressively longer and more attenuated. It can easily be severed with a micromanipulator needle. Spontaneous enucleation also occurs, and many cells can be found without nuclei after exposure to the compound for 24 h. The mechanism later proposed for nuclear extrusion does not account for the complete expulsion of the nucleus. When this occurs it may be dependent on loss of nuclear viability or accidental rupture of the connecting thread. Cells which have lost their nuclei in this way have been found to be viable 24 h later, but no detailed investigation of their ultimate fate has been attempted. Such cells are capable of limited movement if placed in normal medium.

**Mechanism of action.** Cells which have been exposed to cytochalasin *B* at 0.5 µg/ml. are flatter than untreated cells, and each cell occupies a proportionately larger area of glass surface. The cell outline tends to be relatively simple and approximates to a circular shape. This appearance suggests that adhesion between the cell and the glass may be increased. This could indicate that the compound is adsorbed at the cell/glass interface, lowering the interfacial tension between them. Cell adhesion to glass may be regarded as a three way competition for mutual contact between cell, glass and culture medium. It should be kept in mind, therefore, that a reduction in cell/medium boundary tension, or an increase in glass/medium boundary tension, could have the same effect. These possibilities are not mutually exclusive.

Cells which have been dosed with cytochalasin *B* in suspension adhere to a glass surface very much more rapidly than untreated cells. After attachment, however, their rate of spreading is less than that of control cultures. This could mean that adsorption of the compound on the cell surface also increases its surface viscosity.

Cytochalasins *C* and *D* completely prevent cell cleavage at 0.05 µg/ml. A mould filtrate at present under investigation appears to contain an even more active metabolite. Crude extracts of this filtrate have shown cytochalasin-like activity at a dilution of 1/20,000,000. The high potency of these compounds is associated with very rapid action and ready reversibility. Such features fit well with the suggestion that they act as highly specific surface active agents which are preferentially adsorbed on biological membranes.

Several theories have been put forward to account for cytoplasmic cleavage (see review by Wolpert<sup>4</sup>). Whatever the underlying mechanism, it seems likely that if a cleaving cell is attached to glass, the process cannot be properly completed unless the cell membrane is detached from the glass surface along the line of the cleavage furrow. If cytochalasins increase the adhesion between cell and glass, they could slow down or prevent this detachment. The development of a cleavage furrow might also be retarded by an increase in surface viscosity produced by adsorption of cytochalasin. The cleavage mechanism is closely involved with the presence of the mitotic apparatus. The orientation and position of this apparatus determine the subsequent position of the line of cleavage. In order to prevent cleavage, therefore, it may only be necessary to slow down the process of furrowing. The mitotic apparatus is dismantled shortly after nuclear division, and if furrowing is not completed by this time, the relevant mechanism may no longer operate, and the furrow will retreat.

The mechanism of cell motility is poorly understood and many theories have been advanced. The theory of cell movement I have previously put forward<sup>5,6</sup> is particularly

amenable to the interpretation of cytochalasin effects in terms of surface activity. According to this theory, cell movement is basically the result of cell extension by "passive" spread over a surface which can be wetted by the cell. On a heterogeneous surface, the cell moves in a direction which represents an increase in cell to substrate adhesion. This can be shown experimentally using surfaces of graded adhesiveness. Cells move up such an adhesion gradient with improved efficiency and in a highly directional manner. I have suggested the name "haptotaxis" to refer to cell movement in the direction of increased cell to substrate adhesion. Haptotaxis is proposed as the key principle in determining the motile behaviour of tissue cells. The specific application of this idea to the interpretation of contact inhibition, cancer invasion and chemotaxis has previously been discussed<sup>5</sup>. Cell motility on a homogeneous surface can also be interpreted as a result of haptotactic movement. In this case the difference in substrate adhesiveness which determines cell movement is considered to be created by the cell itself. The leading edge of a cell moving on glass is breaking new ground on a clean area of substrate. This could allow the leading edge to maintain a permanent advantage over the trailing edge where the glass surface may be rendered less adhesive through contamination by previous contact with the cell. The theory may be applied to an idealized cell of circular shape moving on a uniform flat surface. The theory then requires that the boundary tension forces tending to move the cell are proportional to its circumference, whereas the frictional forces tending to resist movement are proportional to its area. Area increases as the square of the circumference, so if cell to substrate adhesion is progressively increased, a point will be reached at which the sum of the restraining forces exceeds the forces tending to move the cell. At this point the cell can no longer move. Such a mechanism may underlie the effect of cytochalasin *B* on cell motility. It is interesting to note that "epithelial" cells in culture—usually so called because they tend to be flattened and relatively well spread on glass—are relatively immobile. Cultured cells which normally show rapid movement on glass sometimes produce polyploid giant cells. These cells tend to occupy a disproportionately large area of glass and are generally incapable of movement.

Cytochalasins also inhibit the ruffling movements at the margins of certain motile cells. I have interpreted ruffle formation as the result of a local retreat of the cell margin, causing a piling up of cell material. This retreat is considered to occur in the face of advancing culture medium which is successfully competing for contact with the glass at these points. An increase in the adhesion of the cell to glass due to the adsorption of cytochalasin at this interface would be expected to inhibit ruffle formation if this interpretation of ruffling is correct. If cytochalasins have an effect on surface viscosity as previously suggested, this could also be expected to impede cell movement and ruffle formation.

The explanation for nuclear extrusion follows a similar pattern. In this case, however, it is necessary to postulate adsorption of cytochalasin at an internal cell interface. This might account for the fact that nuclear extrusion requires a higher concentration of compound than the other effects. If cytochalasin is adsorbed on the internal surface of the cell membrane, or on the external surface of the nuclear membrane, it might lower the boundary tension between them. Increased adhesion between these structures would increase the area of any region of chance contact, and tend to make the cell membrane wrap itself closely round the nucleus until it was virtually excluded from the cell. Although it could be almost completely pinched off in this way, the nucleus would remain attached so long as the cell membrane remained intact. The essentially competitive nature of adhesion was emphasized in relation to the adhesion of cells to glass in the presence of culture medium. Similarly, adhesion between nucleus



and cell membrane cannot be fully treated without reference to cytoplasmic fluid acting as a third phase. Again it should be made clear that adhesion between nucleus and cell membrane could involve surface effects at more than one of the three interfaces concerned.

A curious and puzzling observation was made at an early stage in these investigations. Nuclear extrusion occurred more frequently in cells cultured in perfusion chambers than when they were grown in Petri dishes. The significant difference proved to be that cells in the perfusion chambers were attached to the under surface of the cover glass. It seemed unlikely that gravity could play a significant part in nuclear extrusion, as gravitational forces are exceedingly small at the cellular level. The mechanism proposed for nuclear extrusion provides a ready explanation. When cells are growing on the upper surface of a cover glass, the nucleus may fall under gravity within the cell and make contact with the lower cell membrane. In this region the cell membrane is not free to wrap round the nucleus because it is more strongly adherent to the glass substrate.

**Potential value of cytochalasins.** The cytochalasins produce a remarkable range of interesting biological effects. By interfering with specific cell activities such as cytoplasmic cleavage and cell movement, they should prove useful as research tools for investigating these important aspects of cell biology. Their potency, reversibility, and lack of general toxicity are particularly valuable features in this connexion. Their action in preventing cytoplasmic cleavage makes it possible to produce polyploid cells at will. This could be of value, for example, in determining the influence of different, known degrees of polyploidy on specific aspects of cell behaviour

and function. The possible significance of apparent mitotic synchrony in multinucleated cells without nuclear doubling has already been mentioned. The elucidation of this problem could have important consequences for the physiology of mitosis. The use of these compounds for the non-surgical removal of cell nuclei seems to offer new opportunities for investigating the functional relationship of nucleus and cytoplasm. Complete enucleation only occurs in a proportion of cells, but refinements in technique may make it possible to enucleate entire cell populations. When fully extruded, the connecting thread between nucleus and cytoplasm is extremely tenuous. This must restrict any functional interchange between them and may prevent it altogether. If so, it may not be necessary to enucleate the cell completely in order to study some aspects of the interdependence of nucleus and cytoplasm. Because nuclear extrusion is reversible, short-term experiments of this kind may have the additional advantage of allowing the restoration of the nucleus at a later stage.

Many experimental uses for these compounds can be envisaged. Whether or not their usefulness is likely to extend beyond the laboratory will only become apparent when they have been investigated more widely.

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## Is an Ectoproct Possible?

by

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The deficit between predicted and observed rates of oxygen consumption in a small sedentary aquatic invertebrate suggests that there may be circulation of fluid in the body cavity

MEMBERS of the phylum Ectoprocta (Bryozoa) are usually considered to be organisms of sufficiently small volume and large surface area to preclude a selective advantage for specialized respiratory and circulatory mechanisms<sup>1</sup>. Respiratory exchange is believed to occur by means of passive diffusion of oxygen and carbon dioxide across the surface of tentacles. To test the hypothesis that passive diffusion can supply sufficient oxygen to an ectoproct, we compared measured rates of oxygen consumption with those predicted from a model based on ectoproct morphology.

A representative of the class Gymnolaemata, which includes a large majority of all ectoproct species, was chosen. Colonies of *Bugula turrita* (Desor) were collected during late June and July from shallow waters of Eel Pond, Vineyard Sound, Massachusetts, at environmental temperatures of 18°–23° C. Approximately 6,000 healthy zooids were carefully cleaned under a dissecting microscope with fine forceps and brushes, and acclimated to 20° C for 24 h before a series of measurements of oxygen uptake were made. The rate of oxygen consumed by undisturbed animals (lophophores extended) was measured under conditions of constant oxygen concentration at 20° C and

32 ‰ salinity with an adaptation of the Scholander volumetric respirometer<sup>2</sup>. The result was 3.7 ( $\pm 0.7$  S.D.) mm<sup>3</sup> of oxygen/mg dry wt. (excluding skeleton)/h, or  $4.5 \times 10^{-4}$  mm<sup>3</sup>/zoid/h. The only previous measurement of oxygen consumption by an ectoproct of which we are aware is that of Robbie<sup>3</sup>, who gives 2.1 mm<sup>3</sup>/mg of dry wt. (excluding skeleton)/h at 25° C for an unidentified ectoproct from Bermuda.

To calculate the hypothetical rate of oxygen consumption in *B. turrita* that could be maintained by diffusion alone, we considered a model consisting of fourteen small cylinders (lophophore tentacles) mounted on a larger cylinder (tentacle sheath), in turn supported by a rectangular prism (zoecium). External and internal dimensions of the model are given in Figs. 1 and 2. Tentacles and tentacle sheath consist of a single layer of cells surrounding a coelomic space. The zoecium is completely covered by a chitinous layer which is reinforced by calcium carbonate on all but the ventral (frontal) surface. The dry weight of each of the three zoid subdivisions was calculated by assuming that weight is directly proportional to volume (Table 1).

If no circulation of the coelomic fluid occurs, then we

Table 1. SUMMARY OF OXYGEN CONSUMPTION IN A SINGLE INDIVIDUAL OF *Bugula turrita* IN OBSERVED AND HYPOTHETICAL SYSTEMS

Part of animal	Percentage volume	Dry weight (excluding skeleton) (mg $\times 10^{-5}$ )	Hypothetical rate of oxygen consumption (mm <sup>3</sup> /h)		Observed rate of oxygen consumption (mm <sup>3</sup> of oxygen/h $\times 10^{-4}$ )
			Diffusion without circulation $\times 10^{-3}$	Diffusion with circulation $\times 10^{-3}$	
Lophophore	6.1	7.0	23.9	2.1	2.6
Tentacle sheath	6.8	7.8	0.3	0.15	2.9
Zoecium	87.1	100.2	0.04	—	37.0
Whole animal	100.0	115.0	24.2	2.25	42.5

Values for each subdivision of the whole animal are based on an assumption of uniform rate of oxygen consumption in each part.

can use the equation developed by Gerard<sup>4</sup> to estimate the amount of oxygen which can diffuse into each of the fourteen cylinders (tentacles)

$$C_0 = \frac{Ar^2}{4D} \quad (1)$$

where  $C_0$  is the concentration of oxygen at the external surface, measured in atm.,  $A$  is the rate of consumption of oxygen, measured in mm<sup>3</sup>/mg of dry wt./min,  $r$  is the radius of the cylinder, measured in cm, and  $D$  is the diffusion coefficient of oxygen, measured in atm./cm/cm<sup>2</sup>, in tissue. The oxygen saturation value<sup>5</sup> for seawater at 20° C and 32 ‰ salinity (0.29 atm.) was used because *B. turrita* lives in well aerated habitats and because the

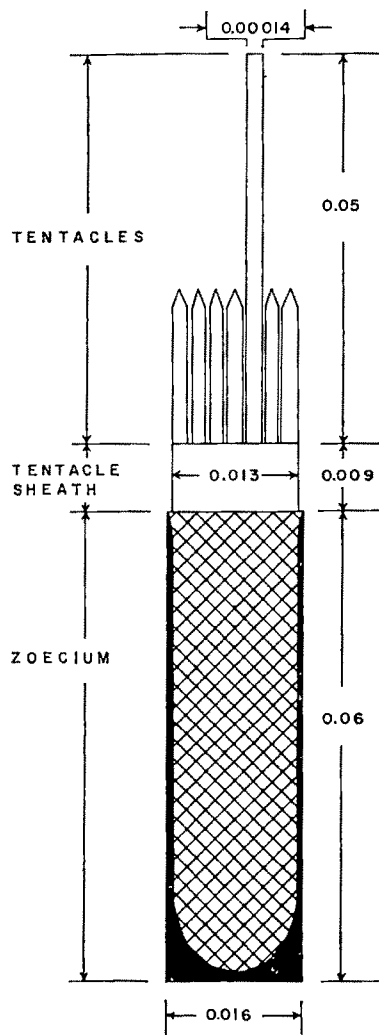


Fig. 1. Ventral (frontal) view of model of *Bugula turrita*. The dark region of the zoecium is covered by chitin and calcium carbonate; cross-hatched region of zoecium is covered by chitin. Dimensions in cm.

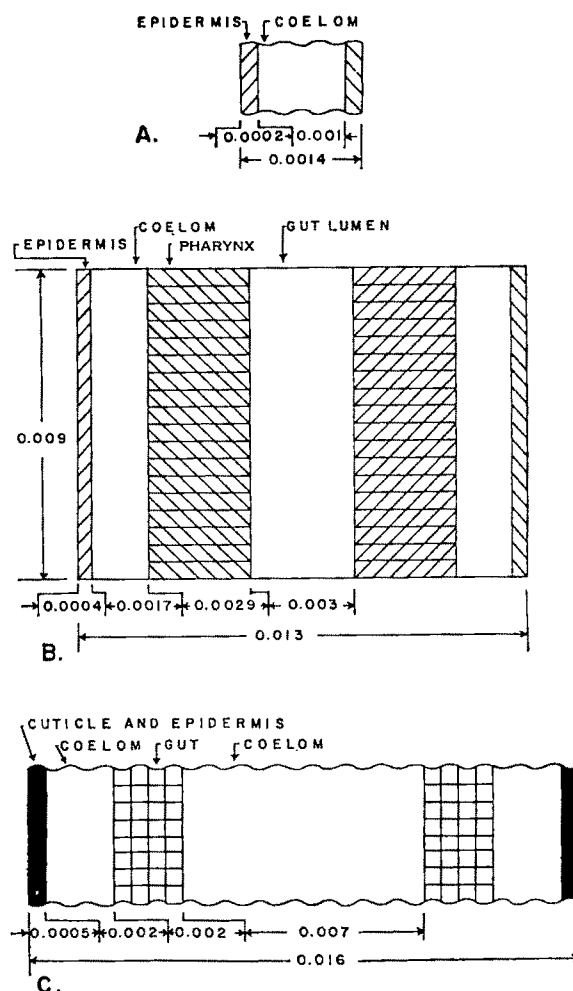


Fig. 2. Internal dimensions of a model of *Bugula turrita* (longitudinal sections). Dimensions in cm. (A) Tentacle. (B) Tentacle sheath. (C) Zoecium.

cilia of tentacles continuously renew the external medium in contact with the surface of the tentacles. The diffusion coefficient<sup>6</sup> ( $D = 2.4 \times 10^{-5}$ ) is the midpoint between the value for oxygen in coelomic fluid (seawater) and that for oxygen in epidermis (muscle and connective tissue). The ratio of coelomic fluid to epidermis in tentacles is 1:1 (Fig. 1).

When the equation is solved for  $A$  the tentacle is shown to be able to support a rate of oxygen consumption in its own tissue of  $3.4 \times 10^2$  mm<sup>3</sup>/mg/h, or the lophophore to support a rate of  $2.4 \times 10^{-1}$  mm<sup>3</sup>/h. The rate observed for the lophophore is  $2.6 \times 10^{-4}$  mm<sup>3</sup>/h, assuming a uniform rate of oxygen consumption in all parts of the animal. The excess of oxygen diffusing into tentacles could, therefore, be approximately a thousand times that consumed by them. If the loss of oxygen in diffusion is limited to that taken up by the epidermis, then the concentration of oxygen in the coelomic fluid of tentacles could be 99.9 per cent of that in the external medium.

The same equation was applied to the tentacle sheath using appropriate dimensions for  $r$  (Fig. 2) and a diffusion coefficient<sup>6</sup> ( $D = 2.3 \times 10^{-5}$ ) calculated from the observed 52 per cent gut and 48 per cent coelomic fluid. The rate of oxygen consumption made possible by diffusion from the external medium is 37.9 mm<sup>3</sup>/mg/h, or  $29.6 \times 10^{-4}$  mm<sup>3</sup>/h for the tentacle sheath. This is approximately ten times larger than the amount consumed by the tentacle sheath (Table 1). The concentration of oxygen in the

coelomic fluid of the tentacle sheath could be 90.2 per cent of that in the external medium.

If the calcium carbonate layer of the skeleton is impermeable, then diffusion from the external medium into the zoecium can occur only through the ventral (frontal) surface which consists of chitin alone (Fig. 1). For the calculation of diffusion into the zoecium, the equation was modified to the form

$$C_0 = \frac{Ad}{D} \quad (2)$$

where  $d$  is depth of zoecium, measured in cm. The diffusion coefficient<sup>6</sup> ( $D = 2.7 \times 10^{-5}$ ) was calculated from observed 70 per cent coelomic fluid, 25 per cent gut and epidermis, and 5 per cent chitin. Solution of the equation gives a possible rate of oxygen consumption of only  $2.9 \times 10^{-2}$  mm<sup>3</sup>/mg/h, or  $2.9 \times 10^{-5}$  mm<sup>3</sup>/h for the zoecium. The observed rate is more than a hundred times larger than the hypothetical value (Table 1).

The amount of oxygen reaching the zoecium could be supplemented, of course, by diffusion within the organism. To calculate the rate of consumption of oxygen that could be supported by diffusion from the tentacles, and from the sheath into the zoecium, the diffusion equation was modified to

$$C_{01} - C_{02} = \frac{Ad}{D} \quad (3)$$

where  $C_{01} - C_{02}$  is oxygen concentration gradient, measured in atm., and  $d$  is distance over which diffusion occurs, measured in cm. For diffusion of oxygen from the coelom of the tentacle to the coelom of the tentacle sheath, the gradient of oxygen concentration is  $0.289 - 0.260 = 0.029$  atm., and the distance,  $d$ , is the height of the tentacle sheath (Fig. 1). When the equation is solved for  $A$  the result is  $6.6 \times 10^{-3}$  mm<sup>3</sup>/mg/h, or  $5.2 \times 10^{-7}$  mm<sup>3</sup>/h for the tentacle sheath. For diffusion of oxygen from the coelom of the tentacle sheath into the coelom of the zoecium, the oxygen concentration gradient is  $0.260 - 0.000 = 0.260$  atm., and the distance,  $d$ , is the length of the zoecium (Fig. 1). Solution of the equation gives  $8.8 \times 10^{-3}$  mm<sup>3</sup>/mg/h, or  $8.8 \times 10^{-6}$  mm<sup>3</sup>/h for the zoecium. Thus, the rate of oxygen consumption that is possible by means of diffusion from the external medium, plus the rate possible by diffusion from other parts of the organism, is approximately three hundred times less than the observed rate for 87.1 per cent of the animal, that is the zoecium (Table 1).

Diffusion of oxygen within the organism could be facilitated by juxtaposition of two physiologically distinct forms of a respiratory pigment in an oxygen transfer system, even if efficient circulation does not occur. The existence of a substance that could transfer oxygen was postulated by Schneider<sup>7</sup>, who found large amounts of iron in ectopros, and his finding has recently been confirmed (unpublished results of Schopf and Manheim). The concentration of ferrous oxide in *B. turrita* is 0.57 per cent of the dry weight (excluding the skeleton), compared with 0.01 per cent to 0.1 per cent in an organ such as the mammalian liver, which is rich in iron<sup>8</sup>.

We attempted to detect an iron metallo-porphyrin by forming a pyridine haemochromagen from crude extracts of *B. turrita*, obtained from 1,288 mg (wet weight) which were homogenized in 4 ml. of seawater. The extract was centrifuged and the supernatant was filtered. The procedure was repeated, using distilled water as the solvent. After addition of 1 ml. of pyridine and sodium thio-sulphate, we were unable to detect the absorption peaks, with a Beckman DU spectrophotometer, of a pyridine haemochromagen in either extract. We have not excluded the possibilities of haemerythrin or an unknown oxygen transfer substance, but we can conclude that significant

quantities of haemoglobin are absent. It seems more likely that iron in *B. turrita* is bound with skeletal components.

Robbie<sup>9</sup> has demonstrated the sensitivity of ectopros to hydrogen cyanide, and so respiration cannot be primarily anaerobic. The remaining alternative is that, contrary to current belief, circulation of the coelomic fluid does occur. This suggestion was tested by calculating the hypothetical rate of oxygen consumption which could be maintained by diffusion combined with effective circulation. A modification of the equation for respiration with circulation, given by Krogh<sup>6</sup> to a form suitable for a cylinder, is

$$C_0 = \frac{ArT}{2D} \quad (4)$$

where  $T$  is thickness of epidermis, measured in cm. Solution of the equation for  $A$  shows that the fourteen tentacles of the lophophore can support a rate of oxygen consumption of  $2.1 \times 10^{-1}$  mm<sup>3</sup>/h, which is two hundred times greater than the observed rate of  $4.3 \times 10^{-3}$  mm<sup>3</sup>/h for the whole animal. Similarly, diffusion through the tentacle sheath, followed by circulation, could supply significant amounts of oxygen to the animal (Table 1). The large excess of oxygen provided by the tentacles and the tentacle sheath easily accounts for the oxygen consumption by the whole animal.

The hypothesis of efficient circulation can explain the observed rate of oxygen consumption, but does it occur? A systematic pattern of circulation of coelomic fluid has been demonstrated in only one gymnolaemate ectoproct, the freshwater species *Paludicella articulata*, in which localized tufts of cilia which line the coelom cause fluid movement<sup>10</sup>. Careful microscopic observation of living individuals provided no evidence of a similar mechanism in *B. turrita*.

A well known rhythmic behaviour pattern of ectopros may be important in effecting the circulation of the coelomic fluid<sup>11</sup>. Periodically, an undisturbed zoid undergoes a sudden, rapid retraction from the extended feeding position to a withdrawn position within the zoecium, which is immediately followed by expansion to the extended state. Distortion of the coelom accompanying the cycle must cause movement of the coelomic fluid. Observation of ten retraction-expansion cycles in each of ten zoids in natural seawater at 23° C indicates that the period of the cycle is 39 ( $\pm 49$  S.D.) sec. This mechanism of circulation would not be completely efficient, but its frequency seems sufficient to explain the observed rate of oxygen consumption.

We conclude that oxygen consumption of the gymnolaemate ectoproct *Bugula turrita* cannot be explained by the diffusion of oxygen from the external medium unless circulation of the internal medium occurs. Coelomic cilia could not be demonstrated, and so we suggest that the rhythmic expansion and withdrawal of the animal causes circulation of the coelomic fluid.

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# Piezoelectricity as a Fundamental Property of Biological Tissues

by

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Piezoelectric effects have been produced in a number of soft tissues, as well as hard, and appear to be associated with the presence of oriented fibrous proteins such as collagen. Thus, piezoelectricity may be a universal property of living tissue, and may play a significant part in several physiological phenomena

In a little less than two decades, evidence has accumulated that points to a previously unrecognized property of biological tissues—an electromechanical phenomenon that closely resembles the well known classical piezoelectric effect in crystalline solids. Although this effect has so far been observed in relatively few different kinds of tissue, chiefly in connective tissue, it seems highly probable that piezoelectricity is a property of most, if not all, tissues in the plant and animal kingdoms. If this is so, it may be possible to account for a number of biological phenomena that appear to be mechanically induced. It may ultimately be possible to trace many phenomena such as bone remodelling, the formation of thrombi due to injury of blood vessels, and the entire range of tactual responses, including the sense of hearing, to this basic biophysical property of tissue.

Piezoelectricity has so far been demonstrated in wood<sup>1,2</sup>, in ramie and silk fibres<sup>3</sup>, in bone<sup>4-6</sup> and in tendon<sup>7,8</sup>. In all these cases the usual criteria for classical piezoelectricity were shown to apply: for example, linearity and reversibility (direct and converse effects). Moreover, the form of piezoelectric tensor was determined or verified in each case. One group of investigators, who made some qualitative observations of electric polarization in bone under bending stress<sup>9,10</sup>, holds that the classical piezoelectric effect is inadequate to account fully for the observed phenomenon. The weight of the evidence, however, strongly supports the classical view.

For completeness it should be noted that perhaps the first indication of a piezoelectric effect in biological tissue was reported in 1941 by Martin<sup>11</sup>, who observed an electric polarization in bundles of wool and hair when subjected to stress. He also observed a pyroelectric effect in these materials under large thermal gradients.

It seems clear that the piezoelectric effect, even in hard tissues, can be attributed to the main organic constituent of the tissue: that is, collagen in the case of bone and tendon, and cellulose in wood. The effect is highly directional, with a maximum for shear stresses and a minimum for pure compressive or tensile stresses. In long bone, for example, where the direction of collagen fibres may or may not be parallel to the axis of the bone, the maximum is found for stresses directed at 45° to the collagen axis. The same is true of the fibrous molecules in wood and tendon.

The piezoelectricity apparently stems from a shearing stress on oriented long chain fibrous molecules, the actual effect being a displacement of charge due to the distortion

of cross-linkages in the molecular structure, probably hydrogen bonds. Thus a requirement for piezoelectricity in living tissue is the presence of a well ordered asymmetric fibrous molecule, cross-linked to form a uniaxial system which can be polarized by a shearing stress.

All connective tissue contains one or more kinds of fibrous molecule such as collagen, keratin, elastin, reticulum, or cellulose, and in general these molecules have a sort of "crystalline" structure in the sense of a short range asymmetry, if not an overall morphological order. It therefore follows, if our understanding of the origin of this effect is correct, that soft tissues should also be expected to have piezoelectric properties.

To test this hypothesis we have investigated the electromechanical effects in several soft tissues. Specimens of skin, callus (stratum corneum) and cartilage were dried, electrodes affixed, and the direct piezoelectric coefficient observed by a simple method which also allows us to determine the form of piezoelectric tensor in each case.

Specimens are normally cut in the form of thin disks, about 1.3 cm in diameter and 0.1–0.2 cm thick, arranged so that the axis of the fibre in each case is essentially in the plane of the disk. They are dried by desiccation for at least 24 h after removal from the host, following which a conductive epoxy is applied to the faces and allowed to harden with thin connecting wires in place. Some of the soft tissues investigated, such as human skin, were an order of magnitude thinner but were otherwise prepared in the same manner.

In the dry state these materials have volume resistivities in the order of  $10^{12}$  ohm cm at room temperature, which places them in the category of fairly good insulators exhibiting intrinsic conductivity with an energy gap of about 1.0–1.5 eV. We have been unable to find evidence of rectification or photoelectric effects in bone, as has been reported by others<sup>10,12</sup>. In fact, a careful search for these effects has revealed a simple experimental artefact that appears to be the explanation for the reported photoconductivity<sup>13</sup>.

Observation of the direct piezoelectric effect is carried out in the following way: a dynamic technique is used to eliminate possible spurious direct current potentials. An electromagnetic force generator, driven at a fixed frequency (generally 50 c/s) by a signal generator, exerts an alternating stress on a clamped specimen. The resulting polarization of the specimen is observed as a surface charge on the electrodes. A high input impedance ampli-

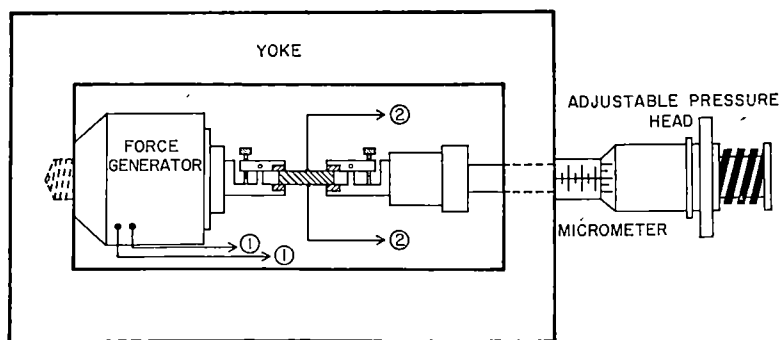


Fig. 1. Yoke assembly for direct piezoelectric measurements showing force generator and clamped specimen (hatched). 1, Input to force generator; 2, output to amplifiers.

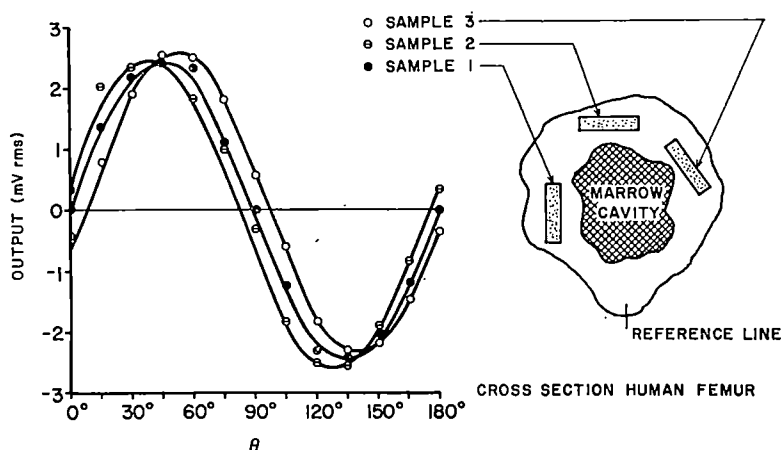


Fig. 2. Relative piezoelectric coefficient ( $d'_{13}$ ) as function of angle for three specimens of human femur. Zero direction is taken along bone axis. Direct effect: input 0.5 amp, 100 c/s, measured at 28° C, RH < 20 per cent.

fier is used for this purpose, followed in those cases where the piezoelectric coefficient may be very small by a tuned amplifier adjusted to the driving frequency\*. The final output is recorded by an alternating current voltmeter and the phase is observed on an oscilloscope or with a simple phase meter†.

A diagram of the yoke assembly used to clamp the specimen and maintain a rigid alignment of force generator and specimen is shown in Fig. 1. (The specimen is shown in edge view.) The micrometer head provides an adjustable stress to the specimen, which is particularly important when soft tissues are used. The entire yoke assembly is suspended in an oven for temperature control and electrostatic shielding. All measurements reported here were made at room temperature (24°–28° C) and about 20 per cent relative humidity.

To determine the angular dependence and ultimately the form of piezoelectric tensor for a given material the sample is rotated stepwise through 180° measured from some arbitrary reference axis. Examples of typical data obtained from hard tissues are shown in Figs. 2 and 3. Fig. 2 shows the variation of piezoelectric coefficient ( $d'_{13}$ ) with angle for three specimens cut from the same human femur. The specimens were disk-shaped and were cut from the same plane just below the neck of the femur; their location in the femur is shown in top view in the figure. The plane of each disk is parallel to the bone axis, which is taken as the zero direction for determining the angular dependence. Each curve shows the sin 2θ

\* This is normally not required in the case of bone, where the maximum piezoelectric effect is about one-tenth that of quartz. When observing the angular dependence, however, the additional gain is useful in determining the points at which the polarity reverses.

† Observing the phase angle between the output voltage and the driving voltage for the force generator permits determination of the sign of the piezoelectric coefficients.

behaviour to be expected of a uniaxial piezoelectric material in which the predominant piezoelectric stress is a shear. Two are, however, displaced from the origin by amounts that reflect the fact that the collagen axis of symmetry in human femur is displaced about 10° from the bone axis; that is, the principal collagen axis, at least as shown by X-ray diffraction patterns<sup>4</sup>, is vertical in the standing position while the femur axis is not. The sensitivity of the technique is readily apparent from this

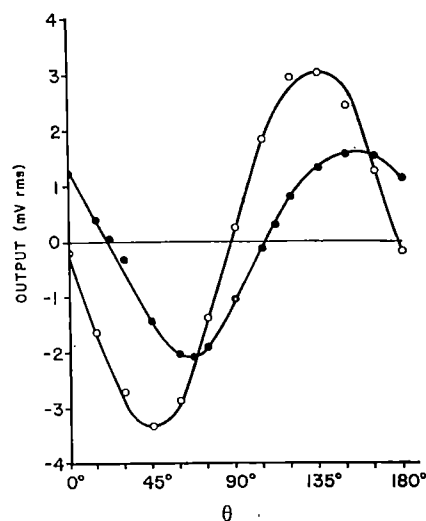


Fig. 3. Relative piezoelectric coefficient ( $d'_{13}$ ) as function of angle for two specimens of human mandible. ●, Sample 1; ○, sample 2. Direct effect: input 0.5 amp, 50 c/s, measured at 24° C, RH 20 per cent.



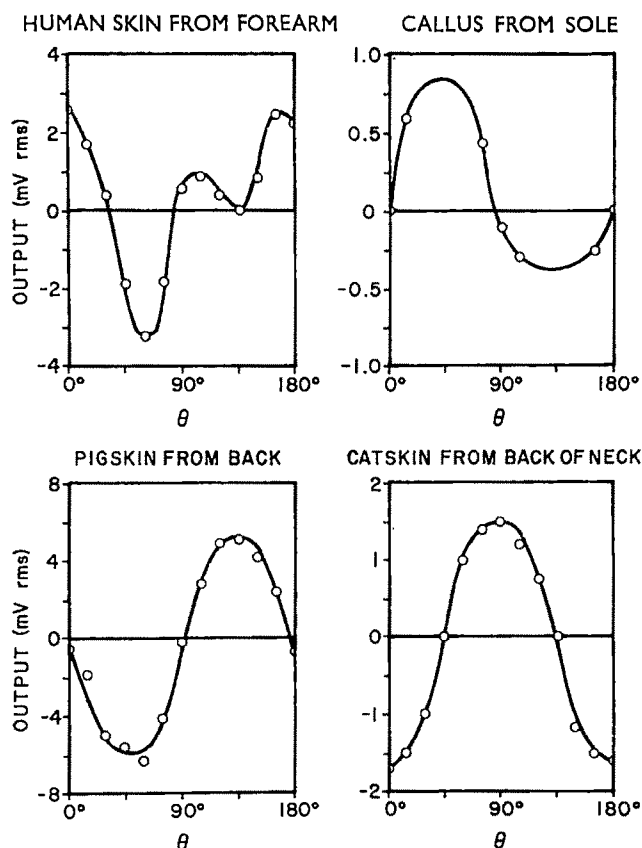


Fig. 4. Piezoelectric effect in various soft tissues. Outputs are shown as function of angle relative to arbitrary zero axis. Direct effect: input 0.5 amp, 50 c/s, measured at 25° C, RH 20 per cent.

set of curves. By way of contrast, no such displacement is found in the humerus.

Fig. 3 shows similar curves from two samples cut from the same human mandible. The shift in these curves is also attributed to the change in orientation of collagen fibres relative to the bone direction at the two sites from which the samples were taken. It is important to note,

incidentally, that we find little or no piezoelectricity in tooth enamel, whereas dentine exhibits a marked effect. This accords with the view that the piezoelectric effect in hard tissues originates in the fibrous molecular constituent rather than the mineral phase.

Fig. 4 shows the results obtained with soft tissues. Here, the same general pattern is observed, indicating a fibrous molecular (protein) structure with a marked uniaxial asymmetry. In one of these (human skin) the shape of the curve suggests a dual orientation, as though the main fibrous structure (collagen and elastin) in adjacent layers of the corium is alternately shifted through 90°. There is some histological evidence to support this picture of collagen orientation in skin<sup>14</sup>. The lack of symmetry between positive and negative portions of the other curves, particularly the callus specimen, is probably attributable to non-uniform thickness of the samples.

Although these observations show only that dried tissues exhibit marked piezoelectric properties, and it remains to extend such investigations to the more physiological state, the apparent universality of the effect suggests that it may play a very basic part in physiology, particularly in regard to homeostasis. A new link may thus be provided between biology and physics.

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## Amino-acid Replacements in Horse Haemoglobin

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Horse haemoglobin contains up to four components which arise by substitution of glutamine for lysine at position  $\alpha 60$  and of tyrosine for phenylalanine at position  $\alpha 24$ . The possible genetic reasons for this heterogeneity are discussed

HORSE haemoglobin consists of two electrophoretically distinct components<sup>1,2</sup>. Previous investigations have failed to reveal the nature of the difference between them<sup>3,4</sup>, but after the recent elucidation of the sequence of amino-acids in the electrophoretically slow component<sup>5,6</sup> it seemed worth while to re-investigate the problem. We find the difference to be a result of the replacement of the lysine at position 60 in the  $\alpha$ -chain of the slow component by glutamine in the fast component.

In addition, of the haemoglobins of twenty-three individual horses investigated, nine had both tyrosine and phenylalanine in position 24 of the fast and slow

$\alpha$ -chains, which gave a total of four haemoglobins; three had tyrosine only and eleven had phenylalanine only in this position.

Horse haemoglobin was converted to globin by the 2 per cent acid acetone method without previous fractionation of the fast and slow components. Separation of the  $\alpha$ -chains and  $\beta$ -chains and conversion of the separated chains to the aminoethylated derivatives was carried out as described by Clegg *et al.*<sup>7</sup>. The resulting fractionation is shown in Fig. 1. Tryptic digestion of the aminoethylated derivatives was carried out for 3 h at 37° C in 1 per cent ammonium carbonate, pH 9, at a protein concentration of 1 per cent and a trypsin to protein ratio of 1:100. The

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digestion mixture was freeze-dried three times—on the two last occasions from water made alkaline by blowing ammonia vapour over its surface.

Fingerprints of the three fractions were prepared by subjecting portions of the digests to electrophoresis at pH 6.5, followed by descending chromatography in BAWP (butanol : acetic acid : water : pyridine (15 : 3 : 12 : 10 by volume)). They were then dipped in 0.25 per cent ninhydrin in acetone and developed at 60° C. Tracings of the resultant patterns are shown in Fig. 2. Additional fingerprints of the three peaks were also stained for histidine, arginine, tyrosine, sulphur and tryptophan<sup>4</sup>.

From a knowledge of the amino-acid sequence of the slow fraction of horse haemoglobin and a comparison with fingerprints of human  $\alpha$ -chains and  $\beta$ -chains, all the chief spots in the three fingerprints were identified (Fig. 2). These identifications were confirmed by the fingerprints stained for specific amino-acids and by amino-acid analysis of the peptides eluted from fingerprints stained with 0.025 per cent ninhydrin in acetone<sup>7</sup>.

The first peak eluted from CM cellulose was shown to be  $\beta$ -chain and the second and third peaks were  $\alpha$ -chain. Thus, the first (that is, the more acidic)  $\alpha$ -chain ( $\alpha_f$ ) to be eluted must have been derived from the electrophoretically fast (that is, the more acidic) haemoglobin component and the second, more basic,  $\alpha$ -chain ( $\alpha_s$ ) from the slow component.

The fingerprint of the  $\alpha_f$  chain is characterized by the presence of a new peptide  $\alpha_f$  7,8 and the absence of peptides  $\alpha_s$  7,  $\alpha_s$  7,8, and  $\alpha_s$  8,9 normally found in fingerprints of the  $\alpha_s$  chain. The compositions of these peptides are shown in Table 1; clearly the data can be explained by the substitution of a glutamic acid (or glutamine) for a lysine at position 60 in the  $\alpha_s$  chain as shown in Fig. 3.

Table 1. COMPOSITION OF PEPTIDES OF THE  $\alpha$  CHAINS

	$\alpha_f$ 7,8	$\alpha_s$ 7	$\alpha_s$ 7,8	$\alpha_s$ 8	$\alpha_s$ 8,9
Lysine	1.1	1.0	1.0	1.0	2.0
Histidine	1.0	1.1	1.0		2.9
Aspartic acid					5.0
Threonine					1.1
Serine					1.9
Glutamic acid	1.0				
Proline					1.0
Glycine	1.0	1.0	1.1		3.0
Alanine	0.9	0.9	1.0		4.2
Valine					2.0
Leucine					6.9

Thus, in the absence of a lysine-lysine bond at position 60–61 in the  $\alpha_f$  chain, the formation of peptides comparable with  $\alpha_s$  7,  $\alpha_s$  7,8, and  $\alpha_s$  8,9 is prevented, with the result that only one peptide ( $\alpha_f$  7,8) is produced from this region of the  $\alpha_f$  chain on digestion with trypsin, instead of the three (and free lysine) normally found in digests of the  $\alpha_s$  chain.

We deduced the substituent to be glutamine rather than glutamic acid, for digestion of  $\alpha_f$  7,8 with "pronase"

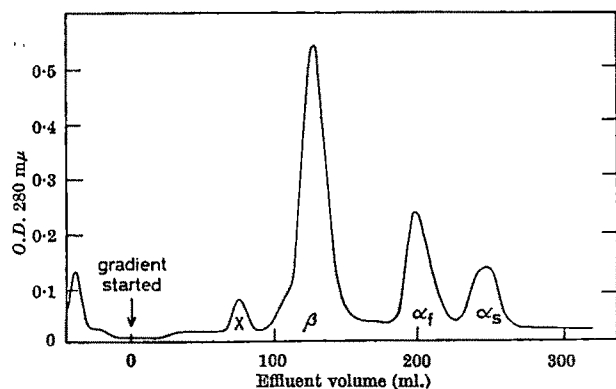


Fig. 1. Separation of the chains from 80 mg of horse globin on a 1 × 10 cm CM cellulose column. The chains were eluted at 1 ml./min by applying a linear Na<sup>+</sup> gradient made by mixing 150 ml. of 0.005 molar Na<sub>2</sub>HPO<sub>4</sub> and 150 ml. of 0.03 molar Na<sub>2</sub>HPO<sub>4</sub>, both adjusted to pH 6.5 with phosphoric acid and made 8 molar in urea and 0.05 molar in 2-mercaptoethanol. Peak X appeared in variable amounts and gave a fingerprint which appeared identical with that of the  $\beta$ -chain.

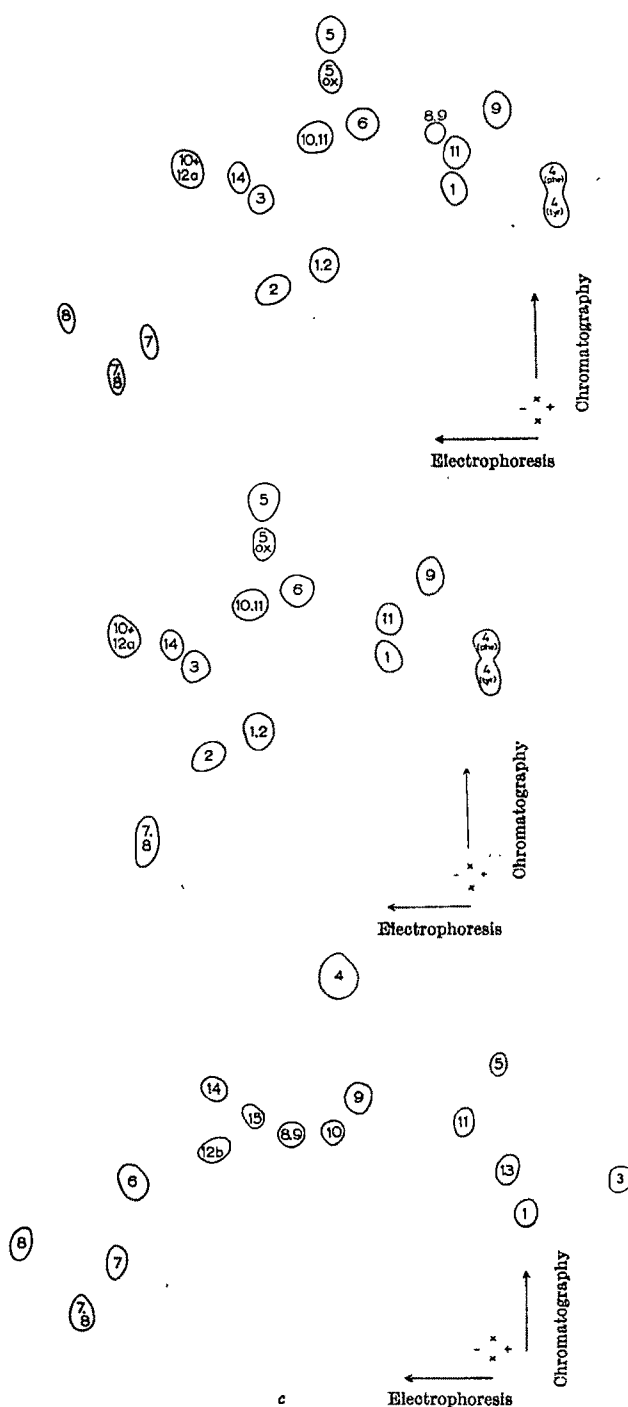


Fig. 2. Tracings of fingerprints of (a) amino ethylated  $\alpha_s$  chain, (b) amino ethylated  $\alpha_f$  chain, and (c) amino ethylated  $\beta$ -chain. We were unable to find  $\beta$ 2 or  $\beta$ 12a in fingerprints of amino ethylated  $\beta$ -chain although the peptides both before and after these in the  $\beta$ -chain were found in good yield. Peptides are numbered sequentially starting from the N-terminal end of the protein chain.

gave a peptide of composition histidine, glycine, glutamic acid (after acid hydrolysis), basic at pH 6.5, and another of composition glycine, glutamic acid neutral at pH 6.5, thus ruling out the presence of an acidic residue.

Our results are consistent with those of Perutz *et al.*<sup>3</sup> and Stockell *et al.*<sup>4</sup>. In fingerprints of whole fast globin the absence of peptides  $\alpha_s$  7,  $\alpha_s$  7,8, and  $\alpha_s$  8 would not have been noticed, for they are identical with the corresponding peptides of the  $\beta$ -chain and thus run in the same place. The only extra peptides seen would be  $\alpha_f$  7,8 in the fast globin and  $\alpha_s$  8,9 in the slow, which probably correspond to peptides 1 and 2 respectively in Stockell *et al.*<sup>4</sup>.

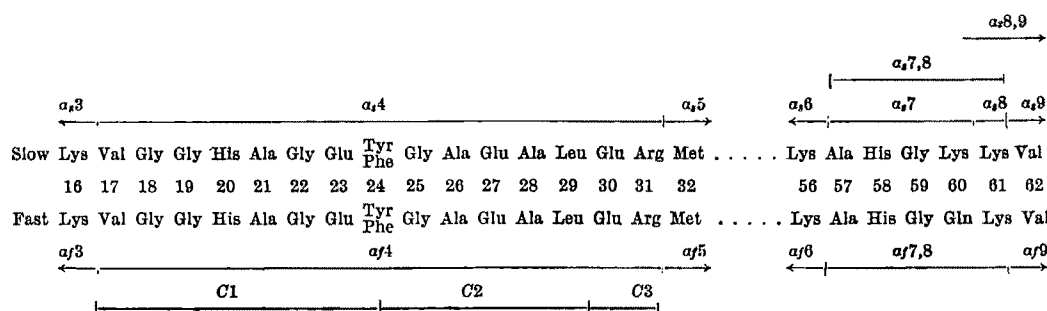


Fig. 3. Amino-acid sequences of portions of the  $\alpha$  and  $\alpha_f$  chains showing the region in which they differ.

During the analysis of the peptides eluted from the fingerprints of the  $\alpha$ -chain, peptide  $\alpha_4$  consistently gave non-integral values for both tyrosine and phenylalanine, in contrast to the reported value of one residue of tyrosine. Analysis of  $\alpha_4$  from the fast and slow  $\alpha$ -chains of several horses with both tyrosine and phenylalanine in this peptide gave 0.4 moles of tyrosine and 0.6 moles of phenylalanine/mole of peptide; these values were the same for both fast and slow chains. The peptide was partially resolved into two components by chromatography in the normal peptide mapping system and completely resolved on a long chromatographic run. The two peptides thus obtained had the normal  $\alpha_4$  composition except that the chromatographically fast component had one residue of phenylalanine and no tyrosine, and the other component had one residue of tyrosine and no phenylalanine. To confirm that the tyrosine and phenylalanine residues occurred in the same position, the mixed peptides were digested with chymotrypsin to give three peptides which separated at pH 3.5 with compositions corresponding to C1, C2 and C3 in Fig. 3. The Pauli-positive peptide C1 was chromatographed in BAWP to give two components, one which contained one residue of tyrosine and the other one residue of phenylalanine/mole of peptide. Digestion of these two peptides with carboxypeptidase A released one residue of tyrosine and one residue of phenylalanine respectively and no other amino-acids. This showed that both the  $\alpha_f$  and  $\alpha_s$  chains give two  $\alpha_4$  peptides on digestion with trypsin, one with tyrosine at position 24 and the other with phenylalanine in the same position.

Other horses were found later in which both the  $\alpha_f$  and  $\alpha_s$  chains had only tyrosine or phenylalanine at position 24. The presence of very low proportions of the second amino-acid cannot be ruled out because some tyrosine is destroyed on acid hydrolysis, and because both residues give rather broad peaks on the amino-acid analyser, making it more difficult to detect very small amounts. Also, the yield of peptides eluted off paper is low (about 20–30 per cent in the case of  $\alpha_4$ ).

The compositions of the remaining homologous tryptic peptides of the  $\alpha_f$  and  $\alpha_s$  chains appear to be identical, although double substitutions of the type  $A \rightarrow B$  and  $B \rightarrow A$  in the same tryptic peptide would not have been detected. The core (residues 105–139) has not been analysed. We have failed to find any other examples of amino-acids which occur in the  $\alpha_f$  and  $\alpha_s$  chains in non-integral amounts comparable to those at position 24.

We conclude, therefore, that there are a total of four horse  $\alpha$ -chains.

While in other mammals multiple electrophoretically distinct haemoglobins have been accounted for by differences in the non- $\alpha$ -chains<sup>8</sup>, in horse the  $\beta$ -chains appear to be identical while multiple  $\alpha$ -chains exist. Both the amino-acid replacements can be explained by the substitution of single nucleotide bases in the corresponding codons of the messenger RNA.

One obvious explanation for the tyrosine/phenylalanine heterogeneity is to assume the existence of two allelic

genes; however, because our experiments would probably not have detected very small amounts of tyrosine in horses thought to contain only phenylalanine at position  $\alpha_{20}$  and vice versa, the evidence in favour of allelic genes is not yet conclusive.

The lysine/glutamine heterogeneity has been thought to be caused by two closely linked non-allelic genes, for no animals homozygous for either lysine or glutamine (that is, slow or fast haemoglobins) have been detected<sup>2</sup>. The simultaneous presence of tyrosine and phenylalanine at position 24 of both the fast and slow  $\alpha$ -chains in some horses makes it difficult to accept this explanation, since it would imply that independent mutations had occurred at the same point in both genes.

An alternative explanation of the lysine/glutamine heterogeneity would be an ambiguity in the translation of a messenger RNA codon as postulated by von Ehrenstein<sup>9</sup> to account for multiple  $\alpha$ -chains in rabbit haemoglobin and by Rifkin *et al.*<sup>10</sup> to account for the inheritance of two mouse haemoglobins in a strain inbred for many generations.

In this context, it is noteworthy that the related donkey has only a single haemoglobin (which can be distinguished electrophoretically in two steps from the two horse haemoglobins because of the substitution of an asparagine residue for the histidine at  $\alpha_{20}$ , shown by unpublished results of Kilmartin and Clegg). The  $\alpha$ -chain of this haemoglobin seems to have only phenylalanine at position 24 and lysine at position 60. Thus, the  $\alpha$ -chain of donkey haemoglobin appears to be under the control of a single structural gene, and it would seem not unreasonable to assume that this is also the case in the horse. A distinction between these alternative interpretations of our results might be possible if a horse with a charged  $\alpha$ -chain variant could be found. If the glutamine/lysine heterogeneity were caused by two non-allelic genes, only one of the two components, either the fast or the slow, should be affected by the mutation. If it is caused by an ambiguity in the reading of the code, both components should be affected.

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# LETTERS TO THE EDITOR

## PLANETARY SCIENCE

### Measurements of the 1.9 cm Thermal Radio Emission from Mercury

MEASUREMENTS of the thermal radio emission from Mercury at 11.3 cm have shown that the brightness temperature is approximately 300° K and does not vary significantly with phase angle<sup>1</sup>. This is not inconsistent with the observed rotation rate of Mercury of  $58.4 \pm 0.4$  days<sup>2</sup> if the thermal inertia of the planet is sufficiently great for the temperature to remain reasonably constant at the depth at which the 11 cm emission originates. It might be expected, however, that a phase effect may exist at shorter wavelengths where the radio emission originates closer to the surface and where the temperature may be more closely related to the instantaneous value of the solar radiation. It is somewhat surprising therefore that Epstein<sup>3</sup>, who observed at 3.4 mm wavelength, claims no phase effect, with an anomalously low temperature of only  $\sim 200^\circ$  K.

We have observed the thermal radio emission from Mercury at a wavelength of 1.9 cm with the 140 ft. radio telescope at the National Radio Astronomy Observatory using a tunnel diode radiometer which had a system noise temperature of 1,200° K and a bandwidth of 2 Ge/s. The root mean square noise fluctuations were equivalent to a source of 0.15 flux units\* ( $0.02^\circ$  K) with an output time constant of 10 sec. The flux density of Mercury varied from about 1.5 flux units near superior conjunction to about 4.4 flux units near inferior conjunction. In order to minimize the effect of atmospheric noise fluctuations, the receiver was switched between the main beam (directed along the electrical axis of the paraboloid) and an offset reference beam pointed 6.5 (about 3 half power beamwidths away).

The observations were made in February and March 1966, and during this time Mercury moved from superior conjunction to just past inferior conjunction. The procedure used was to scan the antenna in hour angle at a rate of 2' arc/min such that the planet moved through a half-power beamwidth in 1 min. The measurements were confined to within  $\pm 1$  h of hour angle to minimize the effect of the decrease in antenna gain with increasing zenith angle. Observations of the source 3C 273 at a nearby declination over a range of hour angles showed that the relative gain was given by

$$G(h)/G(0) = (1 - ah^2)$$

where  $a \approx \frac{1}{2} \times 10^{-4}$  and where the hour angle  $h$  is expressed in minutes of arc.

The direction in which the telescope pointed was known to be sufficiently accurate so that over a period of a few hours near transit positions several minutes of arc apart could be set with a relative accuracy of a few seconds of arc. Because the absolute pointing varied by as much as 30" from day to day, however, we adopted the following procedure. Successive scans in right ascension were made at several declinations over a range of about 2' arc near the expected position of the planet. Examples of several such scans are shown in Fig. 1. At each declination the amplitude of the scan in right ascension was determined and the corresponding value at the meridian was found by correcting for the change in gain of the telescope

with hour angle. These amplitudes were then plotted as a function of relative declination which was corrected for the slight motion of the planet in declination during the course of the observations. The antenna temperature of the planet was determined from the best fitting curve through the measured points. Each day's measurements were referred to a noise tube calibration. The consistency of the noise tube was established from measurements of a number of non-thermal sources which were observed throughout the period. The measured antenna temperatures were converted to flux densities by means of observations of a number of standard sources. We have assumed flux densities of 28, 4.6 and 2.4 flux units respectively for Virgo A, Hydra A, and 3C 161 and disk temperatures of 500° K, 180° K and 200° K for Venus, Jupiter and Saturn respectively. During the course of these observations Mercury moved from declination  $-6^\circ$  to  $+5^\circ$ , in which range the relative gain of the antenna changed by 25 per cent. The uncertainty in this change is probably not more than 5 per cent and we estimate that the overall calibration is not in error by more than 15 per cent.

The equivalent temperature of a black-body disk is plotted in Fig. 2 as a function of planetocentric phase angle,  $\theta$ . A clear phase effect is apparent with a minimum temperature which occurs somewhat before inferior conjunction. The best fitting phase law of the form

$$T_{BB} = T_0 + \Delta T \cos(\theta + \phi)$$

gives  $T_0 = 288^\circ \pm 7^\circ$  K,  $\Delta T = 75^\circ \pm 13^\circ$  K and  $\phi = 38^\circ \pm 17^\circ$ . The quoted errors do not include the uncertainty in the calibration of the flux scale. The mean value of 288° K is in good agreement with the value found at 11 cm and is close to the value expected from solar heating. The positive sign of the phase angle  $\phi$  is consistent with a forward direction of rotation as the minimum temperature occurs before inferior conjunction when the sub-earth point is in the region after midnight on Mercury.

More recent observations at 3.4 mm (Epstein, private communication) give a phase curve the mean temperature, temperature variation and phase lead of which are in substantial agreement with the values we have found at 1.9 cm. This is contrary to the earlier report that there was no significant variation of temperature with phase angle at 3.4 mm (ref. 3). Evidence for a pronounced phase dependence at an intermediate wavelength of 8 mm has also been reported by a group of Soviet observers.

Because of its highly eccentric orbit, the temperature of Mercury at any particular phase angle probably varies

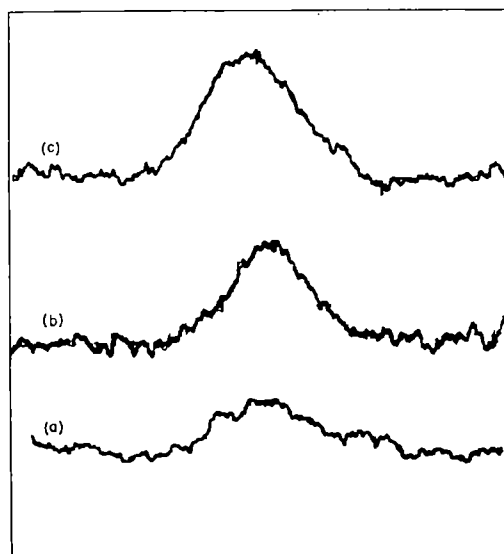


Fig. 1. Scans through the planet Mercury made at a rate of 2 min of arc/min with a 10-sec time constant. a, February 26, 1966; b, March 4, 1966; and c, March 12, 1966.

\* 1 flux unit =  $10^{-26}$  W/m<sup>2</sup>/c.p.s.

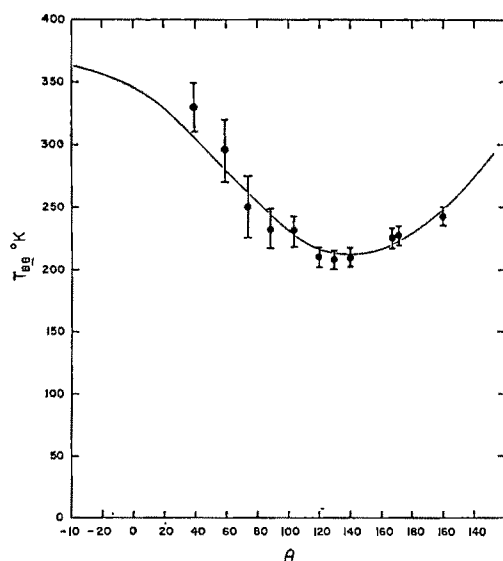


Fig. 2. Measured values of the equivalent black-body temperature,  $T_{BB}$ , of Mercury as a function of the planetocentric phase angle,  $\theta$ . The solid curve is the best fitting sinusoidal curve  $T_{BB} = 238 + 75 \cos(\theta + 38)$ .

from one revolution to another and it may be expected that prolonged observations over many cycles and at several wavelengths will play an important part in determining the thermal properties of the surface of Mercury.

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## Organic Molecules and the Coloration of Jupiter

BOTH naked eye and photographic observations of the visible surface of the planet Jupiter reveal the presence of regions of contrasting and variegated colours. The bands, belts and spots—particularly the Great Red Spot—exhibit characteristic coloration which must indicate differences in molecular composition from place to place at the level of the Jovian clouds. All spectroscopic searches for characteristic spectral features unique to a band, belt or spot have been negative. For example, the Great Red Spot appears spectroscopically as enhanced continuous absorption at short wavelengths with no other identifying spectral features at the resolving powers used. The variable appearance of the Jovian cloud coloration suggests that the molecules responsible are synthesized in certain locales, transported, and dissociated in other locales. Were the coloured compounds produced by a planet-wide thermodynamic equilibrium, the planet should show a generally uniform coloration. Micro-meteoritic infall should provide a small steady-state abundance of certain minerals; however, it seems highly implausible that such minerals would be differentially distributed over the clouds of Jupiter in such a way as to explain the coloration. A more plausible source of chromophores would appear to be the chief atmospheric constituents themselves, thus implying that the coloration is caused by the presence of organic matter at the cloud level of Jupiter<sup>1,2</sup>.

Hydrogen, methane, and ammonia are known to be constituents of the Jovian atmosphere. Because of the high cosmic abundance of oxygen, the impossibility of escape of atomic oxygen from contemporary Jupiter, and the low temperatures of the Jovian clouds, water is expected to be a chief constituent of the atmosphere below (and possibly near) the clouds. In an experiment in which Jovian conditions were simulated, a mixture of hydrogen, methane and ammonia was supplied with energy from a corona discharge producing such simple organic molecules as hydrogen cyanide, methyl cyanide, acetylene, ethylene and ethane<sup>3</sup>. Their interaction products were predicted to be brightly coloured. When water was added, aldehydes were also produced. In experiments relevant to the origin of life on Earth, mixtures of materials that are in effect simulated Jovian environments have been subjected to a wide array of energy sources and organic molecules have been produced in consistently high yield provided that the overall conditions are reducing<sup>4</sup>. Electrical discharges and solar ultra-violet light—which both lead to high electronic temperatures of the excited atoms and are to be expected in the vicinity of the Jovian clouds—will lead to the production of organic molecules by the thermodynamic equilibrium processes at high temperatures and subsequent quenching.

With the pressures, temperatures, and atomic composition prevailing at and below the Jovian clouds we have calculated with an electronic computer the expected distribution of molecular species under local thermodynamic equilibrium<sup>5</sup>. We find that because of the great excess of hydrogen, even such simple organic molecules as ethane are present with volume mixing ratios  $\leq 10^{-15}$ ; with strict thermodynamic equilibrium the coloration of the Jovian clouds cannot be explained by organic molecules.

We have found, however, that with high local temperatures and quenching there is a wide range of pressures and temperatures in which substituted benzene rings and polycyclic aromatic compounds are formed in significant yield. Two sample systems are displayed in Table 1. The first, at a pressure of 1 atmosphere and a temperature of 1,500° K, is intended to represent electrical discharges in the vicinity of the visible clouds; the second, at 10<sup>-8</sup> atmosphere and 1,000° K, is intended to represent a hypothetical Jovian thermosphere from which complex organic molecules rapidly diffuse to lower altitudes. In the former case, by far the most abundant compounds synthesized according to our calculations are just the compounds preferentially produced in the laboratory by corona discharge in the Jovian simulation experiments of Sagan and Miller<sup>3</sup>. In these sample calculations brightly coloured compounds (such as azulene, asphalt and azobenzene) are synthesized, as had also been predicted. More recently, experiments have been performed by Ponnampuruma<sup>6</sup> involving corona discharge in anhydrous mixtures of methane and ammonia at low temperatures. He finds that a reddish-brown polymeric material is

Table 1. PREDICTED EQUILIBRIUM IN THE JOVIAN ATMOSPHERE AT HIGH TEMPERATURES AND MODERATE TO LOW PRESSURES

Pressure	~1 atm.	~10 <sup>-8</sup> atm.
Temperature	1,500° K	1,000° K
Noble gases	0.4	0.4
Hydrogen	0.6	0.6
Methane	4 × 10 <sup>-3</sup>	6 × 10 <sup>-3</sup>
Acetylene	2 × 10 <sup>-4</sup>	2 × 10 <sup>-3</sup>
Ethylene	3 × 10 <sup>-5</sup>	7 × 10 <sup>-7</sup>
Ethane	2 × 10 <sup>-7</sup>	1 × 10 <sup>-3</sup>
Benzene	2 × 10 <sup>-9</sup>	1 × 10 <sup>-7</sup>
Naphthalene	1 × 10 <sup>-13</sup>	4 × 10 <sup>-6</sup>
Asphalt (yellow)	6 × 10 <sup>-23</sup>	1 × 10 <sup>-8</sup>
Nitrogen	7 × 10 <sup>-5</sup>	6 × 10 <sup>-5</sup>
Hydrogen cyanide	6 × 10 <sup>-3</sup>	9 × 10 <sup>-5</sup>
Ammonia	2 × 10 <sup>-7</sup>	2 × 10 <sup>-13</sup>
Methyl cyanide	6 × 10 <sup>-3</sup>	2 × 10 <sup>-10</sup>
Azulene (blue)	5 × 10 <sup>-14</sup>	2 × 10 <sup>-9</sup>
Aniline	3 × 10 <sup>-10</sup>	2 × 10 <sup>-23</sup>
Azobenzene (red)	3 × 10 <sup>-23</sup>	3 × 10 <sup>-23</sup>

The formation of graphite was excluded. High temperatures produced by lightning could permit approach to such equilibria, followed by rapid quenching. Polynuclear aromatics and coloured compounds such as azulene tend to form.



synthesized by reaction between cyanides and hydrocarbons, a result consistent with the conclusions of the present paper. Similar results have been obtained by Lippincott and Pratt<sup>7</sup>, using a plasma discharge of radio frequency. Under conditions consistent with the thermodynamic calculations, bright yellow mixtures of unsaturated high molecular weight compounds form. Specific compounds identified include pyrene, coronene and chrysene. The first two of these compounds are yellow, while the last has a red-blue fluorescence. Thus equilibrium thermodynamics alone tends to produce complex organic molecules—some of them highly coloured—provided that high temperature occurs locally and intermittently.

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### The Mundrabilla Meteorite: a New Discovery in Western Australia

In March 1966, while engaged on a geological survey of a portion of the Eucla Basin in Western Australia, we discovered two large iron meteorites. The two principal masses, lying some 600 ft. apart, are located on the Nullarbor Plain, to the north of the Transcontinental Railway (latitude 30° 47' S., longitude 127° 33' E.). Earlier reports of the sighting of a meteorite by a rabbit-trapper, now deceased, have led to several expeditions which failed, however, to discover these masses.

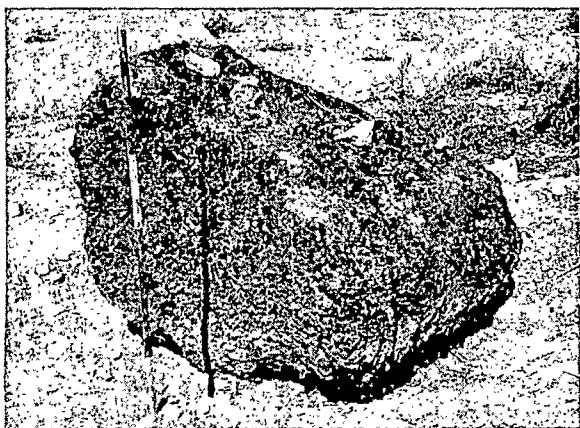


Fig. 1. Large mass of the Mundrabilla Meteorite, showing "basal" surface and "parting" surface.



Fig. 2. Large mass—close-up view of "parting" face showing knobby surface. A matchbox gives the scale.



Fig. 3. Smaller mass of the Mundrabilla Meteorite, showing ablation "furrows" with the "parting" surface to the left of the picture.

Field estimates indicate approximate weights of the masses to be 10 to 12 tons and 4 to 6 tons respectively. Both weights are considerably in excess of any previously recorded Australian discoveries.

The masses lie within slight depressions in clayey soil which overlies Nullarbor Limestone of Miocene age. Excavation has revealed the presence of an underlying irregular crust of finely laminated iron "shale", which rests directly on silicified Nullarbor Limestone. Similar fragmentary iron shale also occurs in an incomplete stellate pattern, distributed for many hundreds of feet, largely eastwards of both masses.

The larger mass tends to have a crude conical to hemispherical shape with the nose partially buried in the soil. In the present position of rest the axis is inclined at an angle of approximately 60° (Fig. 1).

Evidence of fragmentation of a larger mass is afforded by a sharp, angular, vertical face on the larger mass, which matches both in size and shape a similar sharp face on the smaller mass. Another parallel incipient fracture is apparent on the "basal surface" of the larger mass (Fig. 1).

The surfaces of each mass present an extremely "knobby" appearance (Fig. 2), presumably where troilite grains have been selectively ablated during passage through the atmosphere. Atmospheric weathering has undoubtedly modified these features although ablation furrows and striations are still well preserved and provide remarkably clear evidence of stable orientation in passage through the Earth's atmosphere (Fig. 3). Concentrations

of small iron fragments around the bases of each mass are presumed to have weathered from the larger masses since the fall.

The material of several of these smaller fragments has been shown by polishing and etching to belong to the medium-octahedrite subdivision of the iron meteorites. Large troilite grains together with irregular masses of graphite and (?) schreibersite are also present.

By means of a detailed grid of an area of several acres around the larger masses the distribution pattern of small iron and iron-shale fragments has been established. Preliminary investigation of the results indicates that the meteorite came from the west at a relatively low velocity and high angle. No evidence of cratering or bouncing was found and it appears that the meteorite parted from its smaller fragment before impact with the Earth's surface. The process of burning apart along one of two parallel fractures by the torch-like effect of concentrated air-channelling has abraded a great number of smaller fragments, which are distributed along a west to east band for at least 1 mile.

The area of the fall has an extremely arid climate, only a very thin soil cover and is one of very flat terrain with little or no drainage features. Consequently, the present find represents an extremely well preserved record of the fall of a large iron, from which much valuable information in many aspects of meteoritics may be gained.

We thank the managements of Beach Petroleum N.L. and Geosurveys of Australia Pty., Ltd., who have made possible the visits to the area and thus the collection of field data. We also thank Prof. A. R. Alderman and the Meteorite Advisory Committee of Western Australia.

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### Some Geophysical Observations on the Great Glen Fault

EARLIER geologists<sup>1-3</sup> have considered the Great Glen fault as a normal or dip-slip fault, while Kennedy<sup>4</sup> interpreted it as a strike-slip fault, basing his view chiefly on a lithological similarity between the Strontian and Foyers granitic complexes, and he suggested that the former has been moved 65 miles south-west along the Great Glen fault. Shand<sup>5</sup> has not found such characteristic features of strike-slip faults as slickensides or friction-grooves and mylonite. Collette<sup>6</sup> thinks that the fault passes to the south-east of the Shetland Islands. Flinn<sup>7</sup> has examined the submarine topography of the Moray Firth and has outlined a possible course of the fault. Wilson<sup>8</sup> joins the Great Glen fault to the Cabot fault of North America. Riddihough<sup>9</sup> has conducted a detailed magnetic survey off the north coast of Ireland and suggests the presence of two faults which may have some relation to the Caledonian fault systems of Scotland.

The present communication briefly presents the results of a geophysical study of the Great Glen fault<sup>10</sup>. A study of historical earthquake records together with the instrumental determination of seismic epicentres shows three active centres along its length at each of which there has been repeated activity since 1768. All the epicentres except two lie on the southern side of the fault. The Inverness area appears to be the most active region, but no earthquake has been reported since 1934. The region near Fort William has become active since 1924. A probable westward continuation of the fault is suggested by the submarine contours in the region, and the continuation

and the submarine contours are shown in Figs. 1 and 2, respectively.

A survey of the total magnetic field intensity and vertical magnetic field intensity of the fault has indicated magnetic anomalies of deep seated origin over the Strontian and Foyers granitic complexes. Fig. 3 shows the anomalies of total magnetic intensities. The total magnetic anomaly of the Foyers granitic complex is approximately elliptical in plan and seems to reflect the general shape of the granitic complex. It has the steepest gradient towards the north. The maximum amplitude of the

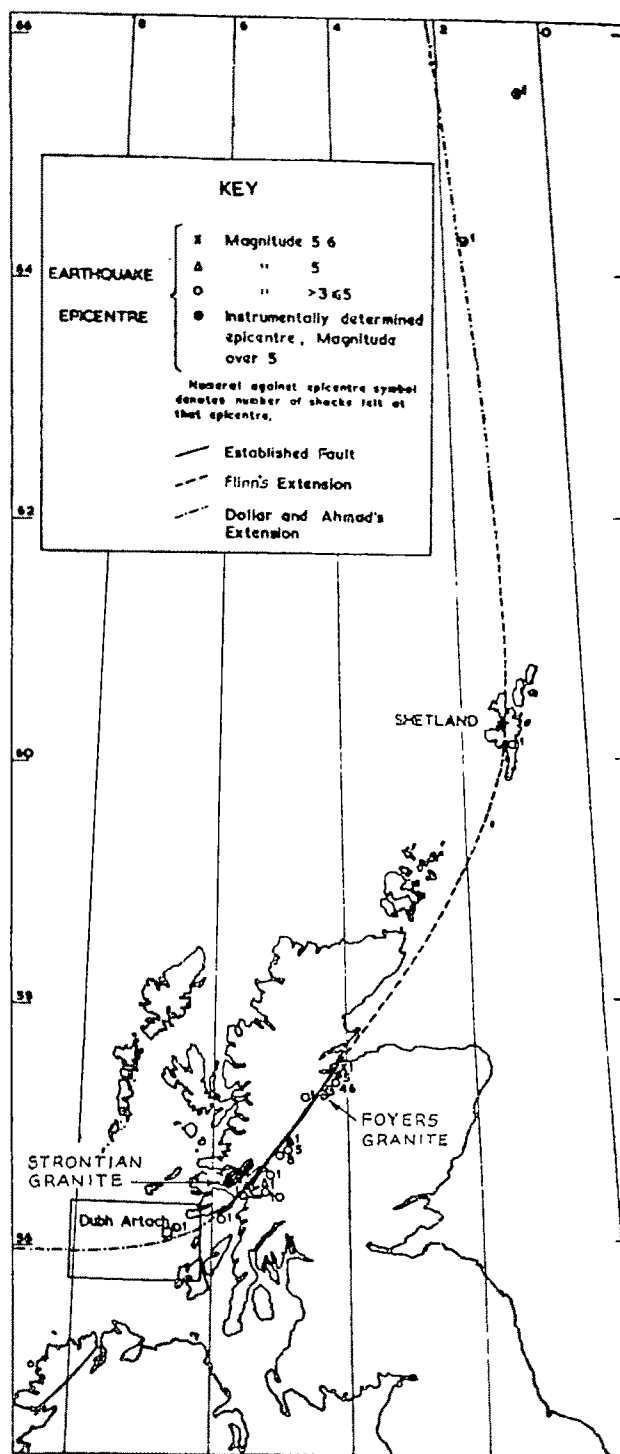


Fig. 1.

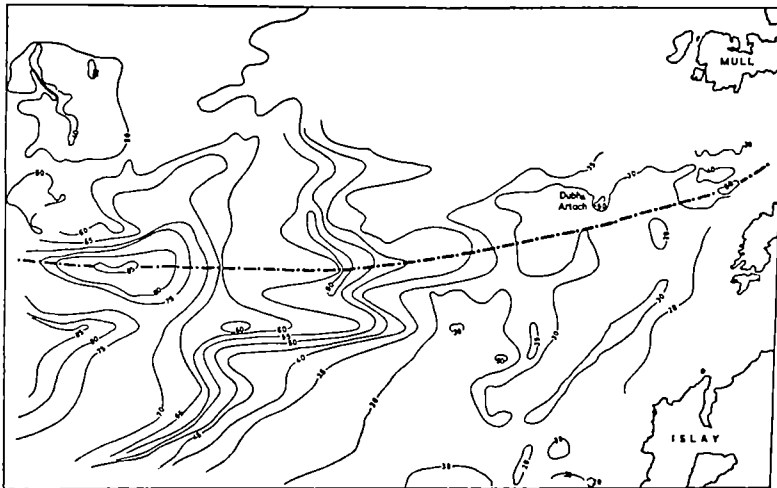


Fig. 2. Submarine contours showing the western extension of the Great Glen fault. Scale: 1 in. = 18 miles.

anomaly is 440 gamma and nearly half of the granitic complex is enclosed by 400 gamma contours. On the basis of the magnetic anomaly, it is suggested that the Foyers granitic complex is a stock-like intrusion about 2 miles wide and 8 miles long, extending to a depth of about 13 miles with the strike of its major horizontal axis N50E, and the stock is deduced to lie well to the south-east of the Great Glen—south of Loch Mhor. The contours of the Strontian total magnetic anomaly also follow the shape of the granitic complex and the steepest gradient is towards the west. The maximum amplitude of the anomaly is 350 gamma and nearly half of the complex is surrounded by 200 gamma contours. One of the probable interpretations of the anomaly is that the Strontian granitic complex is a separate intrusion of a stock-like form about 4 miles wide and 8 miles long extending to a depth of about 13 miles with the strike of its major horizontal axis nearly north-south and its stock is centred south of Kingairloch. Magnetic bodies can extend only to a certain depth in the Earth's crust and 13 miles is considered to be the maximum depth to which matter can remain magnetic. The stock may be deeper than 13 miles, but its real depth cannot be detected by the magnetic anomaly.

By the help of Vacquier<sup>11</sup> theoretical magnetic models in which the direction of magnetization is assumed in the Earth's main magnetic field, susceptibility contrast is determined, and the values are 0.0054 and 0.0023 for the Foyers and Strontian granitic complexes, respectively. The values are rather high. Thirteen thin sections from the Strontian and Foyers complexes were examined, and from them it is observed that the Foyers complex has twice the volume of magnetite than the Strontian complex has. Mould<sup>12</sup> has reported higher values of ferric and ferrous oxide for the Foyers complex compared to the values for the Strontian complex<sup>13</sup>.

A radioactivity field survey has revealed that the Strontian granitic complex has an average natural gamma radiation of about 6  $\mu$ r./h as compared to the Foyers granitic complex of about 3  $\mu$ r./h. No significant change in radioactivity was found to occur between the three members of either the Strontian or Foyers complex. Results

of a laboratory radiometric assay give an average for the Strontian complex of 31 p.p.m. (equivalent octuranium trioxide) as compared with 25 p.p.m. for the Foyers complex.

Foyers granitic complex is more magnetic and less radioactive as compared with the Strontian. Both intrusions extend down to a depth of about 13 miles and have separate stocks and different strikes. Munro<sup>14</sup> has reported structural differences between the two complexes. On the basis of available geophysical and geological evidence, it is considered improbable that the Strontian granitic complex is a part of Foyers, thereby throwing doubt on Professor Kennedy's postulated horizontal displacement of these masses by about 65 miles.

It is postulated that a stress pattern with a dominant vertical component was brought into being at the time of, or as a result of, the 'Newer Granite' intrusions, which gave rise to the Great Glen fault. The seismic and geological evidence is consistent with the dip-slip movement on the fault.

I thank Dr. A. T. J. Dollar for his help and guidance, Professor J. M. Bruckshaw for lending me equipment and for other facilities, and Dr. W. Bullerwell and Dr. D. Ostle for lending me equipment, and the Central

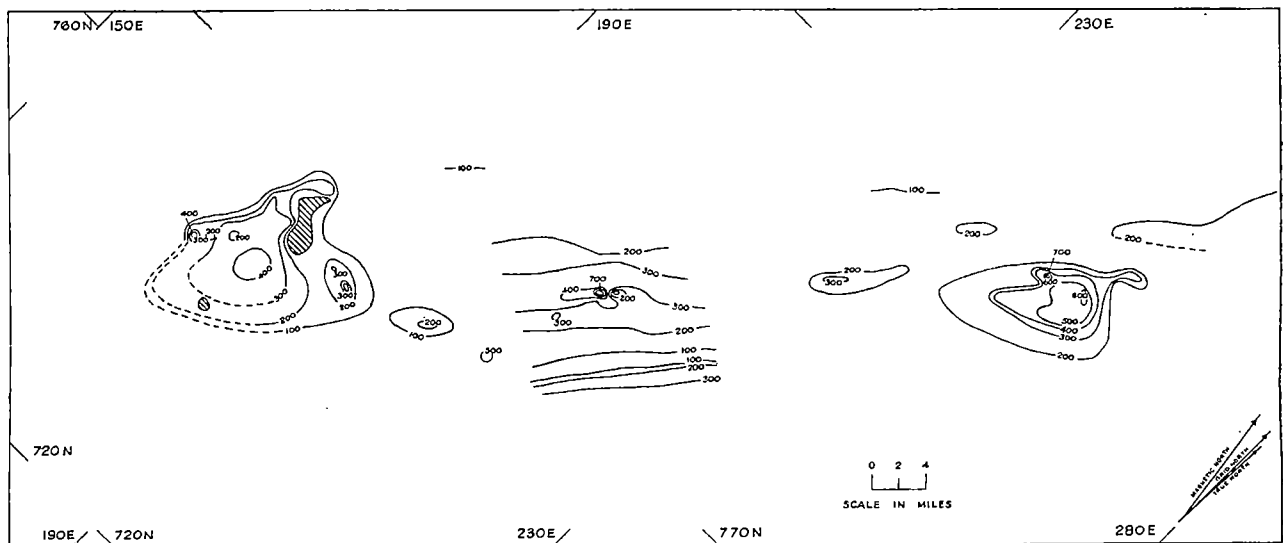


Fig. 3. Total magnetic field anomaly contours.

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## PHYSICS

### Does a Moving Body appear Cool?

P. T. LANDSBERG in an interesting note<sup>1</sup> suggests that the "true" value of temperature of a moving body at relativistic speeds will appear to be the same as the temperature measured in the inertial frame of the body itself. He reaches this conclusion by re-defining temperature in terms of entropy and of internal energy.

I would like to suggest that the whole concept of the apparent temperature of a moving body needs some physical consideration before any mathematics are employed at all.

Suppose that the moving body consists of a box containing gas at about 0° C temperature. Then, if this gas is observed as it moves rapidly past a stationary observer, it will, as is well known, suffer a Fitzgerald-Lorentz contraction in the direction of motion by an amount of  $\sqrt{1-w^2/c^2}$  or  $1/\beta$ , where  $w$  is the velocity of the box with respect to the observer. We know, also, that time measured in the box will move more slowly than time as measured by the observer, the same factor being involved.

This means that if we consider any individual molecule in the gas in the box which happens to be moving in the direction of motion of the box, its velocity will appear to our observer to be reduced by a factor  $\beta^2 \times$  the measurement made relative to the box itself, because the distance which the particle moves between two collisions or other events will be reduced and the time between them increased as seen by the observer.

On the other hand, a molecule moving transversely to the box will appear to the observer to cover exactly the same distance relative to the box between two events, in the  $\beta \times$  increased time, so that in this case its apparent velocity is reduced only  $\beta$ -times.

We have thus, in the view of the stationary observer, a set of particles in the box with a distribution of velocity in one direction quite different from that in the two transverse directions, and any arguments about temperature, which is normally taken to refer to isotropic motions at least in a gaseous system, will be very difficult to apply. The mass of each particle will, of course, appear to be increased  $\beta$ -times to the observer in whatever direction the particles are moving, but nevertheless the mean relative kinetic energy of the particles will appear to be reduced, even if moving transversely.

On the simplest definition of temperature, therefore, the body will indeed look cooler as a result of its motion, even though the question of how much cooler may be regarded as ambiguous. Temperature could, of course, be defined in a number of ways and doubtless each of these

would give a different answer to the apparent change in relativistic conditions, because, as I have tried to show, the whole idea of temperature must be changed drastically when considering such fast moving bodies.

On the general principle that the simplest definition is best, Einstein's original expectation that a moving body would appear to be cool would seem qualitatively valid, while Professor Landsberg's case would seem to be only a selected example of a very much larger class of less simple definitions.

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<sup>1</sup> Landsberg, P. T., *Nature*, **212**, 571 (1966).

## THE SOLID STATE

### Fatigue Crack Propagation in Metals

A RECENT paper by Pearson<sup>1</sup> reports the results of crack propagation experiments on various metals, of which Young's moduli  $E$  range from  $6.5 \times 10^6$  lb./in.<sup>2</sup> to  $30 \times 10^6$  lb./in.<sup>2</sup>. It was found that the fatigue crack propagation rates were sensibly equal when the applied stress  $f$  was a fixed proportion of  $E$ . I have recently obtained theoretical support for this from the (static) analysis of the stresses in the neighbourhood of a crack in a perfectly elastic sheet. Account is taken of the varying geometry of the crack and it is shown, for example, that near the tip of the crack

$$\frac{1}{E} \sigma_y \sim \frac{1}{2} \ln \left( \frac{2f^2 c}{E^2 x} \right)$$

where  $x$  is measured from the tip and in line with the crack,  $2c$  is the length of the crack, and  $\sigma_y$  the direct stress normal to the line of the crack. The important points to notice from this equation are that the stresses are not proportional to the applied load and, furthermore, they depend on the value of Young's modulus itself. A measure of the extent of the stress singularity is given by equating to unity the terms in parentheses, whence

$$\bar{x}, \text{ say} = \frac{2f^2 c}{E^2}$$

The significance of the parameter  $\frac{f}{E} \sqrt{c}$  follows immediately from the assumption that the crack propagation rate depends primarily on the distance  $\bar{x}$ .

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<sup>1</sup> Pearson, S., *Nature*, **211**, 1077 (1966).

### Amorphous Nucleation in Metals

EVIDENCE from metal films grown inside electron diffractometers suggests that the earliest stage of film growth, when metals are deposited on amorphous substrates, is the formation of completely disordered clusters of atoms. In face-centred cubic metals the average thickness for this stage of growth is less than 10 Å, but with some body-centred cubic metals<sup>1,2</sup> the amorphous phase persists to thicknesses more than 50 Å.

After the initial stage, growth can be interpreted from theoretical calculations of the elastically diffracted intensity profiles<sup>3-6</sup>. Fig. 1 shows these for cold lead, assuming randomly oriented identical parallelepipeds<sup>6</sup> each of  $n^3$  atoms;  $2 \leq n \leq 9$  in the successive curves. Fig. 2 shows another process leading to "normal" face-centred cubic diffraction patterns. Here the profiles due to 27-atom and 729-atom assemblies are combined and well developed patterns are reached when most atoms are rearranged in the larger crystals. A combination of the two kinds of process can account for the growths observed<sup>5</sup> after the initial stage.

In gold, silver, 80/20 nickel-iron alloy, nickel and lead, however, the earliest profiles recorded have only three peaks in the range  $0.5 < sa < 4.5$  and these simple profiles cannot be fitted to the profiles of 4- or 8- or 13-atom assemblies because the first ring is too sharp. Profiles of very similar shape are observed in X-ray or neutron diffraction from monatomic liquids<sup>7</sup>.

It is possible to compare the elastic electron diffraction profile of a molten face-centred cubic metal with the profiles observed at the earliest stages of vapour deposited growth, and Fig. 3 shows this comparison for lead. The curve *a* is for liquid lead about 50 Å thick, that of *b* the first stages of a growth leading to the formation of a polycrystalline lead film. The data are scaled to the same relative intensity of the first ring, and plotted on a base of  $sb$ , where  $b$  is the nearest neighbour spacing of the crystalline lattice. The curves *a* and *b* are nearly identical except for a shift outwards of *b* (implying a shorter nearest neighbour spacing). Curve *c* is the earliest stage of a silver growth, leading in the usual way to a polycrystalline diffraction profile. Curves *a*, *b* and *c* are taken from well filtered measurements and the background scattering from the support film of amorphous carbon has been subtracted—an approximation which can be justified with these specimens. Curve *d* is an unfiltered profile of

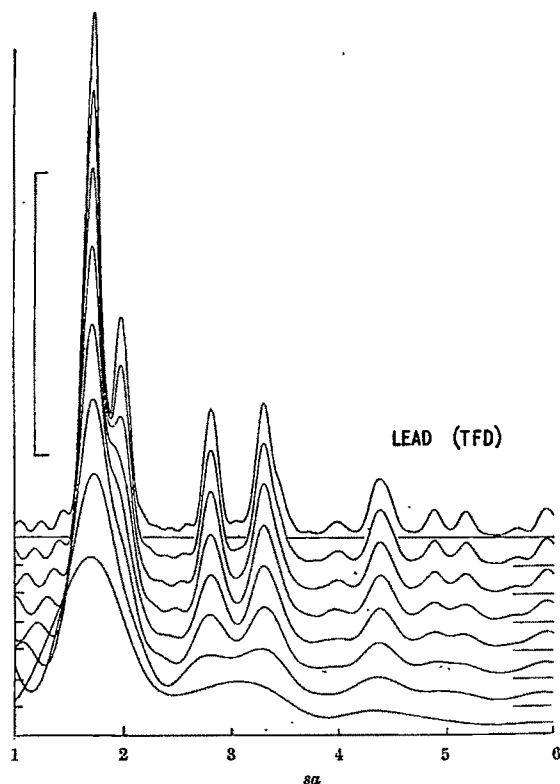


Fig. 1. Development of the theoretical differential scattering cross-sections of lead films consisting of uniform randomly oriented micro-crystals, as the size of crystal is increased. The data for  $f^2$  are taken from the *International Crystallographic Tables*, 3, 1962, and are based on the Thomas-Fermi-Dirac statistical model; interference functions are from (6).  $a$  = face-centred cubic lattice spacing,  $s = 2 \sin \frac{1}{2} \beta / \lambda$ . Successive curves are displaced upwards.

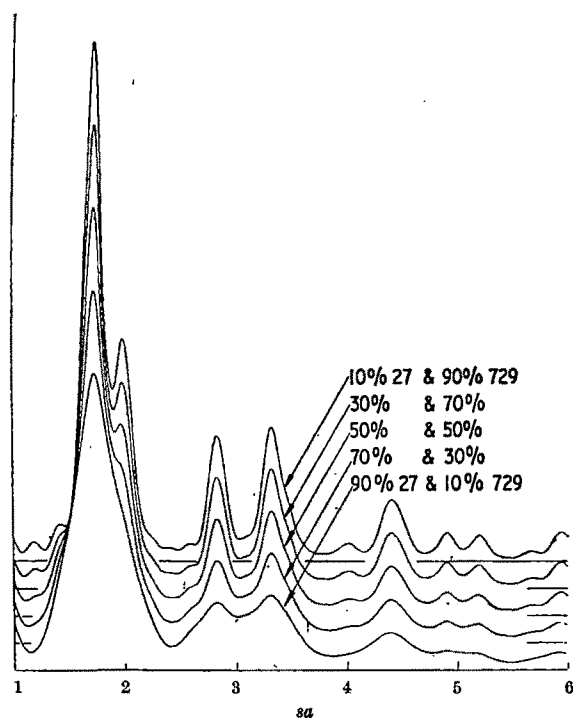


Fig. 2. Development of the theoretical diffraction profile of lead by conversion of 27-atom crystals into 729-atom crystals.

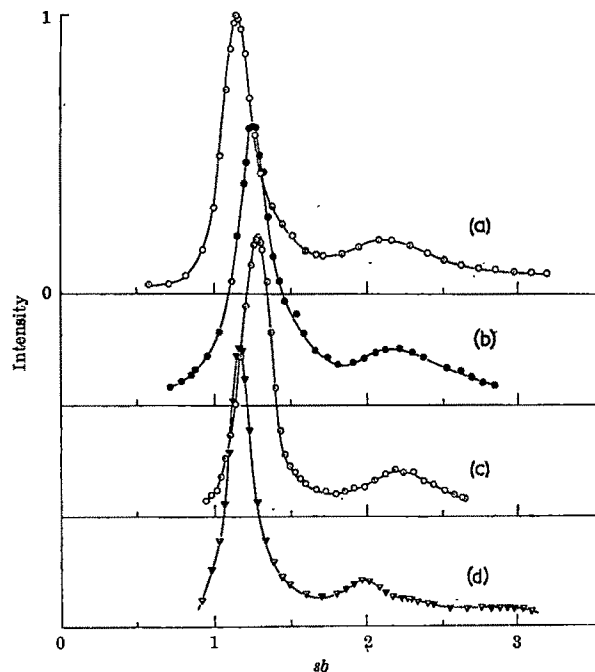


Fig. 3. Electron diffraction profiles of liquid lead (*a*), and of early stages of lead (*b*), of silver (*c*), and of iron (*d*), grown *in situ* in a scanning electron diffraction system. Different diffractometers were used for (*a*), for (*b*) and (*c*), and for (*d*). (*a*), (*b*) and (*c*) are well filtered profiles. The curves are normalized to the inner peak and the dimensionless parameter  $(sb) = 2 \sin \frac{1}{2} \beta / \lambda \times$  nearest neighbour spacing.

amorphous iron (with filtered data the inner ring is sharper).

The profiles *b*, *c* and *d* are closely similar in form to that of the liquid *a*, when plotted against the appropriate dimensionless parameter,  $s \times$  (the nearest neighbour spacing),  $b$ . The values of  $b$  at 20° C (ref. 8) are shown in Table 1; they cover a considerable range. This fact is evidence that the profiles are not due to unknown compounds formed by interaction between residual gas or substrate atoms and the first deposited atoms of the metals.



Table 1 also gives the values of  $sb$  for the inner ring, which have a spread of  $\pm 5.3$  per cent about the median value of 1.22. The spread may result in part from uncertainties in the calibration of the patterns, which was done from the actual specimen when cooled or when fully developed. With curve  $a$  the calibration is from the crystalline phase at an unknown temperature difference from the melt; and in all the curves the lattice spacing value of the calibration may differ slightly from the value taken, that of bulk metal. The important point, however, is the similarity of the profiles.

Table 1. VALUES OF  $b$  AND OF  $sb$  FOR THE INNER RING

Specimen	$b$ (20° C) <sup>a</sup> (Å)	$sb$
$a$	—	1.15
$b$	3.50	1.24
$c$	2.89	1.28
$d$	2.40	1.16

Since scattering differential cross-sections of this form are observed in the initial stages of film growth of many face-centred cubic metals and a number of body-centred metals, one may therefore conclude that the earliest stage of nucleation of metals on to amorphous substrates is clusters of atoms which are amorphous in the strict sense of the disorder characteristic of monatomic liquids.

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## CHEMISTRY

### Effect of Moisture on Liesegang Ring Formation in Ammonia-Hydrogen Chloride System

IF ammonium chloride is formed by counter-current diffusion of ammonia and hydrogen chloride gases in a long tube, periodic precipitation of the product in the form of rings is observed. This phenomenon has been explained by considering that nucleation and deposition of the product occur only when conditions of supersaturation exist, that is, when the concentration product of the reactants greatly exceeds the solubility product of ammonium chloride. By using this concept of supersaturation, Wagner<sup>1</sup> has developed a mathematical model which accounts for the formation of the "Liesegang rings" and which predicts accurately the frequency and separation of the rings.

Uncertainty arises, however, as to the importance of the presence of water vapour in the nucleation of the solid and formation of the rings. Hedges<sup>2</sup> predicted that Liesegang rings could be formed in this system in the absence of water vapour, while Johnston and Manno<sup>3</sup> expressed a contrary opinion. Spatz and Hirschfelder<sup>4</sup> generated the ammonia and hydrogen chloride from their aqueous solutions and postulated that nuclei were formed in the gas by clusters of reactant molecules of not less than a certain critical size, approximating to 100 molecules. Reaction between the molecules in the cluster was assumed to occur instantaneously.

We have investigated the effect of water vapour on the formation of Liesegang rings and its effect on the particle size distribution of the product formed. Initially, the reaction of ammonia and hydrogen chloride under moisture free conditions was investigated. The gases were passed through calcium chloride or phosphorous pentoxide

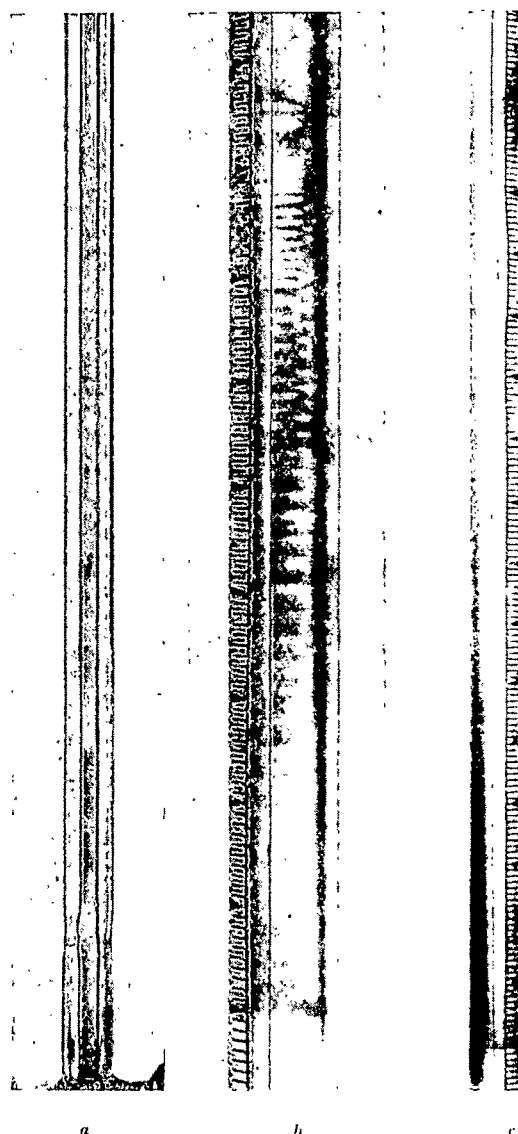


Fig. 1.  $a$  Counter diffusion of 5.12 per cent dry hydrogen chloride in nitrogen and 4.95 per cent dry ammonia in nitrogen in a 6-mm glass tube after exposure to air at 25° C, 75 per cent humidity.  $b$  and  $c$ , Liesegang rings formed by the interaction of ammonia and hydrogen chloride vapours in equilibrium with aqueous solutions at 30° C.  $b$ , 12.26 per cent w/w ammonia with 30.15 per cent w/w hydrogen chloride in an 8-mm glass tube.  $c$ , 14.91 per cent w/w ammonia with 26.15 per cent w/w hydrogen chloride in a 6-mm glass tube.

and molecular sieves. No Liesegang rings were formed, but when the tube was opened to a controlled atmosphere containing water vapour rings were successively formed as the water vapour diffused along the tube (Fig. 1a).

In the second series of experiments where the reacting gases contained water vapour, Liesegang rings formed along the tube (Fig. 1b and c) and the ring frequency and separation were in agreement with those predicted from Wagner's theory. The presence of water vapour is therefore essential for nucleation of the ammonium chloride and formation of Liesegang rings in this system.

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## Kinetics of Low Temperature Oxidation of Coal and Char

At temperatures below 300°C the rate of oxidation of coal and char decreases with increasing time of oxidation, or alternatively with increasing extent of oxidation. Two types of equations have been widely used to represent the experimental curves relating the rate of oxygen uptake  $dq/dt$  to the time  $t$  or the total uptake  $q$

$$\text{Schmidt and Elder}^1, dq/dt = Dt^E \quad (1)$$

$$\text{Elovitch type}^2, dq/dt = F \exp Gq \quad (2)$$

where  $D$ ,  $E$ ,  $F$  and  $G$  are constants for a given set of experimental conditions. Carpenter and Sergeant<sup>3</sup> have observed deviations from the Elovitch type equation and suggested that they result from fresh sorption sites being created by the evolution of the gaseous oxidation products during the oxidation of coal.

Recent work at the Coal Research Establishment on the rate of oxidation of binderless char briquettes made from a low rank coal has shown that neither the Schmidt-Elder nor the Elovitch equation fitted the kinetics curves. The failure of the Elovitch equation is shown by the departure of the experimental points in Fig. 1 from straight lines. The curves shown are derived from a fresh theoretical treatment of the problem as follows.

The rate of oxidation of a char briquette is increased merely by interrupting the oxidation and maintaining the briquette at the same temperature in the absence of oxygen before further oxidation. This suggests that during isothermal oxidation, certain active sites in the char are deactivated by the formation of a surface-oxygen complex and then reactivated by thermal decomposition of the complex. The rate of oxidation decreases with oxygen uptake until it reaches a limiting value at which the rates of deactivation and reactivation are equal.

A theoretical equation for the rate of oxidation can be derived on this basis if certain assumptions are made concerning the dependence of the rates of oxidation, deactivation and reactivation on the number of active sites. Taylor and Thon<sup>4</sup> have discussed the derivation of the Elovitch equation in the kinetics of chemisorption, and have shown that it is consistent with the assumptions that the rate of sorption is proportional to the number ( $n$ ) of active sites remaining, and that the rate of deactivation of active sites is proportional to  $n^2$ . For the present purpose we have adopted the same assumptions for the rates of oxidation and deactivation; we have in addition postulated that the rate of reactivation of sites by thermal decomposition of the oxygen complex is proportional to the number of occupied sites.

These assumptions can be expressed in the following equations, in which the notation is derived from Taylor and Thon:

$$\text{Rate of oxidation} = dq/dt = an/n_0$$

$$\text{Rate of deactivation} = (dn/dt)_1 = -\alpha n^2/n_0$$

$$\text{Rate of reactivation} = (dn/dt)_2 = \beta(n_0 - n)$$

and thus we find

$$\frac{dq}{dt} = A + \frac{AB(A+2B) \exp - \gamma t}{(A+B)^2 - B^2 \exp - \gamma t} \quad (3)$$

where  $A = (\gamma - \beta)/2\alpha$

$$B = a - A$$

$a$  = initial rate of oxidation

$$\gamma = \sqrt{\beta^2 + 4\alpha\beta}$$

The rate of oxidation thus decreases from  $a = A + B$  when  $t = 0$  to a limiting value  $A$  when  $t \rightarrow \infty$ .

With a suitable choice of parameters it is possible to fit the experimental data to within the limits of error

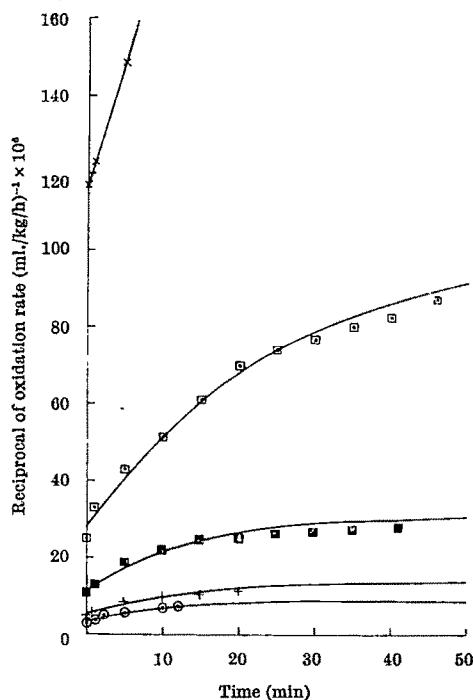


Fig. 1. Experimental and calculated oxidation rates in air of binderless char briquettes. —, Calculated; x, experimental 100°C; □, experimental 150°C; ■, experimental 200°C; +, experimental 250°C; ○, experimental 300°C.

as shown in Fig. 1. On the other hand, the data can be fitted equally well by the equation

$$dq/dt = A^1/(1 - B^1(\exp - \phi t)/(A^1 + B^1)) \quad (4)$$

for which, however, we have been unable to provide a theoretical derivation. Although equation (3) may be preferable for fundamental investigations, it is awkward in form for practical use. Equation (4) and its equivalent

$$dq/dt = A^1 + B^1 \exp(-\phi q/A^1) \quad (5)$$

have therefore been used in the computation of oxidation rates after various time and amounts of oxygen reacted.

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## BIOCHEMISTRY

### Mitochondria and pH

THE concept of intracellular pH depends on the assumption that the cell is either not divided into compartments, or that the volumes of the compartments are large enough to contain a statistically meaningful number of hydrogen ions, the "intracellular pH" in this case being a mean of the pH values in the various compartments. While this may be valid for cells, the concept cannot necessarily be extended to very small intracellular compartments. This can be shown in the case of a mitochondrion of say 0.5μ diameter and 5μ length. The volume of this com-

partment would be approximately  $10^{-12}$  ml. At pH 7.0 the hydrogen ion concentration is nearly  $10^{-7}$  g ions/l., and thus the mitochondrion volume would contain  $10^{-7} \times 10^{-3} \times 10^{-12}$  g ions. If the Avogadro number is  $6 \times 10^{23}$ , then the mitochondrion will contain

$$\frac{6 \times 10^{23} \times 10^{-7} \times 10^{-3} \times 10^{-12}}{2} \text{ hydrogen ions}$$

that is, thirty ions.

Because of random thermal motion, however, the laws of physical chemistry are inaccurate within a probable relative error of the order  $n^{-1}$  where  $n$  is the number of ions or particles that co-operate to bring about the law. Thus in the case of the pH of a mitochondrial volume the probable error involved at pH 7.0 will be  $(30)^{-1}$ , that is, 18 per cent.

There has been considerable discussion<sup>1-4</sup> of the function of "mitochondrial membrane pH gradients" in oxidative phosphorylation, and such a gradient has recently been reported as measured<sup>5</sup>. Claims have also been made for measuring intramitochondrial pH changes of 0.02 and less<sup>6</sup>. In this case it can be seen that the number of hydrogen ions involved in such a change would be too small to have any significance (approximately half a hydrogen ion).

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### Effect of Prednisolone on Synthesis of DNA and RNA by Human Lymphocytes *in vitro*

THE ability of the extract of *Phaseolus vulgaris*, phytohaemagglutinin, to initiate the synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) replication and cytokinesis in human lymphocytes *in vitro* is well known<sup>1-3</sup>. It has recently been shown that the response of normal human lymphocytes to phytohaemagglutinin can be markedly altered by the serum which is used to bolster the culture medium<sup>1</sup>. Of the sera which markedly depressed this response, some were obtained from patients with lymphocytic diseases; of these, many were treated with corticosteroids. We decided, therefore, to investigate the effects of corticosteroids on these cultures. Accordingly, we have measured the *in vitro* responsiveness initiated in normal human peripheral lymphocytes in the presence of increasing doses of prednisolone-21-phosphate by phytohaemagglutinin.

The culture systems which were used have been described in detail elsewhere<sup>1,2</sup>. Normal human blood was obtained by venipuncture, heparinized and allowed to settle for 2 h. The plasma supernatant, rich in leucocytes, was withdrawn and the concentration of leucocytes was ascertained with a clinical haemocytometer. The desired number of cells was then sedimented and the pellet was resuspended in 0.05 per cent trypsin for 6-7 min. After sedimentation the cell pellet was resuspended in a modified Eagle's HeLa medium (EHM)<sup>4</sup> which had been supplemented with 10 per cent bovine serum to give a final concentration of  $1.0 \times 10^6$  cells/ml. The cell suspension was pipetted into 16 mm  $\times$  150 mm test tubes in 3.0 ml. aliquots. Standard solutions were prepared by dissolving 90-100 mg of phytohaemagglutinin-M in 5 ml. of triple

distilled water. One millilitre of this solution was taken as 1.0 U and 0.1 ml. containing 0.1 U of phytohaemagglutinin was added to the culture tubes. Control cultures received 0.1 ml. of triple distilled water. This dose has been shown to be optimal for induction of synthesis of both DNA and RNA in these cultures<sup>1,2</sup>. The cultures were then gassed with 5 per cent carbon dioxide in air and incubated on a slant of 4° at 37° C. After 48 h all cultures received 0.1 ml. of a solution of EHM containing  $3 \times 10^{-5}$  molar amethopterin and  $1.5 \times 10^{-3}$  molar adenosine. Sixteen hours later 0.1 ml. of a solution of EHM containing 30  $\mu$ g/ml. of 2-<sup>14</sup>C-thymidine (S.A. 3.66 mc./mmole) was added and left for 6 h to measure the incorporation into DNA. After each addition the cultures were regassed with 5 per cent carbon dioxide in air before being returned to the incubator. The cultures were collected by sedimenting the cells and extracting the acid insoluble residue by successive treatment with 10 per cent trichloroacetic acid, 80 per cent ethanol, and a mixture of absolute ethanol and diethyl ether (1:1)<sup>1</sup>. The dried residue was taken up in 0.5 ml. of 90 per cent formic acid and 0.2 ml. were added to 20 ml. of  $\alpha$ -naphthylphenyloxazole for counting in a liquid scintillation spectrometer<sup>5</sup>.

To measure synthesis of RNA the cultures were planted in the absence of phytohaemagglutinin. After 42 h of incubation to allow endogenous RNA synthesis to diminish to a basal level<sup>2</sup>, 0.1 ml. of triple distilled water or phytohaemagglutinin (0.01 U) was added to the cultures. Twenty-four hours later the cultures were pulsed for 2 h with 0.1 ml. of an EHM solution containing 1.39  $\mu$ g/ml. (<sup>3</sup>H) cytidine (S.A. 2.34 c./mmole). The cultures were then collected and treated as described above.

Prednisolone-21-phosphate was diluted with triple distilled water and added to the cultures in 0.1 ml. aliquots to give final concentrations of 0-1,000  $\mu$ g/culture. Both RNA and DNA were measured as described after the addition of prednisolone-21-phosphate at the time of planting. Synthesis of RNA was also measured after adding prednisolone-21-phosphate at the same time that the phytohaemagglutinin was added. The results of these experiments are shown in Figs. 1 and 2. It is seen that 0.01  $\mu$ g/culture of prednisolone-21-phosphate had only a minimal effect on the response; however, further increases in dosage effected a logarithmic dose-related suppression of replication of DNA. Inhibition of synthesis of RNA by prednisolone-21-phosphate also occurred at 0.01  $\mu$ g/culture and was logarithmically dose-related up to 100  $\mu$ g/culture. When the addition of prednisolone-21-phosphate was delayed to the time at which the cultures

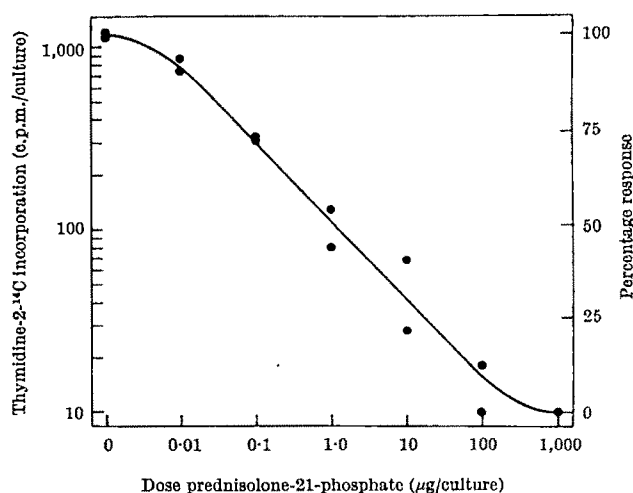


Fig. 1. The effect of prednisolone-21-phosphate on incorporation of labelled thymidine. Normal human lymphocytes were cultured as described in the text and results were expressed as log counts/min/culture versus log dose of prednisolone-21-phosphate. The percentage response is also shown.

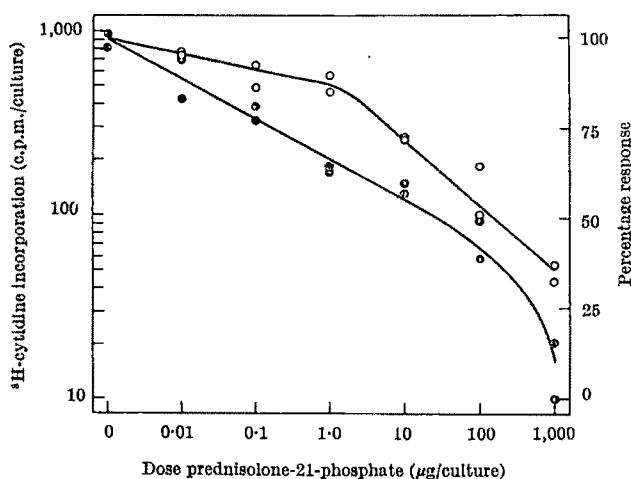


Fig. 2. The effect of prednisolone-21-phosphate on incorporation of ( $^3\text{H}$ ) cytidine. Normal human lymphocytes were cultured as described in the text and results were expressed as log counts/min/culture versus log dose of prednisolone-21-phosphate. The percentage response is also shown. —●—, Prednisolone added at time of planting the cultures. —○—, Prednisolone added after 48 h of incubation, immediately after phytohaemagglutinin was added.

received phytohaemagglutinin, there was less inhibition of the response until a concentration of about 100–1,000  $\mu\text{g/culture}$  was reached. There was no incorporation of label into the cultures which did not receive phytohaemagglutinin.

Our data raise the possibility that the decline in number of blood lymphocytes after treatment with corticosteroids<sup>6,7</sup> may result in part from a decrease in the replication of lymphocytes. It has been reported that hydrocortisone will inhibit transformation in mixed lymphocyte cultures<sup>8</sup>. Other investigators have observed that 100  $\mu\text{g/ml.}$  of prednisolone will prevent transformation and 0.01  $\mu\text{g/ml.}$  will prevent mitosis of lymphocytes stimulated by phytohaemagglutinin<sup>9</sup>. It is noted here that a dose as low as 0.003  $\mu\text{g/ml.}$  begins to suppress the synthesis of RNA and DNA. Accordingly when serum from patients ingesting corticosteroids is used to bolster medium in investigations of the response of lymphocytes to phytohaemagglutinin, the potential effects of the concentration of corticosteroid in serum must be considered, because very small concentrations will interfere with synthesis of DNA and RNA.

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## Extension of the Concept "Vertebrate Epidermal Melanin Unit" to embrace Visceral Pigmentation and Leucocytic Melanin Transport

THE process described by Masson<sup>1</sup> as "cytochrome" implies that the melanocyte is the active partner in the transfer of melanin granules to Malpighian cells. Cruickshank and Harcourt<sup>2</sup> pointed out this implication and advanced evidence that, as judged by the activity of the cell membrane, the epithelial cell appeared to play a more active part in the transfer of melanin granules. Recent investigations indicating that the Malpighian cells may be the active partner in phagocytosing melanin granules led Fitzpatrick and Breathnach<sup>3</sup> to the development of their concept of an "epidermal melanin unit" in which the functional unit of pigmentation is seen as a melanocyte and the pool of epidermal cells to which it is connected. Such units were recently demonstrated in amphibian skin by Hadley and Quevedo<sup>4</sup>, who in addition provided evidence that the rate at which melanin granules are carried away from melanocytes may also be a decisive factor in determining the rate at which they are synthesized. This useful concept is a significant advance in the study of "melanokinetics"<sup>5</sup>, but it does not embrace other cells transporting melanin, such as leucocytes.

Leucocytic melanin transport received much attention up to the end of the nineteenth century<sup>6</sup>, but the observations could not be interpreted before identification of melanocytes as the cellular site of melanogenesis<sup>5</sup>. In view of increasing awareness of visceral melanin deposits<sup>7,8</sup>, leucocytic melanin transport<sup>5,9-11</sup> again assumes importance.

In acute aseptic cutaneous inflammatory reactions of Bantu and Cape Coloured individuals, the first neutrophils to appear clumped melanin granules and these were transferred to lymphocytes which subsequently hypertrophied to macrophages<sup>9</sup>. "Dendritic processes" were frequently seen in this transport (Figs. 1 and 2), but in well filled macrophages vacuoles were common and "dendritic processes" were never seen (Fig. 3).

This transport of melanin was very marked in hyperpigmented individuals, but inconspicuous in Caucasians<sup>9</sup>. In Caucasians with phenothiazine-melanos, however, leucocytic melanin transport could be confirmed<sup>12</sup>.

In normal Bantu males, lymphocytes containing melanin were observed in concentrates of leucocytes from peripheral blood, but not in Caucasians<sup>10</sup>. This could also be observed in Caucasians with phenothiazine-melanos<sup>12</sup>.

The skin-draining lymph nodes in Bantu may show marked melanin deposits in 85–90 per cent of autopsies<sup>13</sup> while the incidence of such melanin deposits were found to be only 20–26 per cent of similar lymph nodes in Caucasians<sup>14</sup>. Melanin deposits in viscera were marked in autopsy series of patients with phenothiazine-melanos<sup>8,12,15</sup>; in both Negro and Caucasians deposits of



Fig. 1.

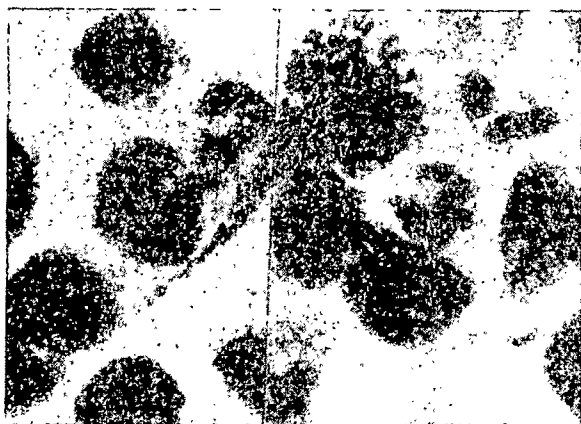


Fig. 2.



Fig. 3.

placental melanin were more frequent in cases with a history of skin injury during pregnancy<sup>16</sup>.

In amphibians especially, but also to a lesser extent in reptiles, leucocytes containing melanin are readily demonstrable in the peripheral blood, and visceral melanin deposits in liver, lungs, heart and kidney are conspicuous in amphibians<sup>10</sup>.

In hyperpigmentation associated with Addison's disease, hyperthyroidism and anorexia nervosa, an absolute lymphocytosis in the peripheral blood is common, and depends on changes in pituitary-adrenocortical function, which is also related to the increased pigmentation<sup>11</sup>. In Bantu and Caucasians, studied at sea level in an area free from malaria and where individuals were selected for normal haemoglobin concentrations and erythrocyte sedimentation rates, the Bantu had a statistically significant higher lymphocyte count and a statistically highly significant neutropenia as compared with the Caucasians. This would appear to depend on lesser adrenocortical activity in the Bantu<sup>11</sup>.

These observations suggest that, if the concept of an "epidermal melanin unit" is extended to all vertebrates justifying reference to a "vertebrate epidermal melanin unit"<sup>4</sup>, the leucocytic transport mechanism should be included. The function of leucocytes appears at present to be integrated with the unit as follows: (i) Disruption of epidermal melanin units, as in our experimental model<sup>9</sup>, allows leucocytes to assume a role in melanin transport. (ii) When epidermal melanin units are filled to capacity as in hyperpigmentation, whether racial or acquired, the leucocytic system apparently acts as an additional transporting system and could help to maintain the existing high rate of melanogenesis. (iii) Hyper-

pigmentation induced by hormones (Addison's disease, hyperthyroidism and anorexia nervosa) is also accompanied by an increase in the number of lymphocytes in the peripheral blood, which suggests that with increased melanogenesis, the additional transport system is simultaneously activated. (iv) The activation of the additional transport system is associated with increased visceral melanin deposition.

Phenothiazine-melanosis shows evidence of (i) increased melanogenesis<sup>15,17</sup>, (ii) leucocytic melanin transport<sup>12</sup>, and (iii) visceral melanin deposits<sup>15</sup>. It has been shown that melanin, as electron acceptor, binds with the electron donor chlorpromazine probably by the formation of charge transfer complexes<sup>18,19</sup>. These findings could be explained if the melanin accepting ability of cells depended on their concentration of electron donors. The transfer of granules would then stop on cancellation of the charge by the formation of charge-transfer complexes. Chlorpromazine as electron donor would thus enhance transfer to epidermal units and leucocytes, increase melanogenesis, and this would result in visceral melanin deposition.

The absence of hair and feathers, and the thin integument of amphibians, could limit the capacity of the epidermal component of the unit. Additional transport through leucocytes would then help to maintain the rate of melanogenesis and increase visceral melanin deposition. Such visceral melanin deposition renders a potent electron acceptor in tissues which may now be vulnerable to free radical formation by ultra-violet radiation as in the case of the skin<sup>20</sup>. The evolution to a thicker integument, leading to a larger epidermal component and enhanced by skin appendages as melanin repositories, would lessen the demand on the additional transport system.

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### Lactic Dehydrogenase Isozymes: Changes during Lens Differentiation in the Chick

THE lens of the eye is an ideal system for the examination of cell differentiation because of its isolation from the blood stream, lack of connective tissue and because it includes only cells derived from ectoderm. Furthermore, with the epithelial cell, the annular pad cell and the cortical and nuclear fibre cell, the lens cell shows a sequence of progressive differentiation. The lens lacks a mechanism for discarding cells, and so has within its structure a complete record of its embryonic and adult differentiation.



The change in lactate dehydrogenase (LDH) isozyme pattern during the development of the whole chick lens has been described<sup>1</sup>. We have analysed the LDH isozyme pattern in correlation with the structural differentiation from epithelial to fibre cell, and at different stages of development.

Eyes were taken from 8, 12, 15, 18 and 19 day old White Leghorn chick embryos, newly hatched chicks, and from 1 day old, 10 day old, 3 week old, 6 week old, 7 week old and 1-2 year old animals. Whole lenses were dissected from surrounding tissues and homogenized in 0.9 per cent saline solution, using 100 mg wet weight of tissue/ml. of solution. The homogenates were centrifuged at 15,000*g* at 4° C for 25 min and the supernatant was used for enzyme analysis. Extracts were also prepared from epithelium, annular pad, cortical fibre and nuclear fibre cells. The lens was viewed under a stereomicroscope and the capsule anterior to the equator was excised circumferentially. The capsule with attached epithelial cells was homogenized in minimal amounts of saline. The annular pad was removed from the lens and homogenized using 100 mg wet weight of tissue/ml. saline. The most

superficial fibres were dissected as cortical cells and the core of the lens as nuclear fibre cells. Intermediate fibre cells were discarded. LDH isozymes were separated by vertical starch gel electrophoresis<sup>2</sup>. The gel was prepared with 0.076 molar *tris*-citrate buffer (*pH* 8.6), and boric buffer (5 g of sodium hydroxide, 37 g of boric acid in 2 l. of distilled water) served as bridge buffer<sup>3</sup>. After electrophoresis at 4° C for 16 h at a field strength of 6 V/cm, the gel was incubated in a medium composed of 45 mg of nicotinamide adenine dinucleotide, 37 mg of *p*-nitro blue tetrazolium salt, 3 mg of phenazine methosulphate, 113 ml. of distilled water, 15 ml. of 0.5 molar *tris*-hydrochloric acid buffer (*pH* 7.4), 7.5 ml. of 0.1 molar potassium cyanide and 15 ml. of 0.5 molar sodium lactate solution (*pH* 7.4). Enzyme activity was also measured on a spectrophotometer at 40° C, at a wavelength of 340 mμ (ref. 4). Pyruvate concentrations of  $3.3 \times 10^{-4}$  molar and  $6.6 \times 10^{-3}$  molar were used to detect the activity of, mainly, LDH 1 and LDH 5, respectively<sup>1</sup>.

The results are shown in Fig. 1. In the 12 day embryonic lens, LDH isozymes 3-5 predominate in epithelial and fibre cells. The nuclear fibre cells show less enzymatic

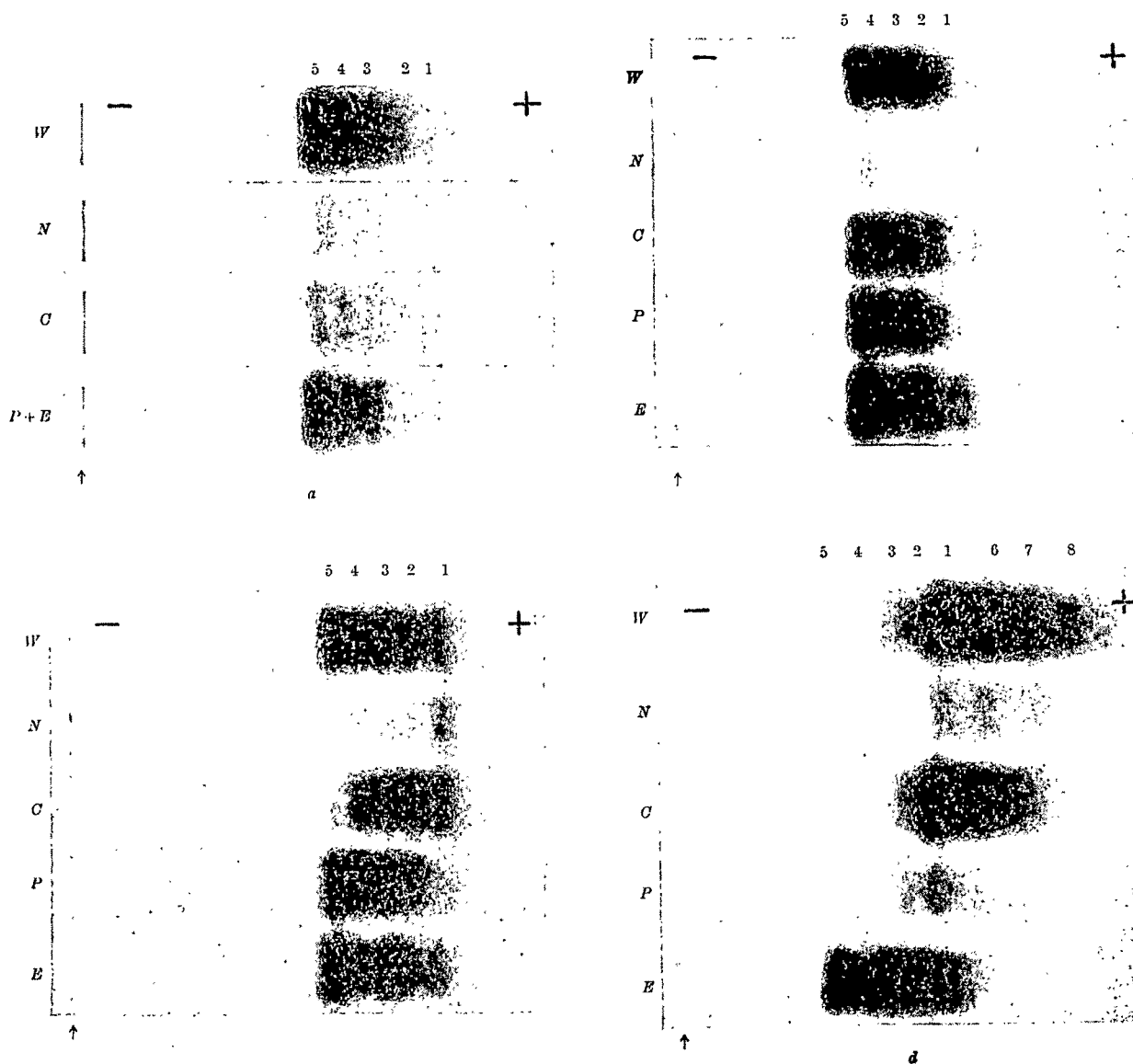


Fig. 1. Isozyme pattern of chick lenses of different ages. (a) 12 day old embryo; (b) 1 day after hatching; (c) 10 days after hatching; (d) 1 yr old adult. The arrows indicate the origin. W, Whole lens; N, nuclear fibre cells; C, cortical fibre cells; P, annular pad cells; E, epithelial cells.

activity of tissue than the cortical fibre cells or epithelial cells. A result of the isozyme distribution is that the 12 day embryonic whole lens shows a predominance of LDH isozymes 3-5. This isozyme pattern was maintained during the hatching period except for slight changes which consisted of an increase of activity at LDH 1 relative to the other isozymes. The epithelium acquires a characteristic isozyme pattern with maximal activity at LDH 5, 3 and 1, while the annular pad cells show a marked intensity at LDH 5 and 3, but not at LDH 1. Thus, the newborn whole lens shows a more uniform distribution of isozymes although LDH 5, 4 and 3 still predominate.

Within a few days of hatching there is an abrupt change in isozyme distribution. LDH 5 and 3 still predominate in the epithelial and annular pad cells of 10 day old chick lenses, but the fibre cells show a marked shift in activity, with heaviest staining at LDH 1 and not at LDH 3-5. Thus the whole lens shows markedly increased activity at LDH 1 over that found in embryonic lenses and LDH 1 and 5 stain with nearly equal intensity.

The isozyme patterns of older lenses show an increasing predominance of anodal isozymes. This is due to an increase of LDH 1 especially in the cortical fibre cells and to a lesser extent in the nuclear fibre cells. There is, at the same time, a progressive decrease of activity at LDH 5, 4, 3 and 2. Additional bands which appear on the anodal side of LDH 1 are found in large quantities only in the fibre cells and appear first in significant amounts in lenses of 6 and 7 week old chickens.

In the lens of 1 year old adult animals, LDH 5 and 3 are still mainly present in the epithelium, but in the annular pad there is a much decreased amount of LDH 5. The fibre cells show the heaviest staining at LDH 1 and the more anodal bands, but LDH 5 and 4 cannot be detected. Thus the whole lens isozyme pattern reflects activity mainly at LDH 1 and the more anodal bands, and only traces of LDH 5 and 3 are discernible. Epithelial cells comprise only a very small part of the total adult lens weight, and so their predominant isozymes, LDH 5 and 3, are greatly diluted in the whole lens extract.

Spectrophotometric analysis of LDH activity confirmed these observations, for LDH 1 acts maximally at low pyruvate levels and LDH 5 at high pyruvate levels. There is a marked increase in enzyme activity with age for the whole lens, cortical and nuclear fibre cells, especially at the low pyruvate concentration. In a 1 year old animal the ratio of activity at low pyruvate concentration to that at high pyruvate concentration was 1.5 for the fibre cells, and 0.85 for the epithelial cells. The annular pad cells showed maximal activity in the 7 week old animals, with more activity/g of tissue than the fibre cells.

We have provided here clear evidence of a biochemical change associated with the differentiation of the epithelial cell into a fibre cell, which is manifested only after hatching. There is no significant difference between epithelial and fibre cell isozyme patterns before hatching, so that the change in isozyme pattern of the whole lens from a predominance of cathodal isozymes in embryonic life to anodal isozymes in adult life is a function of a change in enzyme composition of the fibre cell. The epithelial cell pattern is essentially the same in embryonic and adult life.

Several theories have been proposed to explain the significance of isozymes and the changes in the pattern of distribution that occur during development. These relate to the aerobic or anaerobic nature of respiration<sup>5</sup>, to the presence or absence of a nucleus in the cell<sup>6</sup> and to the rate of mitotic activity<sup>7</sup>. These theories do not satisfactorily explain the change in lens isozyme pattern which occurs within a few days of hatching, and it seems most reasonable to assume that this change is related to metabolic factors, possibly in connexion with the onset of lens function.

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### Lignin and Certain Other Chemical Constituents of *Phylloglossum*

*Phylloglossum drummondii*, Kunze is a small and relatively rare cryptogam of New Zealand and Australia. It is considered to be a lycopod which is reduced and specialized in the adoption of a geophytic habit<sup>1</sup>. So far as we know, it has not been examined for any of its organic chemical constituents.

In view of the fact that two types of lignin are known to occur in *Lycopodium* and that the distribution of these types is in agreement with suggestions regarding the taxonomy of the genus<sup>2</sup>, we considered it of interest to examine *Phylloglossum*, the only other generally accepted living genus in the *Lycopodiales*.

Through the kindness of Mr. W. E. Hillis, 1.2 g of air-dried *Phylloglossum* was made available to us. The main results of our chemical analyses were as follows:

(1) Alkaline oxidation of the extracted wood-meal with copper hydroxide yielded vanillin and *p*-hydroxybenzaldehyde as well as *p*-hydroxybenzoic, vanillic and syringic acids.

(2) Phenols, obtained by alkaline hydrolysis of ethanol insoluble residues, included protocatechuic, *p*-hydroxybenzoic, vanillic ferulic and syringic acid.

(3) As in *Lycopodium*, the chief sugars in aqueous or ethanolic extracts are sucrose, glucose and fructose.

(4) Alkaloids were detected but the limited amounts of material available precluded identification. Chromatograms showed that some of the alkaloid spots agreed in *R<sub>F</sub>* with those from a similar extract of *Lycopodium clavatum*. They differed in their chromatographic behaviour from the fifteen authentic *Lycopodium* alkaloids available to us.

Species of *Lycopodium* which are included in the genera *Lycopodium* and *Diphasium*, as proposed by Rothmaler<sup>3</sup>, yield syringic acid in ethanolic extracts or on lignin degradation whereas species included in the genera *Huperzia* and *Lepidotis* do not<sup>4</sup>. *Phylloglossum* therefore displays a chemical similarity to the latter groups. This may be of some significance in considerations of its phylogenetic relationships. The general absence of syringaldehyde from among the lignin oxidation products of *Phylloglossum* or of other lycopods is curious in view of the condition in *Selaginella*. In *Selaginella*, all species which we have examined so far yield both syringaldehyde and syringic acid on lignin degradation.

Paper chromatographic methods were used for the identification of sugars<sup>5</sup>, phenolic acids and phenolic

aldehydes<sup>2</sup>. Alkaloids were extracted from 0.8 g of plant material<sup>5</sup> and examined by thin layer chromatography on plates of silica gel G made up with 0.1 normal sodium hydroxide. The solvent systems were benzene:chloroform:methanol (8:1:2) and benzene:ethanol (5:6). Spots were visualized with the iodo-platinate reagent<sup>6</sup>.

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### Dissociation and Recombination of a Polygalacturonase Complex during Ion Exchange Chromatography

PURIFICATION and characterization of extracellular microbial polygalacturonases depend on reliable techniques for separation. By ion exchange chromatography, six apparently distinct polygalacturonase components were separated from the culture filtrate of *Fusarium oxysporum* variety 'Lycopersici' on an anion exchanger<sup>1</sup>, and four polygalacturonase components were obtained from *Coniothyrium diplodiella* by similar methods on anion and cation exchangers<sup>2</sup>. We now report the existence of polygalacturonase complexes of different particle sizes and their interconversion during ion exchange chromatography.

Extracellular polygalacturonase was obtained from a shake-culture filtrate of *Fusarium oxysporum* variety 'Lycopersici' (Sacc.) Snyder and Hans. grown in modified Richard's medium<sup>3</sup> containing 1 per cent w/v pectin and adjusted to pH 5.0. After 6 days growth, the cultures were centrifuged and filtered through a 'Millipore HA' filter (0.45 $\mu$ ) to remove all fungal cells. The filtrate was dialysed for 18 h against running tap water and for 8 h against distilled water, concentrated to about one quarter of its original volume under reduced pressure at 30° C in a rotary evaporator, and then frozen until it was used. Polygalacturonase activity was estimated by a cup plate assay<sup>4</sup>, in which zones of enzyme action were linearly related to the logarithm of the concentrations of enzyme.

For anion exchange chromatography, coarse grade DEAE cellulose with an exchange capacity of 0.93 mequiv./g was regenerated by the method of Albersheim and Killias<sup>5</sup>, packed into a column 1.9 cm in diameter, 19 cm high, and equilibrated with 0.01 molar acetate buffer (pH 5.0). The concentrated culture filtrate was placed on the column and polygalacturonase was eluted with 200 ml. of buffer followed by 200 ml. of buffer containing 0.4 molar sodium chloride. Samples of 5 ml. were collected.

Of the total polygalacturonase obtained from the column, about 98 per cent was eluted by buffer immediately after the void volume, and 2 per cent, which was more highly adsorbed, came out with the mixtures of buffer and sodium chloride. Stepwise elution of similar columns with concentrations of sodium chloride below 0.4 molar has given several distinct components of polygalacturonase from the adsorbed enzyme of *Fusarium*<sup>1</sup>.

For cation exchange chromatography, 'Duolite CS-101' obtained from Bio Rad Laboratories as 'Bio-Rex 70', 100–200 mesh, 10.0 mequiv./g of dry wt, was regenerated according to the manufacturer's recommendations, packed into a column 3.0 cm  $\times$  30.0 cm, and equilibrated with 0.01 molar acetate buffer (pH 5.0). The concentrated culture filtrate was placed on the column and polygalacturonase was eluted in samples of 10 ml. by 200 ml. portions each of 0.01 molar and 0.1 molar acetate buffer (pH 5.0) and 1.0 molar sodium acetate. A similar elution schedule was followed by Endo<sup>2</sup>.

Almost all the polygalacturonase was adsorbed on 'Duolite CS-101', and was eluted as a single peak in 1.0 molar sodium acetate. Traces of polygalacturonase were detected in the 0.01 molar and 0.1 molar buffer eluates, but in total these represented less than 0.7 per cent of the enzyme eluted. Elution with intermediate concentrations of buffer and sodium acetate failed to give additional enzyme components.

The fractions which contained the highest polygalacturonase activity in 1.0 molar sodium acetate were combined, dialysed and rechromatographed on 'Duolite CS-101' and DEAE cellulose. Polygalacturonase rechromatographed on 'Duolite CS-101' as a single component corresponding to the original peak, and no enzyme was detected in the 0.01 molar and 0.1 molar buffer eluates. On DEAE cellulose, the 'Duolite CS-101' fraction chromatographed in a manner similar to the enzyme from the culture filtrate, that is, about 98 per cent of the polygalacturonase was eluted immediately after the void volume, while 2 per cent was adsorbed and eluted in 0.4 molar sodium chloride. Adsorption of polygalacturonase on DEAE cellulose indicates the presence of negatively charged enzyme molecules, which, theoretically, should not have been present in the component eluted from the cation exchanger, 'Duolite CS-101'. This deviation from the expected suggests that chromatography of polygalacturonase on DEAE cellulose can produce artefacts.

The relationship of polygalacturonase from the culture filtrate and the chief enzyme components obtained after cation and anion exchange chromatography was determined by gel filtration which permits fractionation of molecules according to their size. 'Sephadex G-200' was prepared for gel filtration according to the manufacturer's recommendations, packed in a column 2.5 cm  $\times$  35 cm and equilibrated with 0.01 molar acetate buffer (pH 5.0). Samples containing enzymes (2 ml.) were placed on the column; it was eluted with the acetate buffer, and 4 ml. fractions were collected and assayed for polygalacturonase activity.

Two distinct components of polygalacturonase were obtained from the culture filtrate (Fig. 1). The first component, eluted mainly in fractions 12–15, contained about 14 per cent of the total polygalacturonase obtained from the column and was probably composed of relatively large particles of enzyme, for molecules generally appear in the effluent which follows gel filtration in order of decreasing size. The main component, with smaller particles of enzyme, contained about 86 per cent of the polygalacturonase eluted.

The main component of polygalacturonase, eluted from a DEAE cellulose column immediately after the void volume, was applied to the column of 'Sephadex G-200'. Two distinct components of polygalacturonase were obtained which corresponded in the size of particles to those in the culture filtrate (Fig. 1). The component with the larger enzyme particles contained, however, about 1 per cent of the polygalacturonase obtained from the column, while the fraction with smaller particles of enzyme contained about 99 per cent of the polygalacturonase eluted. The small quantity (2 per cent) of polygalacturonase, initially more highly adsorbed by DEAE cellulose and thus removed from the main component, was insufficient to account for the relative loss of large particles of enzyme after chromatography on DEAE

cellulose. Dissociation of a complex between polygalacturonase, substrate molecules, and (or) other materials from the culture filtrate, or the fragmentation of polygalacturonase into active enzyme sub-units, are plausible explanations for the loss of large particles of enzyme. The apparent presence of negatively charged polygalacturonase particles in the enzyme component which should have been freed of negative enzyme by adsorption and elution from the cation exchanger may similarly be a result of dissociation of an enzyme complex during chromatography on the DEAE cellulose, as reported for other enzymes<sup>5</sup>.

Modifications of polygalacturonase may be influenced significantly by the method of regenerating the exchanger. For example, when DEAE cellulose was prepared for chromatography by Mandele's method<sup>6</sup> only a trace of polygalacturonase could be eluted from the column. The enzyme probably was inactivated during the exchange reactions, possibly by extreme dissociation.

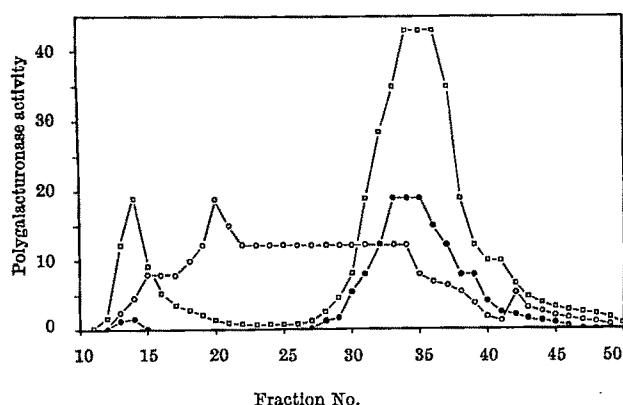


Fig. 1. Fractionation of *Fusarium* polygalacturonase on a column of 'Sephadex G-200' with a void volume of 48 ml. □, Enzyme obtained from the culture filtrate; ●, chief components from a column of DEAE cellulose; ○, chief components from a column of 'Duolite CS-101'. Enzyme activity is expressed in units based on 100 for a 20 mm zone of enzyme activity in the cup plate assay.

The main polygalacturonase component from the culture filtrate adsorbed on 'Duolite CS-101' and eluted with 1.0 molar sodium acetate was dialysed and applied to a 'Sephadex G-200' column. Polygalacturonase was eluted continuously after the void volume with no peaks of activity corresponding to those obtained from the culture filtrate or the chief component from the DEAE cellulose column (Fig. 1). Failure to resolve polygalacturonase into distinct components could not have been due to saturation of the column because relatively small quantities of polygalacturonase were applied, but was probably caused by the recombination of polygalacturonase into complexes with particles of varying sizes. Recombination could be a result of association of enzyme molecules or sub-units to form a series of isoenzymes, or it could result from complexing of the enzyme with other molecules, for example, the pectic substrate that could have been carried along from the culture filtrate.

Knowledge of dissociation and recombination of polygalacturonase components will be helpful in the selection of methods for separation and purification of this enzyme and is a necessity for understanding the properties of polygalacturonases. Natural occurrence of polygalacturonase complexes may explain the heterogeneity of this enzyme from various microbial species, and account for the differences between polygalacturonase from diseased plants and culture filtrates of the pathogen<sup>1,7</sup>.

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### Mechanism of Action of Carboxydismutase

THE mechanism of action of carboxydismutase—the enzyme which catalyses the fixing of carbon dioxide in plants—has been the subject of numerous investigations. The enzyme requires two substrates, ribulose-1,5-diphosphate (RuDP) and bicarbonate, and is found to form a <sup>14</sup>CO<sub>2</sub>-carboxydismutase complex<sup>1</sup>. If the isolated complex is stabilized with diazomethane and then digested with trypsin, the peptides formed can be isolated by ion exchange chromatography. It is found that only one of these peptides is highly radioactive, which suggests that the <sup>14</sup>CO<sub>2</sub> is bound to a specific site in the enzyme<sup>2</sup>.

The enzyme is inactivated by iodoacetamide (IAM) (ref. 3), but it can be protected by RuDP. This suggests a reaction between a sulphhydryl group and RuDP. It has also been concluded that the sulphhydryl group is the active site of the enzyme, and that one step of the overall enzyme reaction is the formation of a hemimercaptolate structure between the sulphhydryl group and RuDP (ref. 4). To determine the validity of this proposal we performed a number of experiments with other sugar phosphates as well as carbamyl phosphate (CBMP), sulphate and phosphate ions, and found that they too can protect the enzyme against inhibition by IAM, thus indicating that there are other interpretations for the findings.

Carboxydismutase was prepared from spinach chloroplasts (*Spinacea oleracea*) as described earlier<sup>5</sup>. The enzyme was incubated with IAM in the presence and absence of protectors. At different intervals aliquots were removed and the enzyme activity measured as before<sup>6</sup>.

IAM was found to inhibit the enzyme in a similar way to that reported by Rabin and Trown<sup>4</sup>. RuDP, fructose-1,6-diphosphate (FruDP), fructose-1-phosphate (Fru1P), fructose-6-phosphate (Fru6P), CBMP, or sulphate and phosphate ions at various concentrations suppressed the IAM inhibition (see Fig. 1). The protective power depends on the concentration of the suppressor and it is greater for CBMP. Fructose (Fru) protects the enzyme slightly at 4 mmoles but not at 1.5 mmoles/l. Glucose-6-phosphate (G-6-P) did not suppress the IAM inhibition at the concentration tested. The effect of sulphate and phosphate ions is quite pronounced, although higher concentrations are necessary. This finding, however, shows that the effect of a protector will be increased when sulphate or phosphate ions are present in the incubation mixture, and this was in fact observed with FruDP. After treatment with sodium sulphate, the calcium salt of FruDP was a much more potent protector than the salt itself. The RuDP used in our experiments was 40 per cent pure. In addition to sulphate ions, phosphate and ribose phosphate were present as impurities, and thus the effect of RuDP in protecting the enzyme from IAM inhibition was enhanced by the contaminants.

Initially, we thought that the effect of the sugar phosphates arose from their structural similarity to RuDP, and that the protection resulted from binding at the active site, thus making impossible alkylation by IAM. If this were so, however, it would be expected that FruDP would be a competitive inhibitor of the enzyme. Such an inhibition was not found up to a concentration of 4 mmoles/l. Even greater protection was provided by CBMP, which also does not inhibit the action of the enzyme up to the concentration tested (3 mmoles/l.). In addition, the enzyme is specific for RuDP (ref. 6). It is therefore difficult to visualize a mechanism in which IAM inhibits the enzyme by alkylating the active site, CBMP, FruDP and RuDP protect it by blocking the active site and do not allow the IAM to react, and yet FruDP or CBMP are not inhibitors or substrates of the enzyme. Moreover, if the protection of the sugar phosphates results from the formation of hemimercaptols with the sulphhydryl group, it would be expected that G-6-P would be a more potent protector than FruDP or RuDP, because aldehydes are more reactive to mercaptans than ketones<sup>7</sup>; however, this is not so. Finally, the pronounced effect of polyvalent ions would only justify an unspecific binding of the protector to the enzyme, and would bear no relationship to binding at the active site.

To obtain more information on the nature of protection of the enzyme by these compounds, the reaction leading to the formation of the  $^{14}\text{CO}_2$ -enzyme complex was studied<sup>1</sup>. Table 1 shows the results of a representative set of experiments. It is evident that IAM inhibits the formation of the  $^{14}\text{CO}_2$ -enzyme complex by about 50–60 per cent. As judged by the enzyme activity found and the  $^{14}\text{CO}_2$ -enzyme complex formed, FruDP protects the enzyme. Moreover, FruDP protects the enzyme from even the slow denaturation which probably occurs during the incubation period and the subsequent dialysis.

FruDP protects the enzyme against inhibition by IAM even for the formation of the  $^{14}\text{CO}_2$ -enzyme complex, and

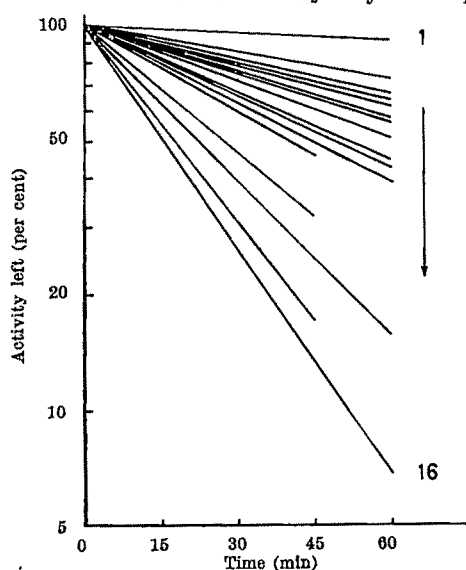


Fig. 1. Time curve for the inhibition of carboxydismutase by IAM at 25°C in 0.1 molar *tris* buffer, pH 8.0, in the presence of different protectors. All reaction mixtures contained: enzyme, 0.9 mg/ml.; IAM, 0.5 mmoles/l.; protector (1) CBMP, 0.5 mmoles/l.; (2) Fru1P, 1.5 mmoles/l.; (3) Fru2P, 1.5 mmoles/l.; (4) RuDP, 0.5 mmoles/l.; (5) Fru1P, 1.0 mmoles/l.; (6) sodium sulphate, 15.0 mmoles/l.; (7) Fru2P, 1.5 mmoles/l.; (8) Fru2P, 1.0 mmoles/l.; (9) Fru1P, 0.5 mmoles/l.; (10) Fru2P, 1.0 mmoles/l.; (11) Fru2P, 0.5 mmoles/l.; (12) sodium monohydrogen phosphate, 15.0 mmoles/l.; (13) Fru 4.0 mmoles/l.; (14) Fru2P, 0.1 mmoles/l.; (15) G-6-P, 2.0 mmoles/l.; (16) control, no protector. Final volume 1 ml. The IAM was added at zero time to the reaction mixture containing the enzyme *tris* buffer and protector and the reaction was allowed to proceed at 25°C. 500  $\mu$ l. portions were removed at predetermined intervals and added at 0°C to tubes containing 50  $\mu$ l. of 0.01 molar cysteine in 0.2 molar *tris* buffer (pH 8.0). Magnesium (II),  $\text{H}^{14}\text{CO}_2$  and RuDP were then added and the reaction mixture was incubated at 25°C for 10 min. The RuDP, Fru1P and Fru2P were prepared from their barium salts<sup>1</sup>. The FruDP and CBMP were the calcium and lithium salts, respectively, and were not treated with sodium sulphate.

Table 1. EFFECT OF IODOACETAMIDE ON THE ACTIVITY OF CARBOXYDISMUTASE AND ON THE FORMATION OF THE  $^{14}\text{CO}_2$ -CARBOXYDISMUTASE COMPLEX, IN THE PRESENCE AND ABSENCE OF FRUCTOSE-1,6-DIPHOSPHATE

Sample	Enzymatic activity (c.p.m./mg protein)	Radioactivity of $^{14}\text{CO}_2$ -enzyme (c.p.m./mg protein)
Native enzyme	416,000	3,630
Native enzyme (No $\text{Mg}^{++}$ )		400
Enzyme + IAM	0	2,000
Enzyme + IAM + FruDP (5 mmoles/l.)	92,000	2,050
Enzyme + IAM + FruDP (10 mmoles/l.)	181,000	2,200
Enzyme + IAM + FruDP (20 mmoles/l.)	512,000	3,550
Enzyme + FruDP (10 mmoles/l.)	509,000	4,000
Native enzyme*	500,000	

Enzyme samples (5.3 mg/ml.) were incubated with IAM (5.0 mmoles/l.) at 25°C for 75 min in the presence or absence of FruDP. The same amount of enzyme but without IAM served as control. After the incubation the samples were dialysed against 0.02 molar *tris*, pH 8.0, at 2°C for 24 h, and then they were assayed for enzyme activity and were allowed to react with magnesium (II) and  $\text{H}^{14}\text{CO}_2$  at 0°C for 1 h to form the  $^{14}\text{CO}_2$ -enzyme complex<sup>1</sup>.

\* Not incubated and not dialysed.

therefore it seems that the protection is due to the formation of a hydrogen bond between the sugar phosphates or CBMP and the enzyme, so that its native form is preserved. The finding that 40 per cent of the  $^{14}\text{CO}_2$ -enzyme complex is still formed although the alkylated enzyme is inactive (Table 1) may also result from the structural alteration of the alkylated protein, the sites of attachment of " $\text{CO}_2$ " and RuDP being separated and thus no phosphoglycerate can be formed. This unfolding of the alkylated protein may also be responsible for the low recovery of the complex from the column, which renders the  $^{14}\text{CO}_2$  bound to the enzyme more easily exchangeable with the anion groups of the resin.

The existence cannot be disregarded of amino-acid residues responsible for the rigidity of the structure of the enzyme, for example, sulphhydryl groups, and which may be alkylated by IAM with a concomitant change in spatial configuration and inactivation. The protector may thus be bound on the same structure determining site (or sites), therefore making "attack" by IAM impossible and keeping the enzyme active. This site, however, cannot be considered to be "the" active site because that would be highly specific for the substrate.

Mention should be made of the effect of polyvalent anions and polyanions which can inhibit the spectral shift effect of 8 mmolar urea on ribonuclease<sup>8–10</sup>. Optical rotatory changes which occur during the denaturation of ovalbumin by urea are similarly influenced by anions<sup>11</sup>. Full activity in the presence of such denaturing agents results from refolding of the protein caused by the polyvalent anions. That the activity of carboxydismutase depends to a considerable extent on the folding over of the protein, which probably results in superimposition of the two active sites of the enzyme, is also evident from the activating effect of a magnetic field on the enzyme<sup>12</sup>; hydrogen bonding was considered to be the reason for the activating effect. The part played by sulphate and phosphate ions in preventing the inhibition of carboxydismutase by IAM may thus be understood.

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## Chemical Form and Physiological Availability of Iodine in Lettuce (*Lactuca sativa*)

LITTLE information is available concerning the particular chemical form in which iodine occurs in plants<sup>1,2</sup>. It is known that the concentration of iodine in plant materials is directly related to that of the soil and may be affected by soil pH (ref. 1). Whether the iodine is organically bound in the plant, however, has not been properly established; furthermore, no physiological function has been found for this element in plants<sup>1,2</sup>.

The physiological availability of inorganic elements in plant materials has received a great deal of attention, and it has been shown that the availability of certain elements is related to the chemical form in which they are present. For example, there is evidence that phosphorus as inositol phosphate (phytic acid)<sup>3</sup> and iron as iron phytate are not well utilized<sup>4</sup>. The chemical form in which iodine is present in foods of plant origin may be of significance in areas of endemic goitre where vegetables represent the chief dietary source. It is conceivable that one of the contributing factors to the endemicity may be the physiological unavailability of plant iodine; that is, the iodine may be chemically bound in a non-utilizable form.

The present work was undertaken to investigate the chemical form in which radioactive iodine was incorporated into growing lettuce (*Lactuca sativa*, var. *longifolia*). In addition, the physiological availability of this iodine was studied in rats. Lettuce was chosen because of its rapid growth and because it is reputed to be a good source of iodine<sup>1</sup>.

Young, vigorous lettuce seedlings 8–10 cm high were transferred from soil into culture bottles containing a nutrient medium<sup>5</sup>. On the second or third day, radioactive iodine in the form of a solution of carrier-free Na<sup>125</sup>I was added to each container at the desired level of radioactivity. The plants were kept in a warm and well lit place for 7–25 days, depending on the type of experiment.

To investigate the uptake of radioiodine, plants were kept for 7 days in three different levels of radioactivity. At the end of the period, radioactivity was measured in leaf samples using a well type crystal scintillation counter; the results are shown in Table 1. It is evident that, at the levels of radioactivity used, uptake by the plants was in proportion to the amount of radioactivity present in the medium.

Table 1. UPTAKE OF IODINE-125 BY LETTUCE PLANTS GROWING IN VARIOUS LEVELS OF RADIOIODINE\*

Level of radioactivity in medium (μc.)	Radioactivity in plant (c.p.m./g fresh material)
16	650,000
32	1,100,000
64	2,430,000

\* Plants were kept in nutrient medium for 7 days.

The chemical form of the radioiodine in lettuce leaves was investigated in plant extracts using paper chromatography, column chromatography and paper electrophoresis. In addition, radioactive plant extracts were dialysed for the detection of possible non-dialysable forms of iodine. For the preparation of the extracts, plants were grown for 25 days in the culture medium in the presence of iodine-125 (20 μc./plant). The fresh leaves were then thoroughly washed with distilled water and homogenized with a minimum of distilled water. Clear extracts were collected as supernatant after centrifugation of the homogenates at 3,600 r.p.m. for 30 min; hereafter this material will be referred to as the "extract".

A portion of the extract was dialysed against distilled water for 3 days; the water was changed each day. At the same time a control solution of carrier free Na<sup>125</sup>I, containing the same level of radioactivity as the extract, was dialysed under the same conditions. In both the control and the extract, most of the radioactivity was found in the dialysate at the end of 3 days. The daily

portions of the plant dialysate were pooled and concentrated by flash evaporation to a volume of 10 ml. This concentrate, hereafter referred to as "dialysate", was used for further investigations.

**Paper chromatography.** The clear extract and dialysate were subjected to uni-directional chromatography on Whatman No. 1 paper with the solvent descending; a sufficient amount was applied so that the level of radioactivity at the origin was at least 5,000 c.p.m. In addition, a known concentration of Na<sup>125</sup>I was run alone on each chromatogram. Two solvent systems were used: (1) the top layer of a mixture of *n*-butanol, glacial acetic acid and water (4:1:5 v/v); (2) the top layer of a mixture of *n*-butanol, dioxane and normal ammonium hydroxide (4:1:5 v/v). Iodine is organically bound to certain proteins and amino-acids in the body of an animal, and therefore we endeavoured to determine radioactive areas on the chromatograms which were sensitive to ninhydrin. Each chromatogram was developed with a chloroform solution of 0.1 per cent ninhydrin and autoradiographs were prepared by exposing the chromatograms to X-ray film for 7 days. When the films had been developed, *R<sub>F</sub>* values were calculated for the blackened spots.

One radioactive spot appeared with both solvent systems on the chromatograms of extracts and dialysate. In butanol-acetic acid, the *R<sub>F</sub>* of the spot coincided not only with that of the standard iodine-125 but also with an area sensitive to ninhydrin (Table 2). In the dioxane-ammonia system, however, no ninhydrin spot developed at the peak of radioactivity (Table 2). The fact that, with one solvent, a radioactive area sensitive to ninhydrin was detected made necessary further work to establish the presence or absence of organically bound iodine.

Table 2. *R<sub>F</sub>* VALUES OF RADIOACTIVE PEAKS ON PAPER CHROMATOGRAMS OF LETTUCE PLANT EXTRACT AND DIALYSATE CHROMATOGRAPHED IN TWO DIFFERENT SOLVENT SYSTEMS

Material	Butanol-acetic acid	Dioxane-ammonia
Plant extract	0.187*	0.260
Dialysate	0.189*	0.264
Iodine-125	0.185	0.271

\* Coincided with an area sensitive to ninhydrin.

**Column chromatography.** For column chromatography, 200–400 mesh 'Dowex 50 WX8' resin (hydrogen form) was packed under water to a depth of 6 cm in a column 1 cm in diameter. This resin is known to bind organically bound iodine preferentially<sup>6</sup>. Similar amounts of radioactivity in the form of either plant extract, dialysate or Na<sup>125</sup>I were added to each of three columns; the columns were eluted with 2 ml. portions of deionized water. In all three instances negligible radioactivity appeared after the sixth fraction of eluent and the majority was eluted in fractions 2–5 (Fig. 1). The radioactivity in extract and dialysate left the columns more slowly than did the

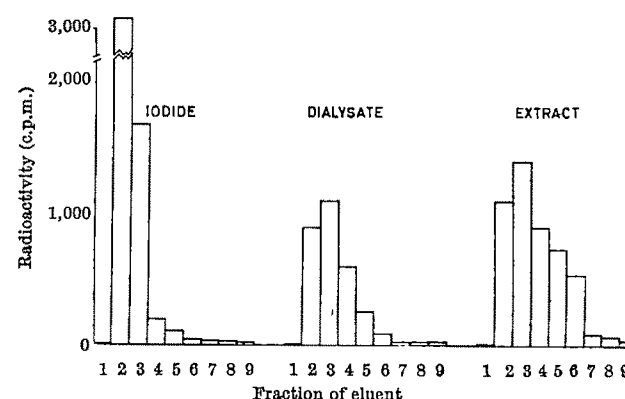


Fig. 1. Peaks of radioactivity eluted from columns to which were added radioactive iodide (control), and dialysate and extract of lettuce grown in a medium containing Na<sup>125</sup>I. The resin used was 'Dowex 50WX8' (hydrogen form); the eluent was distilled water.

control, perhaps because in the former samples there was a certain amount of cellular material which may have hindered the movement of the iodine; however, the peaks for all three samples occurred consistently between fractions 2 and 6. It was found that, with all samples (even the control), about 10 per cent of the radioactivity remained on the resin and could not be removed by successive washings. These results strongly suggest that there was little or no organically bound iodine in the plant material.

**Paper electrophoresis.** Plant extract, concentrated dialysate and iodine-125 were electrophoresed on Whatman No. 3 paper at 300 V and pH 8.6. After electrophoresis, the papers were dried, sprayed with ninhydrin and autoradiographs were prepared. Initially, when the material was electrophoresed for 10 h, no radioactive spots were detected on the exposed film. By decreasing the time to 2 h, however, it was found that, as with the paper chromatograms, there was one peak of radioactivity in the extract and dialysate and that the peaks moved at the same rate as the iodine-125 standard. The relative positions of the three radioactive areas corresponding to iodine-125, lettuce extract and dialysate are shown in Fig. 2. All three radioactive spots moved similarly at a rapid rate toward the anode and were far out in front of the areas sensitive to ninhydrin. The results of the electrophoretic investigations indicated that all the iodine-125 taken up by the plant remained in the unbound inorganic form.

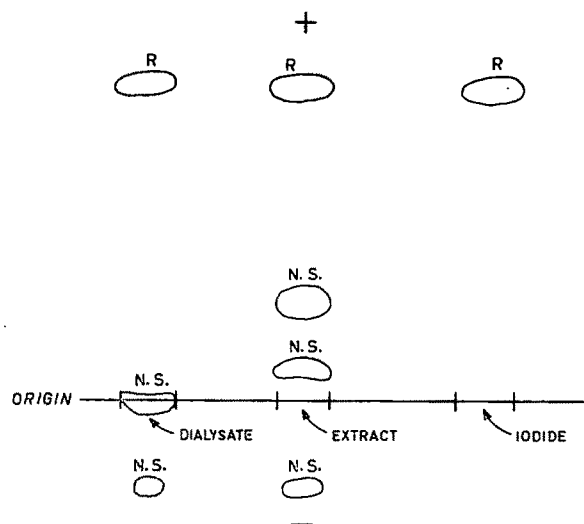


Fig. 2. Relative positions of radioactive (*R*) and ninhydrin-sensitive (*N.S.*) areas after paper electrophoresis of radioactive iodine and extract and dialysate of lettuce grown in a medium containing  $\text{Na}^{125}\text{I}$ . (2 h; pH, 8.6; 300 V.)

It was presumed that the radioiodine was in the water soluble fraction of the plant material. To investigate the possibility that some was present in the lipid fraction, however, a dried sample of radioactive plant material was continuously extracted with ether for 16 h. No radioactivity was recovered in the ether extract.

The following experiment was performed to see if the iodine as found in lettuce was physiologically available. Two growing male rats were rendered deficient in iodine by keeping them on a low iodine diet for 4 weeks. Each animal was then given, by gelatine capsule, about 5,000 c.p.m. of radioactivity in the form of dried powdered lettuce leaves. The animals were placed in metabolism cages so that urine and faeces could be collected separately; after 24 h, the rats were killed with chloroform and the thyroids removed. The results of radioactivity measurements in the urine, faeces and thyroids are shown in Table 3. These data suggest efficient utilization of the iodine because a large portion of the radioactivity was

Table 3. UPTAKE AND EXCRETION OF RADIOIODINE IN LETTUCE BY IODINE DEFICIENT RATS\*

Material	Radioactivity	
	Rat No. 1	Rat No. 2
Thyroid (c.p.m.)	2,330	1,870
Urine (c.p.m./ml.)	100	120
Faeces (c.p.m./g)	<100	<100

\* Each animal received 5,000 c.p.m. of radioactivity in the form of iodine-125 incorporated into lettuce; thyroids were removed 24 h after the animals received the radioiodine.

found in the thyroids while very little was detected in the urine and faeces.

In summary, it was concluded that, under the experimental conditions used, radioactive iodide was taken up readily by lettuce plants and appeared to remain in the plant in the unbound, inorganic form. Radioiodine incorporated into lettuce was readily absorbed and utilized in iodine deficient rats.

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### Esterase and Phosphatase Activity in the Opisthosomal Region of Spiders

We have been able to demonstrate the presence of widespread intracellular and intercellular esteratic activity in the opisthosomal region of the dictynid species *Ciniflo fenestralis* (Stroem.) and the argiopid species *Zygiella atrica* (C. L. Koch). We used sections 5 $\mu$ –14 $\mu$  thick embedded in wax or frozen, and applied the 5-bromo-indoxyl acetate method<sup>1–3</sup>. The sections were fixed in acetone and incubated for 30–60 min in a medium containing 1.3 mg 5-bromo-indoxyl acetate dissolved in 0.1 ml. of ethanol. The alcoholic solution of 5-bromo-indoxyl acetate was used in a fresh solution containing 2.0 ml. of 0.1 molar 2-amino-2-hydroxymethyl-1,3-propanediol/hydrochloric acid buffer, 1.0 ml. of 0.05 molar potassium ferricyanide, 1.0 ml. of 0.05 molar potassium ferrocyanide, 1.0 ml. of 0.1 molar calcium chloride, and 5.0 ml. of water.

Generalized esterase activity was found in the cells of the interstitial tissue, the silk glands and in the haemolymph. This may be interpreted as demonstrating the widespread presence of esterases equivalent to the A, B and C esterases of vertebrates.

In contrast to this widespread distribution of generalized esterase activity the use of a modification of Gomori's calcium/cobalt method for alkaline phosphatase showed that phosphatase activity occurred predominantly at the periphery of the cells, especially the sericeogenous epithelia. The sections were incubated for 30–60 min at 37° C in a medium containing 10 ml. of 3 per cent sodium  $\beta$  glycerophosphate, 10 ml. of 2 per cent sodium diethyl barbiturate, 5 ml. of distilled water, 20 ml. of 2 per cent calcium chloride and 1 ml. of 5 per cent magnesium sulphate.

After rinsing in cold water they were treated with 2 per cent cobalt nitrate for 2 min and then, after rinsing, with a dilute solution of ammonium sulphide for 1 min.

Opisthosomal homogenates were subjected to electrophoresis on polyacrylamide gel columns for 45 min, and then incubated in the glycerophosphate solutions of Gomori's method. They showed a single moiety of low mobility with alkaline phosphatase activity. The moiety could be seen as a white disk after incubation. This rendered subsequent treatment with cobalt nitrate and ammonium sulphide unnecessary. Incubation of comparable gel columns using Barka's<sup>4</sup> method for acid phosphatase showed the presence of phosphatase activity at acid pH. It is worth noting, however, that in neither case did the inhibitors of vertebrate phosphatases, 0.01 molar sodium fluoride and 0.01 molar potassium cyanide, have any effect on these reactions.

Both acid and alkaline phosphatases have long been known to occur in insects<sup>5-8</sup>, and alkaline phosphatase is particularly common in epithelia secreting silk. It has been suggested that at this latter site the alkaline phosphatase may be involved in the release of silk from an intracellular nucleoprotein complex. It is clear that it may have a similar function in the silk glands of spiders.

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### Topically Applied *n*-Decyl Acetate as a Precursor for Metabolic Investigations in Insects

WHEN small insects are injected with labelled metabolites trouble is caused by mechanical damage, excessive bleeding, clogging of the needle, and the serious distortion of metabolism which results from the sudden administration of up to 20 per cent of the insect's weight of water plus 10–60  $\mu$ moles of metabolite/g of live weight. One way to overcome these difficulties would be to make a fat-soluble acetate precursor which could be applied directly to the cuticle in the same way as an insecticide. The use of *n*-decyl 1-<sup>14</sup>C-acetate prepared by direct esterification of *n*-decyl alcohol has been investigated. No effect on mortality, pupation or emergence was found to result from topical application of acetone solutions, or even up to 1  $\mu$ l. of undiluted *n*-decyl acetate, to larvae of the blowfly *Calliphora erythrocephala*.

Each member of batches of eight blowfly larvae 3–4 days old was treated with 1  $\mu$ l. of undiluted and labelled *n*-decyl acetate, and the batches were placed in wire gauze cages. Each cage was suspended in a 15-ml. glass-stoppered tube containing 10 cm<sup>2</sup> of filter paper saturated with 10 normal potassium hydroxide. The experiment was conducted at 20° C. Every 30 min the cage was transferred quickly to a fresh tube and 3 h after treatment it was dropped into liquid nitrogen. The cage was then washed thoroughly in ethanol at –10° C and re-frozen. The larvae were extracted with perchloric acid<sup>1</sup>, and three times with chloroform and methanol (1 : 1) at room temperature, and the residue was heated for 24 h at 100° C in a sealed tube with 2 ml. of 50 per cent potassium hydroxide. The insoluble residue of chitin was washed with water and dried.

About one fifth of the activity recovered was metabolized and was found in carbon dioxide, fat-soluble, or water-soluble extracts. Small amounts of activity (less

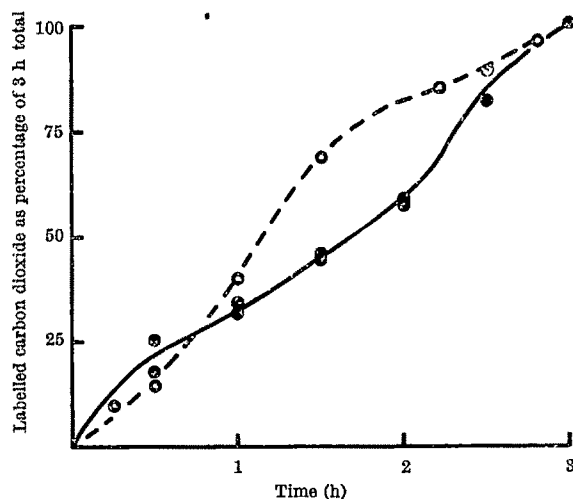


Fig. 1. Production of carbon dioxide labelled with carbon-14 by insects. ○ — ○, Adult houseflies after injection with labelled acetate (ref. 2); ● — ●, larval blowflies after topical application of labelled *n*-decyl acetate.

than 0.1 per cent of the total recovered) were found in the alkaline digest of the residue and similar small amounts in chitin. Chromatography of fat-soluble extracts on thin layers of silicic acid with benzene and ether (60 : 40) as solvent failed to reveal any intact *n*-decyl acetate. This thin layer system was the only one out of many that were tested which separated *n*-decyl acetate from all the detectable insect fat fractions.

The production of labelled carbon dioxide at various times after topical application of the *n*-decyl acetate is compared with earlier data<sup>2</sup> from adult houseflies injected with labelled acetate (Fig. 1). There was no sign of a lag period between application of the ester and the evolution of <sup>14</sup>C carbon dioxide and it was concluded that *n*-decyl acetate is rapidly hydrolysed, and the free acetate produced further metabolized, as soon as it is absorbed by the insect.

These results demonstrate that the use of a fat-soluble precursor permits a water-soluble metabolite to be given to an insect, however small, in a dose smoothly applied over several hours without loss of blood, risk of mechanical damage or unnecessary metabolic disturbance.

I thank Mr. F. P. W. Winteringham, who first suggested that a fat-soluble acetate precursor might be a valuable tool.

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### Absorption of Exogenous and Endogenous Biliary Copper in the Rat

THE daily intake of copper in man exceeds normal requirements<sup>1</sup>, but abnormally high body stores have only been recorded in Wilson's disease<sup>2,3</sup>. Knowledge of the exact mechanism of the absorption of dietary copper is incomplete<sup>4</sup> and even less is known about the absorption of endogenous biliary copper or its entero-hepatic circulation<sup>4,5</sup>. This report concerns the relative intestinal absorption of exogenous copper-64 in two chemical forms (ionic cupric acetate and chelated copper (II-EDTA)) and the absorption of endogenous biliary copper and caeruloplasmin copper.

Adult male Sprague-Dawley rats were given an intrapyloric dose of copper-64 either as cupric acetate or as copper (II-EDTA) in a dose range of 0.50–1,000  $\mu\text{g}$  of copper. The animals were killed after 24 h, the entire gastrointestinal tract was resected and the total radioactivity remaining in the carcass, liver and blood was used as the index of absorption.

For the absorption of biliary copper, bile was first obtained, through an external biliary fistula, from animals previously given an intravenous dose of 100  $\mu\text{g}$  of copper. Known amounts of copper in the biliary fractions taken at 0–4 h, 4–8 h and 8–24 h (both undialysed and dialysed) were then injected intrapylorically and absorption during 24 h was determined similarly. Finally, the absorption of 2.5  $\mu\text{g}$  of caeruloplasmin copper (prepared from donor rats) was measured.

The results are shown in Tables 1 and 2. A relatively constant fraction of the injected copper (about 40 per cent) was retained at 24 h when cupric acetate was given intrapylorically in the dose range of 0.50–10.0  $\mu\text{g}$ . With larger doses less was absorbed and with doses of 200 to 1,000  $\mu\text{g}$  of copper only 10 per cent was retained. Similar results were obtained with labelled copper (II-EDTA).

When biliary copper was given intrapylorically in doses of 0.50 to 10.0  $\mu\text{g}$ , 10 per cent to 15 per cent was absorbed by 24 h. At a constant dose of 2.5  $\mu\text{g}$  of endogenous undialysed bile copper, absorption decreased progressively when the fractions of bile taken at later times were injected (15 per cent absorption for the 0–4 h fraction and 9 per cent absorption for the 8–24 h fraction). This occurred when the dialysed bile was used. After an intrapyloric dose of 2.5  $\mu\text{g}$  of caeruloplasmin copper approximately 10 per cent of the label was retained.

Table 1. PERCENTAGE ABSORPTION OF COPPER ACETATE AFTER 24 H

Dose ( $\mu\text{g}$ )	No. of experiments	Percentage absorption (mean $\pm$ S.D.)
Cupric acetate		
0.5–10.0	19	38.2 $\pm$ 13.6
50.0	3	19.8 $\pm$ 7.2
200	3	8.7 $\pm$ 1.8
500	3	10.17 $\pm$ 4.0
1,000	2	11.5
Copper (II-EDTA)		
2.5	3	27.9 $\pm$ 3.1
200	2	16.73

Table 2. PERCENTAGE ABSORPTION OF BILIARY COPPER AT 24 H

Dose ( $\mu\text{g}$ )	Bile fraction	No. of experiments	Percentage absorption (mean $\pm$ S.D.)
Cupric acetate			
2.5–10.0	0–4 h	5	16.7 $\pm$ 3.9
0.5–5.0	4–8 h	6	16.20 $\pm$ 5.5
2.5	0–4 h*	5	15.63 $\pm$ 2.8
2.5	4–8 h*	6	12.30 $\pm$ 4.8
2.5	8–24 h*	3	9.64 $\pm$ 2.9
Copper (II-EDTA)			
2.5	0–4 h	3	21.6 $\pm$ 4.2
2.5	4–8 h	2	12.73
2.5	8–24 h	2	7.59

\* Dialysed fractions.

With increasing amounts of exogenous copper, there is a progressive reduction in the absorption of biliary copper irrespective of which of the two chemical forms was used. Endogenous biliary copper is poorly absorbed even with small doses, which contrasts with the relatively greater absorption of exogenous copper (Fig. 1). This difference is probably due to the indiffusibility and protein binding of biliary copper<sup>3</sup>. The differing degree of absorption in the three bile fractions (0–4, 4–8, 8–24 h) when identical amounts (2.5  $\mu\text{g}$ ) of bile copper is used, when the bile had been dialysed in each case, suggests a change in the copper protein complex with increasing time after intravenous administration.

The results of these investigations in the rat might explain the zero copper balance in man despite a relatively high intake of dietary copper. The excessive exogenous copper is poorly absorbed, but experiments in the rat show this to be quantitatively significant. The subsequent biliary excretion<sup>4</sup> and poor reabsorption of biliary copper,

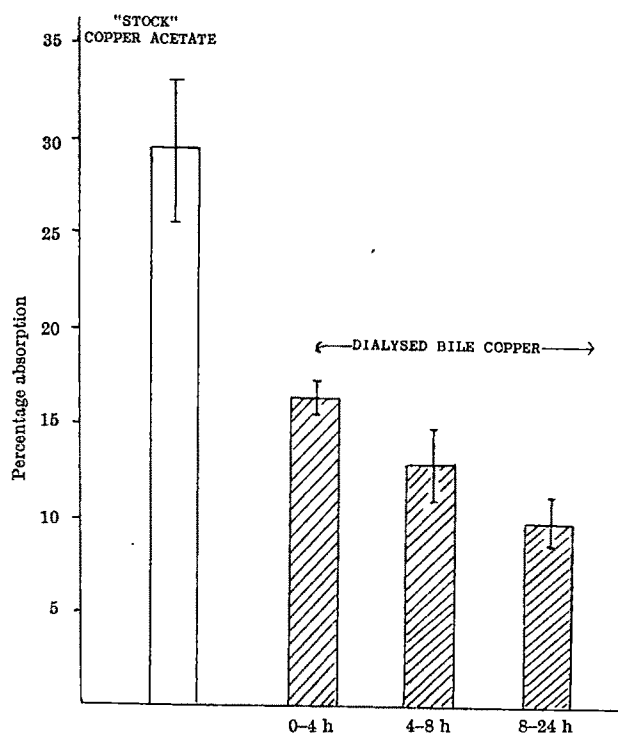


Fig. 1. Percentage absorption of 2.5  $\mu\text{g}$  of labelled copper acetate, showing stock and dialysed bile copper.

that is, lack of significant entero-hepatic circulation, may represent the chief pathway for the elimination of copper from the body.

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### Chromatography of Gibberellins on Silica Gel Partition Columns

ALTHOUGH there are many chromatographic methods for separating the various gibberellins from each other, there appears to be no report of the use of silica gel partition columns. Columns are more useful for preparative separations than thin layer and paper chromatographic methods. Methods using absorption columns made with charcoal and silicic acid have been described<sup>1,2</sup>. Partition columns are usually considered to provide especially mild conditions for chromatography, which may be important with compounds that tend to be somewhat labile, such as the gibberellins.

We have used two silica gel partition column techniques. The first technique, which employs gradient elution, uses a column constructed from silicic acid which has been washed with 3 N hydrochloric acid, then water, and finally dried to constant weight at 100° C. Eight grams of a 100–200-mesh fraction of this silicic acid (silica gel)

was hydrated with 5.0 ml. of 0.5 molar formic acid and thoroughly mixed until a free-flowing powder was obtained. The hydrated silica gel was then slurried with *n*-hexane that had been saturated with 0.5 molar formic acid, and poured into a 15-mm internal diameter glass column with a stopcock on one end. The column was packed with air pressure from a hand squeeze bulb while excess hexane was drained from the column. Residues containing gibberellins may be applied to the top of the column by various means<sup>3,4</sup>, but the technique used here consisted of first transferring the gibberellins dissolved in alcohol to a small paraffin boat (capacity about 5 ml.), evaporating the alcohol, then applying the paraffin boat with residue to a few millilitres of acid-saturated *n*-hexane contained on top of the column. After the paraffin had dissolved it was washed through the column with about 30 ml. of *n*-hexane, leaving behind on the column the gibberellins and most other plant constituents. The gibberellins were eluted from the column by using increasing concentrations of ethyl acetate in *n*-hexane. The solvent gradient was established with a 3-chambered 'Varigrad' similar to the device described by Peterson and Sober<sup>5</sup>. The first chamber of the 'Varigrad' was filled with 100 ml. of *n*-hexane saturated with 0.5 molar formic acid, while the other two chambers each contained 75 ml. of ethyl acetate saturated with 0.5 molar formic acid. After elution of the column, the 10-ml. fractions were dried in a device especially designed for rapid evaporation of solvents from test-tubes at low temperatures. The residues were analysed for gibberellins either by thin layer chromatography<sup>6</sup> or by the lettuce hypocotyl bioassay<sup>7</sup>. More detailed description of the use of these columns may be found in earlier papers describing their use for the chromatography of indoles<sup>3,4,8</sup>.

Table 1. GRADIENT ELUTION PATTERNS FOR GIBBERELLINS  $A_{1-5}$  FROM SILICA GEL PARTITION COLUMNS

Fraction No.	Gibberellins eluted
1-4	$A_4, A_3, A_2, A_1, A_5$
11-15	$A_4, A_3, A_2$
18	$A_4$

The results are shown in Table 1. The least polar gibberellins ( $A_4$  with no hydroxyl groups, and  $A_4, A_3, A_2$  and  $A_1$ , each with one) were eluted in the first four fractions. Gibberellins  $A_1, A_2, A_3$ , each with two hydroxyl groups, emerged in fractions 11 to 15.  $GA_4$ , with three hydroxyl groups, was found in fraction 18. This technique, therefore, separates gibberellins  $A_{1-5}$  into certain groups with similar polarity, but, perhaps more important, it can be used to separate many of the other plant constituents from the gibberellins. Polar substances such as many of the common organic acids, sugars, amino acids and some pigments are not eluted. A number of non-polar substances, however, such as the lipids and some of the indoles, are eluted in the same general region as the gibberellins. The lipids may be separated from the gibberellins by partitioning between acetonitrile and hexane, the gibberellins being dissolved into the acetonitrile.

The other silica gel column technique employed stepwise elution of the gibberellins from a column identical with that described for the gradient elution technique. When the column was eluted with 25-ml. volumes of a series of solvents with increasing concentrations of ethyl acetate in *n*-hexane (all solvents were saturated with 0.5 molar formic acid) most of the gibberellins ( $A_{1-5}$ ) were separated from each other. As with the first method, many of the chief plant constituents are left behind on the column. The eluting solvents used, and the elution pattern for the gibberellins, are summarized in Table 2. This method does not give complete separation of  $GA_4$  and  $GA_1$ , nor of  $GA_1$  and  $GA_2$ , but the results suggest that some improvement might be obtained by either modifying the percentage of ethyl acetate in the fractions where better resolution is desired, or by increasing the length of the column.

Table 2. STEPWISE ELUTION PATTERNS FOR GIBBERELLINS  $A_{1-5}$  FROM SILICA GEL PARTITION COLUMN

Per cent ethyl acetate in <i>n</i> -hexane	Gibberellin eluted
0	
1.0	
3.0	
6.0	$A_4$
9.0	
12.0	
15.0	$A_4$
16.5	$A_4, A_1$
18.0	
19.5	$A_4$
21.0	
22.5	
24.0	$A_4$
25.5	
27.0	
30.0	
33.0	
36.0	
39.0	
40.5	
42.0	$A_3, A_1$
43.5	$A_3, A_1$
45.0	$A_3$
46.0	$A_2$ (trace)
48.0	
51.0	
54.0	
57.0	
60.0	$A_4$
63.0	
66.0	

These two techniques have proved useful in our gibberellin investigations of plant tissues. The first is a rapid one (2-3 h) for obtaining considerable purification of the gibberellins, and gives the investigator some idea of the types of gibberellins with which he may be dealing. The second method, though more time consuming (5-6 h), permits better characterization of the gibberellins. Four recovery experiments with  $GA_4$  gave more than 90 per cent recovery in each case<sup>9</sup>. The quantities of gibberellins used in this work ranged from a fraction of a microgram to 10 micrograms. We have not had the opportunity of comparing our methods with the gradient elution silica gel adsorption column technique for gibberellins recently announced by Khalifah *et al.*<sup>2</sup>, with which they were able to separate seven acidic gibberellins with good recovery.

The authors are indebted to Prof. P. W. Brian for supplying the gibberellins used in this work.

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## MICROBIOLOGY

### Effect on the Infectivity of Various Viruses by the Intestinal Factor of Normal Mice which inactivates Murine Hepatitis Virus

THE capability of the intestine of normal mice to inactivate murine hepatitis virus (MHV-3) (ref. 1) has been reported<sup>2</sup>. The inactivating factor is present mainly in the first section of the mucosa of the small intestine of the adult mouse<sup>3</sup>; it is present in the intestine of germ-free mice<sup>4</sup>, and its interaction with the virus *in vitro* is dependent on temperature and incubation time<sup>5</sup>. The factor is inactivated at 70° C for 10 min, is not dialysable and is not present



in acetone-dried powders of mouse intestine<sup>5</sup>. The present paper deals with the effect of the partially purified inhibiting factor on the infectivity of several viruses.

The inhibiting factor was prepared in the following manner. Small intestine of normal mice 3 months old and weighing about 30 g was excised from the animals, washed to remove any faecal debris, and mechanically homogenized in three volumes of buffered saline. The suspension was then centrifuged at 30,000*g* for 60 min at 4° C and the sediment was discarded. The supernatant was saturated with ammonium sulphate and the precipitate was collected by centrifugation and dissolved in a minimum volume of distilled water. The resulting solution was dialysed against a 40 per cent saturated ammonium sulphate solution, and the precipitate formed was discarded by centrifugation. The supernatant was then dialysed against a 70 per cent saturated ammonium sulphate solution. The precipitate was again collected by centrifugation, dissolved in a minimum volume of 0.05 molar *tris*-hydrochloric acid buffer pH 7.4, and thoroughly dialysed against the same buffer; this preparation was used in all the experiments.

Titration of the partially purified inhibiting factor was carried out as follows. Progressive dilutions of the inhibitor in a volume of 2.0 ml. were incubated for 2 h at 37° C in a water bath with an equal volume of a suspension of *MHV*-3 containing 10<sup>6</sup> LD<sub>50</sub>/0.1 ml. Groups of ten 4 week old Swiss mice, weighing 12 g each, were injected intraperitoneally (0.2 ml./mouse) with each sample and mortality was recorded. The value of 1 unit was assigned to the amount of inhibitor contained in the highest dilution which neutralized 10<sup>6</sup> LD<sub>50</sub> of *MHV*-3.

Table 1. EFFECT OF PARTIALLY PURIFIED MOUSE INTESTINAL FACTOR ON THE INFECTIVITY OF VARIOUS VIRUSES

Virus	Strain	Inhibitor units	Log <sub>10</sub> LD <sub>50</sub> neutralized	Host-cell system
Murine hepatitis virus	<i>MHV</i> -3	1	0.00*	Mouse
Influenza A	PR 8	1	1.23	EE
Influenza A	PR 8	5	2.75	EE
Influenza A	FM 1	1	1.16	EE
Influenza A	Singapore	1	1.83	EE
Influenza B	Lee	1	1.20	EE
Influenza B	Lee	5	2.50	EE
Newcastle disease virus	B 1	1	0.00	EE
Newcastle disease virus	Local	1	0.00	EE
Infect. bronch. chicken	Beaudette	1	1.50	EE
Measles	Edmonston	1	0.83	HeLa
Rubella	M 33	1	≥3.50†	GMKC
Poliomyelitis:				
Type 1	Brunhilde	5	0.00	GMKC
Type 2	MEF-1	5	0.00	GMKC
Type 3	Saukett	5	0.00	GMKC
ECHO-11		1	0.00	GMKC
ECHO-11		5	0.66	GMKC
Coxsackie A-9		1	2.00	GMKC
Coxsackie B-5		1	0.00	GMKC
Encephalomyocarditis	EMC	5	0.00*	Mouse
Simian virus 40		1	1.16	GMKC
Variola	Lister	1	1.00	HeLa

\* LD<sub>50</sub>; † interfering dose<sub>50</sub>.

EE = Embryonated eggs; GMKC = green monkey kidney cells.

Each mouse was injected intraperitoneally with 0.2 ml. of the different mixtures. Each tissue culture tube or egg (allantoic route) received 0.2 ml. of the mixture. For each virus the experiment reported in Table 1 is one of three which gave similar results.

In order to compare the activity of the inhibiting factor on the infectivity of various viruses the following procedure was used. Serial ten-fold dilutions of each virus preparation were made up in buffered saline solution; each virus dilution was dispensed into two series of tubes containing (a) an equal volume of buffered saline, or (b) an equal volume of inhibitor in the proper concentration, respectively. The two series were incubated at 37° C for 2 h and frequently shaken. The inhibiting effect was evaluated by inoculating the mixtures into susceptible host-cell systems, and by determining the LD<sub>50</sub> according to the method of Reed and Muench<sup>6</sup>.

From the data which are summarized in Table 1, a weak inhibitory effect is apparent on the infectivity of influenza, measles, infectious bronchitis of chicken, coxsackie A-9, *SV*-40 and variola viruses. With the exception of rubella virus, the inhibitory effect seems to be highly specific for *MHV*-3, at least for the viruses tested. No effect on the haemagglutinating activity of influenza and measles viruses was observed when 1 unit of inhibitor was tested against 4 haemagglutinating units of the respective viruses.

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### Facilitated Uptake of Streptomycin by Kupffer Cells during Phagocytosis

PHAGOCYTOSIS and intracellular digestion of bacteria have been investigated primarily with blood leucocyte or peritoneal macrophages derived from experimental animals. Many attempts have been made to isolate the intracellular events by the addition of bactericidal concentrations of streptomycin to the extracellular medium after the phagocytic cells have ingested the bacterial particles. The assumption made is that streptomycin by virtue of its relative impermeability exerts no bactericidal or bacteriostatic effect on those bacteria which are established intracellularly. Data concerning penetration of streptomycin into mammalian cells, however, are not consistent and seem to depend on the types of cell and the methods of assay for streptomycin which are used. The general conclusion is that the antibiotic is not concentrated intracellularly in quantities large enough to be bactericidal<sup>1-3</sup>. A critical examination of the data, however, shows that such a conclusion may not be entirely warranted in all cases<sup>4-7</sup>, and consequently the extent of bactericidal activity which streptomycin exhibits for intracellular bacteria remains conjectural.

During an investigation to measure the kinetics of phagocytosis of viable bacteria by the isolated, perfused rat liver<sup>8</sup>, the presence of streptomycin in the perfusing fluid caused an apparent increase in inactivation of phagocytosed organisms; that is, greater than that which could be accounted for by phagocytic digestion alone. This observation suggested several alternative mechanisms which could account for the decreased recovery of viable bacteria. (1) The fixed macrophages of the liver may possess properties of permeability to streptomycin different from those of the blood leucocytes and mobilized macrophages. (2) A significant number of bacteria were adsorbed to the surfaces of capillaries and Kupffer cells and these could not be washed out mechanically before the addition of streptomycin. (3) The process of phagocytosis itself facilitated the uptake of streptomycin in a fashion analogous to the 'piggy back' phagocytosis phenomenon<sup>9</sup>, described when anti-metabolites which were ordinarily impermeable accumulated intracellularly if the cells were allowed to phagocytose latex particles in a medium containing the impermeable compounds.

This communication describes an experiment which was designed to distinguish between these possibilities and which provides tentative evidence supporting a hypothesis. Rat livers were surgically removed and perfused with serum as described previously<sup>2</sup>. Three sets of experimental conditions were imposed during the first 30 min of perfusion; (a) livers were perfused with normal serum containing killed *Salmonella enteritidis* in a concentration of  $1 \times 10^8$ /ml.; (b) livers were perfused with normal serum containing 1 mg/ml. of streptomycin sulphate (this concentration of antibiotic was far greater than the minimal inhibitory concentration); (c) livers were perfused with normal serum containing the non-viable salmonellae plus 1 mg/ml. of streptomycin sulphate. After the 30 min period, the three groups described were treated identically for the duration of the perfusions. The livers were washed thoroughly with warm sterile physiological saline to remove any extracellular non-viable salmonellae and (or) reduce the concentration of streptomycin to a non-bactericidal level of 0.5 µg/ml. The circulation through the livers was then re-established with normal serum containing viable *Salmonella enteritidis* in a concentration of  $1 \times 10^8$ /ml. After 2 h of perfusion warm saline was again allowed to flush out any non-phagocytosed organisms. The number of viable bacteria within the Kupffer cells was then determined by plating methods immediately after the perfusion.

The data (Table 1) suggest that streptomycin does not penetrate the Kupffer cells of the liver in quantities sufficient to exert a bactericidal effect on organisms later phagocytosed. On the other hand, the recovery of only 40 per cent of the viable salmonellae phagocytosed during 2 h of the secondary perfusion in the group which had been perfused initially with killed bacteria and streptomycin<sup>3</sup> provides evidence that the presence of particles in the perfusing menstrium allowed the intracellular accumulation of the antibiotic.

Table 1. RECOVERY OF VIABLE PHAGOCYTOSED *Salmonella enteritidis* FROM PERFUSED RAT LIVERS FOLLOWING PRETREATMENT WITH STREPTOMYCIN AND/OR NON-VIABLE SALMONELLAE

Primary perfusion (30 min)	Constituents in perfusate Secondary perfusion (120 min)	Percentage recovery of viable bacteria*
Group I (controls)		
Non-viable <i>S. enteritidis</i> ( $1 \times 10^8$ /ml.)	Viable <i>S. enteritidis</i> ( $1 \times 10^8$ /ml.)	131
Group II		
Streptomycin (1 mg/ml.)	Viable <i>S. enteritidis</i> ( $1 \times 10^8$ /ml.)	121
Group III		
Non-viable <i>S. enteritidis</i> ( $1 \times 10^8$ ml.) and streptomycin (1 mg/ml.)	Viable <i>S. enteritidis</i> ( $1 \times 10^8$ /ml.)	40

The probability that differences observed between group III and groups I and II are a result of chance is less than 1 in 1,000 ( $P < 0.001$ ). Differences between groups I and II are not statistically significant.

\* Percentages were calculated on the basis of results of three separate experiments for group I, two experiments for group II, and four experiments for group III.

Several speculations can be made as to the mechanism by which this phenomenon can be explained. The most plausible is that the streptomycin sulphate which is usually impermeable to the phagocytic cell gains entrance by a process similar to the "piggy back" process already described. An alternative mechanism is that the antibiotic is bound to receptor sites on the outside surface of non-viable bacteria and is then carried in with the bacteria as they are phagocytosed. It must in this case be assumed that the bound streptomycin is still functional and free to act on other bacteria once inside the Kupffer cells. The possibility that the decreased recovery of salmonellae may be a result of the killing of organisms which are not phagocytosed but adsorbed instead to surfaces of cells and capillaries cannot be ignored. No matter how thoroughly the livers were rinsed, we invariably obtained small numbers of viable bacteria in the wash fluids. Histological examination of sections prepared at the termination of

each experiment, however, showed that the bacteria were located intracellularly. Bacteria were detected outside phagocytic cells only in rare instances.

The results of this experiment cannot be considered definitive, but clinical evidence provides the most compelling argument that streptomycin can be concentrated within phagocytic cells by some mechanism. If this were not the case it would be difficult to explain the efficacy of the antibiotic in the treatment of intracellular infections such as tuberculosis, brucellosis and tularaemia.

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### Mycostasis in Soils recently Exposed by a Retreating Ice-cap

SOIL mycostasis is a familiar and widespread phenomenon which has recently been reviewed in some detail<sup>1,2</sup>. Its nature and mode of action are obscure, but it is considered to be of microbial origin and has been found to be pronounced where microbial activity is high and less where microbial activity is low. Studies have usually been made on agricultural soils or those which at some time have supported vegetation and, apart from a study of some subsoils, investigations of "virgin" soils with few traces of plant residues have not often been made.

In an attempt to obtain information on the appearance of mycostatic effects in natural soils, a study was made of soil polygons from near the western edge of the Drangajökull in north-west Iceland. Extensive polygon fields occur here in an area which has been exposed by the retreating ice during the past 50 yr.

Where soil movement is not pronounced, the polygon margins are colonized by higher plants providing there is some shelter from the desiccating winds blowing from the ice-cap and good drainage of surface water. The polygon centres are free of vegetation, as marked solifluction, exposure to desiccating winds, and periodic waterlogging preclude the establishment of seedlings<sup>3</sup>.

Soil samples of about 500 g each were taken from polygons at 400, 800 and 1,000 m from the present ice limit. At each site three samples from different polygon centres were taken and three separate samples from around the roots of the three common colonizers of the margins: *Armeria maritima* (Mill.) Willd., *Deschampsia alpina* (L.) Roem. and Schult., and *Oxyria digyna* (L.) Hill. Samples were collected in August 1965 and mycostasis assessed in the laboratory in November of the same year. The method used was the agar disk technique<sup>4</sup> with week-old conidia of *Penicillium frequentans* Westling as test spores. Soils were first passed through a 1 mm mesh sieve and their moisture content adjusted with distilled water to almost 100 per cent of their total water holding capacity. Spore germination was scored after 24 h incubation at 25° C.

The numbers of fungi present in each sample were estimated using the dilution plate method<sup>5</sup>, and organic matter determined by loss on ignition. Average results are shown in Table 1.

Both the content of organic matter and fungal numbers were consistently higher at the margins than in the centres of the polygons. At the uncolonized centres mycostasis was invariably high, yet here the soil contained little organic material and its fungal population, and probably the population of other soil micro-organisms, was relatively small. The fungi and other components of the microflora were presumably mainly present as propagules and not actively growing in the soil.

Table 1. AVERAGE PERCENTAGE GERMINATION OF *P. frequentans* CONIDIA, FUNGAL POPULATION AND LOSS ON IGNITION FOR POLYGON SOILS

	Distance from ice (m)					
	400		800		1,000	
	Margin	Centre	Margin	Centre	Margin	Centre
Fungal/g of oven-dry soil, in thousands	291	45	74	72	115	18
Per cent germination conidia	12.6	0	32.0	0	11.3	3.6
Per cent loss on ignition	4.3	2.6	4.2	4.1	5.4	3.1

Average values from each sample site are from three non-colonized (centre) and three colonized (margin) soils.

Where the three colonizers were present there was an alleviation of mycostasis at the margins although the level of this was not consistently the same at each sampling site. At the margins there was a higher level of organic matter as a result of the return of root and foliage material to the soil and microbial numbers were relatively high and presumably reflected a higher level of activity, yet as much as 87 per cent germination was recorded on disks in contact with soil from around the roots of *D. alpina*.

In natural soils amelioration of mycostasis is observed when nutrients are added to them<sup>6</sup>. Mycostasis is regarded as having survival value for soil fungi since sensitive spores will lie dormant until stimulated by contact with soluble nutrients so that spore wastage caused by random germination is reduced or eliminated. The effect is not generally considered to be a result of nutrient deficiency in either the spore or the soil but more of the presence of an inhibitor or group of inhibitors produced as a result of microbial activity. It has been pointed out, however, that lack of nutrient is still a possible basis for some mycostatic effects because conditions for germination in soil might be much more exacting than those under artificial conditions<sup>7</sup>.

It appears that such a situation exists at the place described where, during the early stages of the colonization of a natural "virgin" soil by both higher plants and micro-organisms, mycostasis does occur as a result of factors other than those which arise as a consequence of microbial activity. Mycostasis at polygon centres might be caused by a nutrient lack while at the margins nutrients are more abundant but microbial activity is not so high as to produce a mycostatic effect through the release of metabolites to the soil. It is suggested that a more extensive study of the appearance of mycostasis in freshly exposed soils might lead to a better understanding of the basis of the phenomenon.

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## PHARMACOLOGY

### Effect of Tetrodotoxin on Membrane Currents in Mammalian Cardiac Fibres

THE action potential of cardiac fibres differs from that of most other cell types: the fast rising phase is followed by a plateau lasting a few 100 msec. The results of voltage clamp analysis in Purkinje fibres<sup>1</sup> show that a depolarizing voltage step elicits a large sodium current, which has a slow component which declines for several hundred milliseconds. The potassium current falls after depolarization with a similar time constant. The slow decline of both currents can explain the plateau of the cardiac action potential. The sodium current was identified by comparing the membrane currents in Tyrode solution with those in a sodium-free solution<sup>2</sup>. An independent check of this procedure would be useful; we therefore investigated the effect of tetrodotoxin (TTX) on short Purkinje fibres of sheep. TTX at concentrations of  $10^{-8}$  to  $10^{-7}$  g/ml. is known to block selectively the excitatory inward sodium current in invertebrate axons<sup>2,3</sup>. We recorded membrane potentials in fourteen preparations and passed current through a second intracellular glass microelectrode. Details of the preparation and of the electronic apparatus are given elsewhere<sup>4</sup>.

In a concentration of  $10^{-8}$  to  $10^{-6}$  g/ml. TTX surprisingly had no effects on the rate of rise and duration of the action potential of the Purkinje fibre. Specific effects of TTX were observed in the range of concentrations from  $10^{-5}$  to  $3 \times 10^{-5}$  g/ml. At  $10^{-4}$  g/ml. TTX, the preparation was depolarized to about  $-20$  mV. The effective concentrations of TTX are peculiarly high compared with those required in other tissues<sup>2,3</sup>.

TTX in a concentration of  $10^{-5}$  g/ml. often led to a progressive slight depolarization, and the rate of rise of the action potential was always slowed and its plateau phase simultaneously shortened. In Fig. 1 (dashed curves) the action of the drug after 7 min has reached a steady state. The "action potential" in the presence of TTX has an ill defined high threshold. The rate of rise in Fig. 1B is reduced to one-tenth, and the regenerative component of the potential rise is small. The low rate of rise under the action of TTX cannot be explained by the depolarization of the resting potential from  $-102$  to  $-89$  mV<sup>5</sup>. The repolarization phase in TTX is fast, but not altogether passive. It is remarkable that the pacemaker potential at the end of the action potential is not affected by TTX. All the effects of TTX on the action potential, including the rate of rise, are reversible within 10 min of washing. More quantitative information on the action of TTX can be obtained through voltage clamps. In Fig. 2 the membrane was depolarized in four rectangular steps starting from a holding potential of  $-104$  mV. The same steps were repeated in  $10^{-5}$  g/ml. TTX. Since the resting membrane is depolarized in TTX (Fig. 1), increased negative currents were necessary at the holding potential. In the controls of Fig. 2 B-D, a negative current appears at the beginning of the depolarization which rapidly subsides. This is the equivalent of the excitatory sodium current. In TTX the record of the current assumes a nearly rectangular shape, and most of the sodium current seems to be blocked. The current amplitude at the end of the depolarization step is, however, little affected by TTX. The declining current following repolarization of the voltage change is also uninfluenced by TTX, except that the threshold at which this current component appears is shifted under TTX from 20 mV depolarization (Fig. 2A) to 40 mV depolarization (Fig. 2B). This current flowing after repolarization is the equivalent of the pacemaker potential.

If TTX blocks the sodium current, one would expect essentially the same membrane currents in the presence of TTX as in the absence of sodium. This is, however, not the case. In a sodium-free solution at the beginning

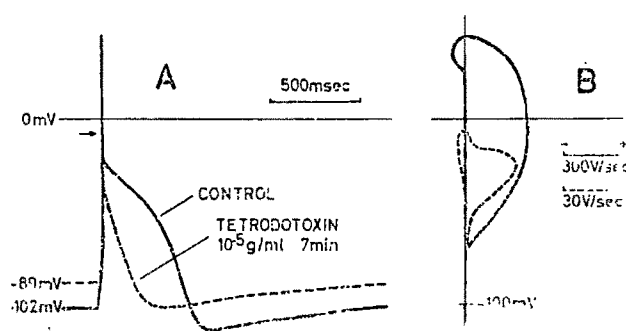


Fig. 1. *A*, Intracellularly recorded action potential; *B*, rate of change of this potential. Continuous curves represent controls in Tyrode solution, dashed curves measured in presence of  $10^{-5}$  g/ml. tetrodotoxin. The horizontal arrow in *A* denotes the peak of the dashed action potential. The stimulating current pulse was 20 msec long, its amplitude (threshold) was  $3 \times 10^{-7}$  amp in the control, and  $5 \times 10^{-7}$  amp in TTX. Ordinates in *A* and *B* represent membrane potential; abscissa in *A* represents time, in *B* rate of change of potential. The latter value was measured without distortion using an operational amplifier. Note the change in calibration in *B*.

of the depolarization step the positive current is much larger than in TTX, but falls, within a few 100 msec, to the same steady level as in TTX (compare ref. 1, Fig. 1; and ref. 6, Fig. 2). The clamp current in sodium-free solution is not affected by TTX. It seems that one of two possible conclusions can be drawn: (a) TTX does not completely block the excitatory sodium current, and has relatively little influence on a slowly declining component. (b) The

falling positive current component is only generated in sodium-free solution and is not contained in the current measured in Tyrode solution. We think that several lines of evidence tend to favour the first alternative. Experiments to clarify this issue are in progress.

All our results on the action of TTX can be explained if, on an analogy with other excitable cells, this drug inhibits inflow of sodium during depolarization. Thus our experiments are further evidence for the assumption that the fast inflow of sodium ions initiates the cardiac action potential<sup>5</sup>. The results may suggest some independence of an early, rapidly declining, and a later, slowly falling, component of the sodium inward current. The later component of the sodium current may be less sensitive to TTX than the early one. Hagiwara and Nakajima<sup>6</sup> have reported effects of TTX on frog ventricular fibres which are similar to our results shown in Fig. 1. They found that the rate of rise of the action potential slowed, whereas the plateau phase was less affected. The authors concluded that considerable inward flow of calcium maintains the plateau phase of the cardiac action potential, and this calcium current is insensitive to TTX. We do not think that these results should necessarily be explained by a calcium current. An alternative explanation is a slow component of the sodium current less influenced by TTX. In Purkinje fibres the relative insensitivity of slow negative current components towards TTX cannot be due to a calcium current. In this tissue it has been shown that no appreciable calcium current flows during the plateau phase of the action potential<sup>7</sup>. We do not believe, therefore, that inward currents insensitive to TTX are necessarily calcium currents.

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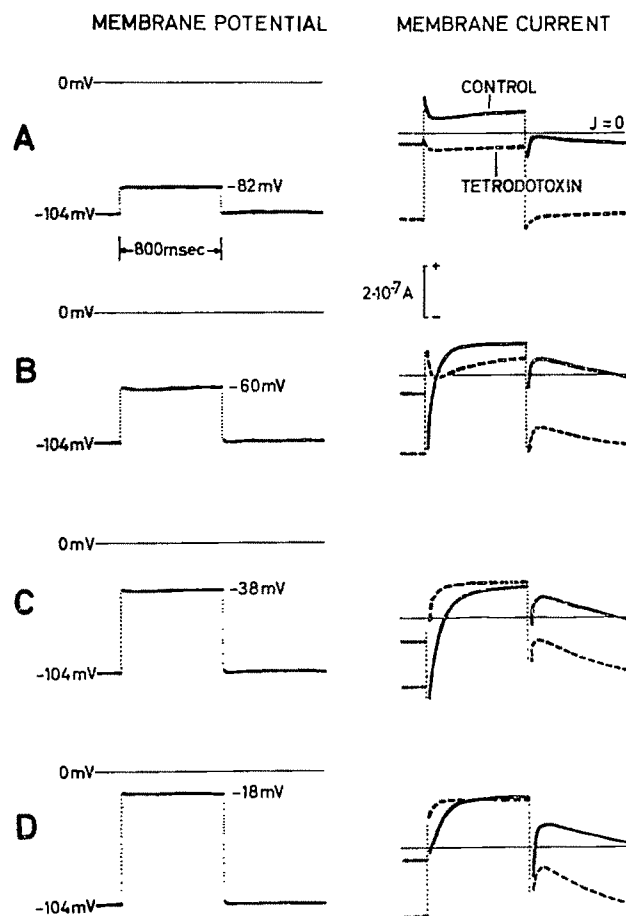


Fig. 2. Clamped changes of membrane potential (left column) and simultaneously measured clamp currents (right column). Continuous curves represent control, dashed curves measured in presence of  $10^{-5}$  g/ml. tetrodotoxin. The thin horizontal reference lines represent zero potential and zero current. Same preparation as in Fig. 1.

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### Inhibition of the Extraneuronal Metabolism of Noradrenaline in the Isolated Heart by Adrenergic Blocking Agents

ADRENERGIC blocking agents inhibit the response of effector cells to noradrenaline and related amines<sup>1</sup>; several of these drugs also act as inhibitors of the uptake of noradrenaline into sympathetic nerves<sup>2,3</sup>. This communication describes a novel action of adrenergic blocking agents, the ability to inhibit the extraneuronal metabolism of noradrenaline in a peripheral tissue.

Hearts from adult male Sprague-Dawley rats were perfused by the Langendorff technique for 10 min with Krebs-Henseleit bicarbonate solution at 37° C (ref. 4). Tritiated noradrenaline, cocaine and adrenergic blocking agents were added to the perfusion medium. Cocaine was used to reduce the uptake of noradrenaline by sympathetic nerves. At the end of the perfusion the tissue was analysed for tritiated noradrenaline and its metabolites by methods previously described<sup>5,6</sup>. In other experiments the effects of adrenergic blocking agents on the *in vitro* activity of the enzymes catechol-O-methyl transferase<sup>7</sup> and monoamine oxidase<sup>8</sup> were studied in heart homogenates.

Table 1. EFFECT OF ADRENERGIC BLOCKING AGENTS ON THE EXTRANEURONAL METABOLISM OF NORADRENALINE IN THE ISOLATED PERFUSED RAT HEART

Drugs administered	Normetanephrine	Deaminated* catechols	O-methylated† deaminated	Noradrenaline
NA	180 ± 31	25 ± 2.5	**	3,000 ± 310
NA C	280 ± 18 ‡	15 ± 1.7 ‡	56 ± 5.6	240 ± 46 §
NA C Phentolamine 10 <sup>-4</sup> molar	20 ± 2.3 ¶	3.2 ± 0.46 ¶	14 ± 1.8 ¶	45 ± 7.4
NA C Phenoxybenzamine 10 <sup>-4</sup> molar	10 ± 3.3 ¶	1.4 ± 0.31 ¶	14 ± 3.1	29 ± 3.2
NA C Dichloroisoprenaline 10 <sup>-4</sup> molar	110 ± 14 ¶	4.1 ± 0.40 ¶	27 ± 6.0	79 ± 3.9
NA C Pronethalol 10 <sup>-4</sup> molar	124 ± 10 ¶	5.3 ± 0.63 ¶	26 ± 3.6	80 ± 10

Rats were perfused with  $3 \times 10^{-8}$  molar tritiated noradrenaline (NA),  $10^{-4}$  molar cocaine (C) and adrenergic blocking agents for 10 min and the hearts were then assayed for noradrenaline and its metabolites. Results are expressed in  $\mu$ curies of noradrenaline or its metabolites per gram of heart  $\pm$  standard error of the mean. Five hearts were used in each group.

\* Deaminated catechols include 3,4-dihydroxy-mandelic acid and 3,4-dihydroxy-phenylglycol.

† O-Methylated deaminated metabolites include 4-hydroxy-3-methoxy-mandelic acid, 4-hydroxy-3-methoxy-mandelic aldehyde and 4-hydroxy-3-methoxy-phenylglycol.

‡  $P < 0.05$  compared with control hearts.

§  $P < 0.001$  compared with control hearts.

||  $P < 0.05$  compared with cocaine treated hearts.

¶  $P < 0.001$  compared with cocaine treated hearts.

\*\* Too low to be measured accurately in the presence of a large excess of unchanged tritiated noradrenaline.

In hearts perfused with tritiated noradrenaline alone almost all the radioactivity in the heart was present as unchanged tritiated noradrenaline, which confirms previous findings<sup>9</sup> (Table 1). Small amounts of the O-methylated metabolite tritiated normetanephrine and traces of tritium deaminated catechol metabolites were found. When cocaine was added to the perfusing medium to block the uptake of noradrenaline into sympathetic nerves, the accumulation of tritiated noradrenaline was reduced to less than 10 per cent of that in control hearts. In the presence of cocaine, however, the concentrations of tritiated normetanephrine were generally higher than those in control hearts (Table 1). In control hearts tritiated normetanephrine represented only 5 per cent of the total radioactivity, but in the presence of cocaine, tritiated normetanephrine accounted for about 50 per cent of the total radioactivity. Similar increases in the relative importance of O-methylation in the inactivation of tritiated noradrenaline in the heart have been reported under conditions in which the uptake of noradrenaline into sympathetic nerves was diminished by surgical denervation of the sympathetic nerve supply<sup>10</sup> or in the tissues of immunosympathectomized rats<sup>11</sup>. In the presence of cocaine, tritium deaminated catechol metabolites were reduced to 60 per cent of the control values. These findings are consistent with the hypothesis that the O-methylation of noradrenaline occurs at extra-neuronal sites, whereas deamination occurs at both intra- and extra-neuronal sites<sup>12</sup>.

When hearts were perfused with cocaine and adrenergic blocking agents, the amount of tritiated noradrenaline accumulating in the tissue was still further reduced (Table 1). Under these conditions, however, the concentrations of all the metabolites of tritiated noradrenaline were also markedly reduced. The  $\alpha$ -adrenergic blocking agents, phentolamine and phenoxybenzamine, reduced the concentrations of metabolites to levels only 5–25 per cent of those in hearts perfused with cocaine alone. The  $\beta$ -adrenergic blocking agents dichloroisoprenaline and pronethalol reduced all metabolites by about 60 per cent.

The reduced metabolism of tritiated noradrenaline caused by the adrenergic blocking agents occurred only in the intact tissue. When the drugs were examined as inhibitors of catechol-O-methyl transferase or monoamine oxidase in heart homogenates *in vitro*, using concentrations of tritiated noradrenaline and drugs similar to those in the perfusion experiments, the adrenergic blocking agents did not reduce the metabolism of tritiated noradrenaline by either enzyme.

These results indicate that adrenergic blocking agents can inhibit the extraneuronal metabolism of noradrenaline by preventing the access of noradrenaline to the metabolizing enzymes. Because both monoamine oxidase and catechol-O-methyl transferase are intracellular enzymes, the adrenergic blocking agents may act by preventing the substrate noradrenaline from entering the cells which contain these enzymes. It has been demonstrated that a specific transport mechanism exists for the entry of extracellular catecholamines into sym-

thetic nerves<sup>3,4</sup>; it is possible that the entry of noradrenaline into postsynaptic structures is also mediated by a specific transport mechanism. If the adrenergic blocking agents were to act at such a site, they could prevent the access of noradrenaline to metabolizing enzymes and possibly to intracellular adrenergic receptors.

The effect of a wide variety of pharmacological agents on the extraneuronal metabolism of tritiated noradrenaline is at present under investigation.

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## CYTOLOGY

### Points of Attachment of Pachytene Chromosomes to the Nuclear Membrane in Mouse Spermatocytes

At the present time the only chromosomal element which is readily identifiable on electron microscopic examination is the "synaptonemal complex"<sup>1</sup> of the male primary spermatocyte. In the course of a study of the mode of attachment of this structure to the nuclear membrane in a variety of mammals, we noted that, in the mouse, two distinct types of endings were seen near the point of attachment. In one type the bivalent appeared to be condensed around the synaptonemal complex to form a heterochromatic "basal knob"<sup>2</sup> while, at the other type of ending, the complex was not surrounded by condensed material. The appearances are shown in the electron micrograph (Fig. 1) in which, by a fortunate chance, the entire length of the synaptonemal complex of a bivalent is seen in one section. Since it is now generally agreed that the mitotic chromosomes of the mouse are telocentric with truly terminal centromeres<sup>3,4</sup> and the heterochromatic regions of mouse spermatogenic autosomes are thought to be located adjacent to the loci of the kinetochores<sup>5</sup>,





Fig. 1. Primary spermatocyte from mouse testis. Electron micrograph  $\times 7,200$ . A, "Centromeric" end of bivalent. B, "Distal" end of bivalent. SV, sex vesicle.

there seems to be justification for identifying the attachment labelled A in Fig. 1 with the centromeric end of the pachytene chromosome and that labelled B with the distal end.

Suitably fixed and embedded mouse testis was then sectioned at between 200 and 300  $\mu$  and examined under the electron microscope and the attachments of the synaptonemal complexes to the nuclear membrane were recorded in 100 cells. Cells were only included in the series when the sex vesicle (SV in Fig. 1) was clearly visible. In 100 cells the number of centromeric (type A) ends encountered was 80, the number of distal (type B) ends was 91. The further observation was made that 68 of the 80 (85 per cent) centromeric ends were located in the half of the cell which contained the sex vesicle, and 79 of the 91 non-centromeric ends were situated in the other half.

The findings of the investigation therefore suggest that in the mouse primary spermatocyte at pachynema the centromeric and distal ends of the bivalents are separately identifiable and that the "pachytene bouquet" is formed by the clustering of the centromeric ends of the bivalents around the sex vesicle with the distal ends stretching to be attached in the majority of cases to the nuclear membrane of the opposite side of the cell.

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### Fine Structure of Platyhelminth Sperm Tails

ELECTRON optical studies made during the late fifties led to a general acceptance of the 9 + 2 pattern in the axial filaments of cilia, flagella and sperm tails. In 1961 an exception to the universally accepted pattern was reported in the sperm tail of the trematode *Haematoloechus medio-plexus*<sup>1</sup>. The tail was found to contain one rather than two fibrils in the central unit, giving a 9 + 1 rather than the

9 + 2 pattern. Subsequently (1962), while commenting on the evolutionary stability of the 9 + 2 pattern, one author noted that "even the *Haematoloechus* observation can be explained as an unusual reaction of the central pair (and sheath) to the fixation procedure"<sup>2</sup>. Also in 1962, Klima<sup>3</sup> identified the 9 + 1 pattern in the sperm tail of the turbellarian *Dendrocoelum* and the 9 + 2 arrangement in the cilia of the flame cells—the first record of the two patterns in the same organism. In 1964 Silveira and Porter<sup>4</sup> reported the 9 + 1 pattern in the sperm tails of three other turbellarian species, *Dugesia trigena*, *Bdelloura candida* and *B. propinqua*, but they found the 9 + 2 pattern in the cilia. In 1966 four additional trematode species—*Gorgoderia amplicava*, *Cotylophoran cotylophorum*, *Alaria arisaemoides* and *A. mustelae*—were reported with 9 + 1 sperm tails<sup>5</sup>.

A model of the cross-section of a sperm tail (Fig. 1) can be described as follows: on the outside the enclosing plasma membrane (G) supports a system of microtubules (H) which vary in number from four to a maximum of thirty-six (ref. 1). The remainder of the cross-section is the axial fibre bundle which resembles a wheel; the hub is the single central filament (A) which is connected to the rim of nine doublet filaments (F) by spokes (E). Three distinct areas can be recognized in the single axial filament; the centre is a dense core (B) enveloped by a homogeneous cortex (C) of lesser electron density both of which are encased by a sheath the density of which is similar to that of the core. The diameter of the axial fibre bundle is 150  $\mu$ , while that of the entire sperm tail is approximately 200  $\mu$ .

The recognition that four turbellarian and five trematodian species have the 9 + 1 patterns in their sperm tails suggests that a similar pattern might exist in the remaining group of the platyhelminths, the cestodes. We have not come across any references to electron microscopy of the sperm tails of cestodes. Fortunately, however, in a paper concerned with the macromolecular structure of glycogen<sup>6</sup> there are micrographs of sperm tails of *Hymenolepis diminuta* which show the 9 + 1 pattern (ref. 6, Fig. 16). Thus, this finding—even though for a single species—extends the 9 + 1 sperm tail pattern into the cestodes. Interestingly enough, another micrograph in the glycogen study (ref. 6, Fig. 19) shows the 9 + 2 pattern in the flame cells of the same cestode.

The first reports of the 9 + 1 sperm tail pattern for both the trematodes<sup>1</sup> and the turbellarians<sup>3</sup> were for single species which were extended to other species<sup>4,5</sup> as a result of additional studies. The 9 + 1 fibrillar arrangement is very probably a normal pattern for the sperm tails of these animals. Similarly, the same may apply to the cestodes, especially in view of the close phylogenetic

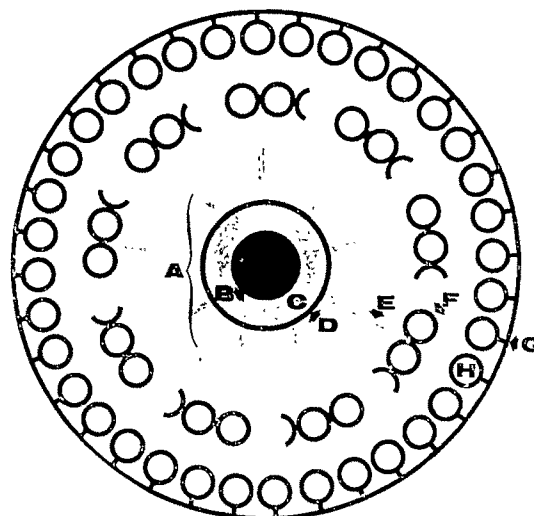


Fig. 1. Generalized cross-section of a platyhelminth sperm tail.

relationship of the three classes. Studies of additional cestodian species are needed, however, before a definite conclusion can be reached. For the moment we can only conclude that the sperm tails of all platyhelminths studied by electron microscopy show a 9+1 pattern in the axial fibre bundle. It is interesting that the 9+2 pattern occurs in the axial fibre bundles in the extensions of flame and epithelial cells in the turbellarians<sup>3,4</sup> and the cestodes<sup>5</sup>, the only classes of the platyhelminths so far investigated.

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### Chemical Composition of the Cell Wall of *Caryophanon latum*

THE "giant micro-organism" *Caryophanon latum* was first described by Peshkoff as the "missing link" between the blue-green algae and the bacteria. He isolated the micro-organism from cow manure near Moscow, noting its unusual morphology including "its long rod forms containing a varying number of nuclei"<sup>1</sup>. Others have since isolated this organism in England and in the United States<sup>2,3</sup>. Pringsheim and Robinow have described this micro-organism. *Caryophanon latum* Peshkoff as "a very large Gram negative, peritrichously flagellated bacterium of unusual structural complexity"<sup>2</sup>. On the other hand, Provost and Doetsch reported the organism to be a Gram positive bacterium, susceptible to egg-white lysozyme and able to form protoplasts readily<sup>4</sup>. The work described here was designed to obtain further evidence as to the bacterial nature of the micro-organism by analysing the isolated and purified cell walls of *Caryophanon latum* for its constituent amino sugars, amino acids and monosaccharides.

The method of analysis was that of Becker *et al.*<sup>5</sup> except that the organism was grown on cow dung extract agar, then gently rinsed into a beaker with 95 per cent ethanol, and the cell disruption was accomplished with a 'Heat Systems Sonifer'.

Our results showed large amounts of glucosamine, muramic acid, alanine, glutamic acid and lysine, typical of cell walls of Gram positive bacteria. Diaminopimelic acid, which often appears in place of lysine in the cell wall mucoprotein, was absent. No monosaccharides were found. These results provide further evidence of the bacterial nature of *Caryophanon latum*. Although the finding of a small number of amino-acids in the cell wall indicates a Gram positive nature, we found the organism to stain Gram negative.

This work was done in the Biology Department of Hamilton College. We thank Dayna McDaniel for the culture of *Caryophanon latum*.

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## HAEMATOLOGY

### Erythropoietic Response of Bone Marrow Cells cultivated in Diffusion Chambers

It is well known that cultivation of bone marrow by traditional *in vitro* methods does not sustain normal morphogenesis for prolonged periods of time<sup>1-4</sup>, and although synthesis of DNA and haem and some erythropoietic activity have been demonstrated *in vitro*<sup>5-9</sup>, these processes are maintained only for extremely short periods.

An alternative approach to the *in vitro* method is the technique of cultivating haematopoietic cells in diffusion chambers in the peritoneal cavity of host animals. With this method it has been demonstrated<sup>10-12</sup> that mouse marrow cells divide, differentiate and undergo maturation in a much more normal fashion for a considerably longer period than in strictly *in vitro* systems. Attempts to demonstrate morphologically the effect of the humoral agent, erythropoietin, on erythropoiesis in chambers by myself and other investigators<sup>13,14</sup> have, however, been unsuccessful. In view of the ability to demonstrate morphologically erythroblasts in unstimulated marrow cultures<sup>10</sup>, two questions arose as to why marrow in diffusion chambers did not respond to erythropoietin. Had cells in the diffusion chambers become refractory to erythropoietin, or were the methods utilized to reveal a possible effect adequate? In an attempt to resolve these questions, experiments were devised in which modified diffusion chambers and a non-morphological end point for demonstrating erythropoiesis were used.

In the first experiment a well-type (solid bottom) diffusion chamber was used.  $15 \times 10^6$  cells from a pool of C57/bl-6 mouse marrow were placed in chambers containing 0.1  $\mu$ c. of iron-59 and 0.1 ml. of either saline, normal serum or serum rich in erythropoietin. The latter was obtained from mice which had been bled 12 h earlier. Chambers sealed by membranes with a pore of 0.3  $\mu$  diameter were placed into the peritoneal cavity of normal mice. At intervals after implantation, chambers were taken from animals and cells were removed by treatment with hyaluronidase. Cells were washed three times with sterile saline to eliminate unincorporated iron-59 and were assayed for cellular incorporation of iron-59 in a well-type scintillation counter. The results obtained are summarized in Table 1, and are expressed in terms of incorporation of iron-59 as a percentage (10<sup>6</sup>) of the original amount of iron-59 in the chamber. Values for the saline and normal serum were essentially the same and are presented as pooled controls. Within 24 h there was seven times as much incorporation of iron-59 in those cells cultivated in serum rich in erythropoietin as in the control group. By day 3 incorporation of iron-59 in the cultures rich in erythropoietin reached a maximum value approximately four times greater than on day 1 and fifteen times greater than the controls. A drop in the incorporation of the isotope was noted between days 3 and 5 and between days 7 and 9. The value on day 9 is essentially the same as that on day 1 and still represents a fivefold increase over the controls.

The data obtained indicate that precursors of erythrocyte are present in diffusion chambers which are capable of responding to an erythropoietic stimulus. The failure to demonstrate a cumulative increase in incorporation of iron in the group with serum rich in erythropoietin could result from the utilization of erythropoietin by erythroid precursors which has been suggested<sup>15</sup>, or from a depletion

Table 1. INCORPORATION OF IRON-59

	1	Days after Implantation			
		8	5	7	9
Serum rich in erythropoietin	29.0 (6)*	100.7 (8)	39.5 (6)	47.3 (6)	24.5 (6)
Controls	4.2 (12)	6.6 (12)	8.8 (12)	8.7 (12)	5.0 (12)

The percentage of iron-59 in each chamber is multiplied by 10<sup>6</sup>.

\* Figures in parentheses denote the numbers of animals used.

of the precursor(s) on which erythropoietin acts. The decreased incorporation between days 3 and 5 and 7 and 9 in the group rich in erythropoietin suggests that a cyclic destruction of cells containing iron-59 may occur and be followed by loss of the isotope by diffusion out of the chambers.

The possible reason for the failure to demonstrate increased erythropoiesis in chambers bound by double membranes is derived from the results of a second series of experiments. Bone marrow cells suspended in saline with identical amounts of iron-59 were placed in well-type diffusion chambers, but were separated from serum rich in erythropoietin and from normal serum by membranes. These chambers were treated in the same fashion as the previous experiment. No effect of serum rich in erythropoietin was found. This suggests that erythropoietin even if present in the peritoneal cavity of host animals is inactivated when it passes through the membranes. This is substantiated by the demonstration that erythropoietically active urine is rendered inactive when sterilized by passage through 'Millipore' membranes<sup>18</sup>.

In addition to demonstrating the response of cultivated bone marrow cells to an erythropoietic stimulus, these results also suggest that the cessation of erythropoiesis in chambers bound by double membranes by its inactivation when diffused through the membranes may leave more stem cells in the implanted marrow free to differentiate into other cell types. This would account for the length of time granular-cytopoiesis ensues in chambers and for the numbers of histiocytes which eventually appear with time of cultivation<sup>19</sup>.

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### Effect of Prolonged Exercise on Platelet Adhesiveness

It has been established that changes in platelet adhesiveness occur in a variety of physical and physiological conditions. A close investigation of the various physical conditions that affect adhesiveness showed considerable variations in the degree of adhesiveness in eighteen identical observations on the same subject<sup>1</sup>; there was also slight increase in adhesiveness after feeding. Many methods have been devised to measure stickiness of platelet, and there has been considerable variation in the observations made. This could mean that different methods are influenced by a number of factors concerned in adhesiveness<sup>2</sup>. We have investigated the effect of prolonged exercise on platelet adhesiveness. The study

was carried out on a group of sixty healthy young adults who were walking from London to Brighton, about 50 miles. The exercise lasted for about 20 h.

Platelet adhesiveness was measured using two techniques in parallel, the adenosine diphosphate (ADP) method<sup>3</sup> and a 'Celite' method (diatomaceous earth)<sup>4</sup>; the results before and after exertion have been compared in forty-five subjects.

Adhesiveness was recorded on fifty-one subjects in the 2 weeks before the exercise and on nine subjects 2-3 weeks after the walk. Forty-five of the walkers completed the 50 miles and the adhesiveness was measured immediately on arrival.

The subjects were not anaemic and their platelet counts were within normal limits. Blood was taken from a vein in the antecubital fossa using a disposable syringe and needle and by a standard technique in each case. Aliquots of 2 ml. of blood each were added to polystyrene bottles (2.5 in. × 1 in.) containing 2.4 mg of ethylenediamine tetraacetic acid (EDTA) and 2 mg of heparin, respectively. This gave concentrations in the blood of more than 4 mmolar EDTA and 1 mg/ml. of heparin. The platelet counts were made as described previously<sup>5</sup>. Independent observers took the average of duplicate counts, which were repeated if there was a discrepancy of more than 15 per cent. All tests were carried out at room temperature. The specimens were mixed on a Matburn wheel at 30 r.p.m. In Eastham's method 0.04 ml. of ADP was added to the heparinized blood (25 mg in 100 ml. of saline stored at -20° C in small aliquots). The blood was returned to the mixer for exactly 30 min, and then transferred to a similar polystyrene bottle containing EDTA and mixed for a further 20 min. Counts of this specimen were made and also of the corresponding original sample of EDTA, and the differences between the two were taken as the adhesive platelet count which was expressed as a percentage.

In the 'Celite' method, platelets were counted in the blood collected into EDTA, after mixing, then 0.5 g of 'Celite 560' was added and mixed for 45 min at 30 r.p.m. on the wheel.

The bottle stood for 2 min to allow the 'Celite' to settle and a further platelet count was performed from the 'Celite'-free upper layer. Platelet counts of EDTA blood samples allowed to stand in this manner showed no significant difference from counts of samples made immediately after mixing. The difference in the platelet counts before and after the addition of the 'Celite' was taken as the adhesive platelet count, which was expressed as a percentage.

By the ADP method (Fig. 1) before exercise the average adhesiveness was 62 per cent with a standard deviation of 7.8 per cent. We included in the normal range variation from the average mean of twice the standard deviation. After exertion the adhesiveness had decreased in thirty-



Fig. 1. Platelet adhesiveness before and after exercise, determined by the ADP method. ●, Male before exercise; ■, female before exercise; ○, male after exercise; □, female after exercise.

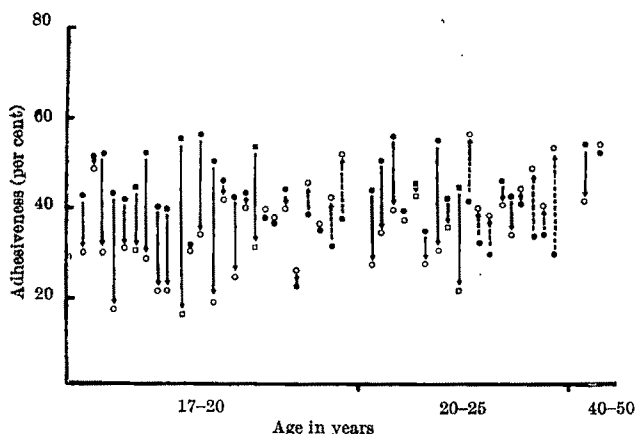


Fig. 2. Platelet adhesiveness before and after exercise, determined by the 'Celite' method. Symbols are the same as in Fig. 1.

three subjects, increased in twelve; thirteen of the former observations were lower than the normal range.

The differences between the results before and after exertion were analysed using Gosset's *t* test and found to show a significant fall in platelet adhesiveness ( $P < 0.001$ ).

By the 'Celite' method (Fig. 2) the average adhesiveness before exercise was 42.5 per cent with a standard deviation of 16 per cent. We took a normal range of twice this standard deviation.

After the walk, adhesiveness had decreased in thirty and increased in fifteen. The results from ten of those with reduced adhesiveness were below the calculated normal range. The difference between the observed adhesiveness before and after exertion was analysed. It was found that after the exercise adhesiveness was significantly reduced  $0.005 > P > 0.001$ .

The results for each technique indicated the same type of change in thirty-six of the forty-five subjects investigated. In twenty-seven, the platelet adhesiveness decreased when measured by both methods and in nine cases it increased. The remainder gave opposing results, but in each case the values fell within our normal range. There appears to be a reasonable correlation between the results of both procedures.

It is clear from these results that there was a fall in platelet adhesiveness in the majority of our subjects. In those persons in whom a rise was observed this was within our experimental range. No attempt was made to correlate the results with the sex of the walkers. Only five women are included in the series, and the significance of any difference of the adhesiveness of platelets would be difficult to interpret in such a small number.

There were several variable factors which are difficult to assess. No one undertook special training for the walk, but some were accustomed to more exercise than others, not, however, to regular exercise of a degree comparable with a 50 mile walk. It was impossible to control the amount or type of food and drink taken by the walkers just before or during the exercise, but questioning revealed that the diet was adequate in all cases and contained chiefly carbohydrate and fluids. Many of the people took drugs during the walk, usually paracetamol, but a few also took codeine or aspirin.

Our results are at variance with others<sup>6</sup> but may not be strictly comparable, because the type of exercise was not the same. In other investigations an increase in platelet adhesiveness was observed after volunteers had been subjected to almost exhaustive muscular exercise for 6 min. In the present tests the exercise was less severe but greatly prolonged.

It seems likely that the marked fall in platelet adhesiveness is the result of the change in environment of the platelets rather than any alteration in the platelets themselves. The platelet counts on the subjects before and

after the walk showed no definite pattern of change (twenty-nine were virtually the same, ten were raised and six were lowered). This would eliminate the possibility that after the exercise we were assessing an altered platelet population, caused by mobilization of platelets in the hyperdynamic state.

While counting the platelets after the exercise it was noted incidentally that the white cell count appeared to be raised to approximately twice the normal in each case. It was probable that there was prolonged increase of adrenaline during the stress of this continuous exercise. Adrenaline has produced increased platelet adhesiveness *in vitro*<sup>7,8</sup>. An increased platelet count associated with increased adhesiveness after adrenaline has been described<sup>9</sup>. In view of these observations, our findings suggest that physiological effects of the prolonged exercise have nullified changes that might have been expected, because of prolonged production of adrenaline.

Although food was taken during the walk, endogenous glucose was probably mobilized because of the severity of the exercise. A fall in platelet adhesiveness when endogenous glucose was mobilized by smoking has been shown<sup>10</sup>. Other changes in the blood may also have contributed to the alteration in the adhesiveness. A relationship between platelet stickiness and changes in fatty acids has been shown *in vitro*<sup>11</sup>.

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## Erythropoiesis in the Regenerating Spleen

Till and McCulloch<sup>1</sup> have observed that cells obtained from mouse bone marrow can produce colonies of cells with haematopoietic competence when injected into the spleens of irradiated mice. Their findings verify the earlier reports of other workers, notably Jacobson *et al.*<sup>2</sup> and Cole *et al.*<sup>3</sup>, who observed that the partial shielding of either bone marrow or spleen, or the injection of homogenates of these tissues from normal animals into irradiated animals, would prevent deaths normally associated with the haematopoietic syndrome.

More recently McCulloch<sup>4</sup>, using an ingenious split-dose technique to label the chromosomes of donor stem cells, has found that individual spleen colonies derived from a single clone can produce blood cells of more than one line. Differentiating cells of erythrocytic, granulocytic, and megakaryocytic lines were found side by side within the same spleen colony.

While this work appears to indicate that individual stem cells are not completely predetermined to one specific line of differentiation, certain questions about the effects of the source of the donor tissues on the subsequent behaviour of the stem cells in the host remain outstanding. For example, McCulloch and Till<sup>6</sup> have reported that ten times as many spleen cells as marrow cells are required to obtain the same number of colonies. Numerous workers<sup>6</sup> including ourselves have observed that spleen nodules can vary greatly in size, but may still be easily divided into two extreme groups of large and small nodules. In addition, Gurney<sup>7</sup> reports that the large colonies seem to develop predominantly along erythroid lines, while the small colonies tend to develop neutrophils, and that the injection of erythropoietin or leucopoietin can affect the relative numbers of large and small colonies.

We have attempted to investigate the effect of the source of donor tissue on erythropoiesis in regenerating spleens containing colonies derived from either spleen or bone marrow. We used an iron-59 labelling technique<sup>8,9</sup> to measure erythropoiesis. There is often a wide divergence in colony size within the spleen and between spleens, and we concluded that the spleen-weight method recommended by Popp *et al.*<sup>10</sup> would be a more reliable method of measuring spleen regeneration than adding up the number of colonies. This method also allowed us to compare directly the erythropoietic ability of the regenerating spleen with that of the spleens of normal animals and of untreated, irradiated control animals.

For these experiments Swiss mice were irradiated with 950 rads, using a 250 kVp. X-ray unit. In each experiment, the age of the donors was identical with the age of the recipients. To reduce deaths due to bacteraemia, all the animals were fed neomycin *ad lib.* in their drinking water for 5 days before irradiation. Combiotic was also injected with the cells at a concentration of 0.003 mg/mouse. The spleen or bone marrow cells were derived from pooled tissue homogenates obtained from two or more animals, suspended in normal saline, and injected into the tail vein (0.5 ml. per animal). The irradiated controls were sham-injected with saline and combiotic only.

In the experiments reported in Table 1, all the mice receiving donor tissue were given  $10^8$  cells within 4 h of the completion of X-irradiation. Female mice 8–10 weeks old were used and the period between irradiation and killing was 10 days. 24 h before killing, each mouse received 0.5  $\mu$ c. of iron-59 as ferrous chloride by intra-peritoneal injection. All the spleens were immersed in Bouin's solution after excision to make the nodules more easily visible. All weights were determined as wet weights. All spleen weights were normalized to the approximate weight of the normal spleen (100 mg). The average weight of the regenerating spleens was roughly 60 per cent that of the normal spleens, regardless of the source of donor tissue. The spleens of the irradiated control mice were approximately 20 per cent of the normal controls in weight. Before assaying for iron-59 content, and before being immersed in the fixative, the spleens were washed thoroughly in normal saline. The uptake of iron-59 was measured by a scintillation counter equipped with a pulse-height analyser and an automatic sample changer. The results are given as mean per cent uptake of iron-59 per 100 mg of spleen tissue, for each experiment. The results in the last line of Table 1 are the combined

Table 1. PERCENTAGE UPTAKE OF IRON-59/100 MG OF SPLEEN

Experiment No.	Controls (not irradiated)	950 rads + spleen	950 rads + bone marrow	950 rads only
1	3.15(9)*	—	—	1.61(8)
2	2.99(7)	—	9.69(15)	2.30(5)
3	4.12(5)	—	10.74(7)	1.53(4)
4	3.01(7)	4.17(5)	—	2.08(8)
5	3.85(6)	6.48(15)	—	1.91(3)
Mean	3.37 $\pm$ 1.12 (34)	5.90 $\pm$ 1.51 (20)	10.03 $\pm$ 4.34 (22)	1.89 $\pm$ 1.22 (28)

\* Numbers in parentheses are numbers of animals in each part of the experiment.

Table 2. PERCENTAGE UPTAKE OF IRON-59/100 MG OF SPLEEN

Experiment No.	Controls (not irradiated)	950 rads + spleen	950 + bone marrow
6	2.69 $\pm$ 1.43 (8)	2.57 $\pm$ 1.67 (5)	6.60 $\pm$ 2.41 (8)

means for all animals in each column, together with a standard deviation computed from the uptake values of individual animals.

The results show that while spleens containing colonies derived from bone marrow tissue were more active in erythropoietic than normal spleens by a mean factor of 3.1, those with colonies derived from spleen cells were more active by a mean factor of only 1.8. Both types of regenerating spleens and the normal spleens showed significantly more iron-59 activity than the irradiated controls.

In the second experimental group (Table 2), we were concerned with both the effects of age and the relative number of colony forming cells in the spleen and bone marrow<sup>5</sup>. In these experiments female mice 4–6 months old were used. The mice receiving bone marrow were given  $4 \times 10^6$  cells; those receiving spleen were given  $4 \times 10^7$  cells, in accordance with the colony-forming ratios given by McCulloch and Till<sup>6</sup>. In this experiment also the ages of the donors matched those of the recipient animals. The other conditions were also similar to those of the preceding experiments.

The results show that the relative erythropoietic activity of colonies derived from spleen and bone marrow is not a function of the number of cells administered. Moreover, it appears that in addition to a general lessening of erythropoietic ability with age in all groups, regenerating spleens derived from spleen cells no longer have the ability to exceed the erythropoietic activity of the spleens of normal animals (as was seen in the younger animals in Table 1).

We conclude from these results that colonies derived from bone marrow tissue are more effective erythropoietically in regenerating spleens than those derived from spleen tissue. It also appears that age is an important factor in determining erythropoietic activity in the regenerating spleen. The results show that by the age of 4–6 months the capacity for erythropoiesis of tissue from regenerating spleen is no longer greater than that of normal mice. On the other hand, though reduced, the erythropoietic ability of tissue derived from bone marrow is still markedly greater than that of normal spleen. These findings are strong evidence that the source of the donor haematopoietic tissue influences its subsequent initial activity in the irradiated host.

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## IMMUNOLOGY

## Simple Method for Preparation of Haemagglutinating Arbo-A Virus Antigens from Brains of Suckling Mice

HAEMAGGLUTINATING (HA) arbovirus antigens are usually prepared from the brains of suckling mice. This preparation is frequently performed by the acetone and ether extraction method of Casals and Brown<sup>1,2</sup>. That method is rather long and costly, however, so that the development of a method which avoids these disadvantages seems desirable. We thought that treatment of infected suckling mouse brains with 'Tween 80' and ether could provide a method by which HA antigens could be obtained simply. This assumption was based on our finding<sup>3</sup> that a haemagglutinating component can be released from the viral envelope of a group A arbo virus (Sindbis) by treatment of "complete" and "incomplete" virus with 'Tween 80' and ether.

In the work reported here the following three methods for the preparation of HA antigens were compared. (Method I) Acetone and ether extraction by the method of Casals and Brown<sup>1,2</sup>. (Method II) Infected suckling mouse brains were frozen and homogenized with 9 volumes of chilled borate buffer, pH 9.0. The homogenate was centrifuged for 5 min at 3,000 r.p.m. in the cold. The supernatants were collected and 'Tween 80' was added to give a concentration of 2 per cent; in addition, peroxide-free ether was added in an amount equal to the volume of the supernatant. The mixture was shaken for 20 min at room temperature and again centrifuged for 10 min at 5,000 r.p.m. in the cold. After centrifugation, the water phase was collected and residual ether was removed by bubbling nitrogen through the mixture. By this method, a supernatant of a homogenate of infected suckling mouse brains which had been treated with 'Tween' and ether furnishes the HA antigen. (Method III) To 1 volume of frozen and infected suckling mouse brain 9 volumes of borate buffer, pH 9.0, 10 volumes of ether, and 'Tween 80' in an amount to give a final concentration of 1 per cent were added. The whole mixture was homogenized, centrifuged in the cold for 5 min at 5,000 r.p.m., and the turbid water phase was collected and treated with nitrogen as described above. This method can be carried out very rapidly because homogenization and treatment with 'Tween' and ether are carried out in a single step.

Three day old mice were infected with a mouse-adapted Sindbis virus strain AR86 and 6 day old mice with a Western equine encephalitis (WEE) virus strain. Inoculation of the mice and removal of their brains were carried out as described by Clarke and Casals<sup>2</sup>. Infectivity of the HA antigens was assayed by the plaque technique using chick embryo cells<sup>3</sup> (P.F.U., plaque forming units). The techniques described<sup>3</sup> were applied in the haemagglutination and haemagglutination-inhibition tests carried out in test-tubes. Haemagglutination was allowed to proceed at 37° C, usually at a pH of 6.0. The haemagglutination titre is expressed in HA units/ml.

Table 1 summarizes the results of experiments with WEE and Sindbis viruses. It can be seen that in the case of WEE virus methods II and III resulted in a yield of haemagglutinin sixteen times greater than that given by method I; in the experiment with Sindbis virus, the yield was four times as great as for method I. HA antigens obtained by method III contained more P.F.U. than those of methods II and III. Similar results were

obtained in additional experiments. The specificity of the HA antigens of all three methods was determined by the haemagglutination-inhibition test. Haemagglutination-inhibition titres of a virus specific anti-hyperimmune serum were the same for 4 HA units of the three different preparations. It should be emphasized that there were slight differences between the antigens of methods I, II and III with regard to the optimal pH values of the HA reaction. Optimal condition for HA reaction of a WEE antigen obtained by method I was at pH 6.0, whereas WEE antigens of methods II and III gave highest titres at pH 5.8 and 6.0. With Sindbis virus, pH optimum for an antigen of method I was 6.0, and for antigens of methods II and III optimum pH was 6.0 and 6.2. Stability of antigens at 4° C was tested over a period of 18 days. Antigens of method I remained stable during this period, whereas haemagglutinating activity of antigens of method II decreased twofold and of method III sixteenfold.

These results suggest that methods II and III are superior to the acetone and ether extraction method I, in that they can be carried out more rapidly, with less material, and give higher yields of haemagglutinin and contain less infective units. They are inferior to antigens of method I with regard to their stability. For practical use, preparation of HA antigen according to method II is recommended, because it is more stable and less infectious than antigen prepared by method III.

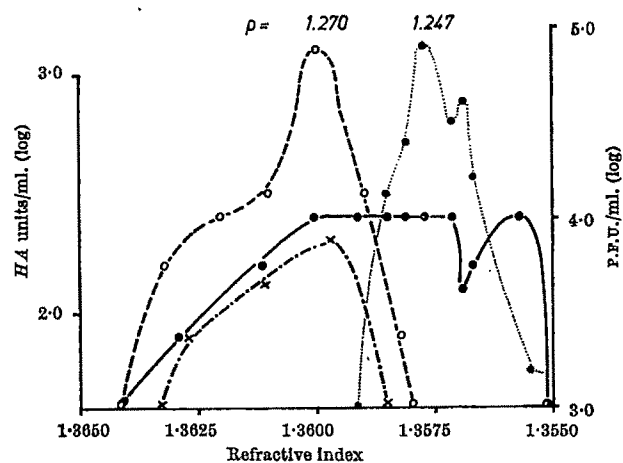


Fig. 1. Distribution of haemagglutinating activity after caesium chloride density gradient centrifugation of antigens obtained by method I (●—●); method II (×—×); method III (○—○); (●—●), distribution of infectivity of the antigens obtained by method I ( $\rho$  = buoyant density).

Finally, the distribution of haemagglutinating activity after caesium chloride density gradient centrifugation of antigens obtained by methods I, II and III was determined. For this purpose, antigens prepared by methods I and III were diluted 1:2, and antigens prepared by method II were diluted 1:4, in borate buffer, pH 9.0. The diluted antigens were mixed with the required amounts of caesium chloride. Centrifugation, sampling of fractions and density determinations were performed as described previously<sup>3</sup>. Fig. 1 shows the results of such an experiment. The greatest haemagglutinating activity of antigens from methods II and III was found in a fraction with a buoyant density of 1.270. Apparently this activity was shown by a component which was also isolated by treatment of purified "complete" and "incomplete" Sindbis virus with 'Tween 80' and ether<sup>3</sup>. On the other hand, haemagglutinating activity of a method III-antigen was distributed in a density range of 1.270–1.225. This indicates that haemagglutinating activity of an antigen obtained by extraction with acetone and ether is associated with components of different densities. One of these components, detected in the 1.270 fraction, seems to be identical with the above.

Table 1. HAEMAGGLUTINATION AND INFECTIVITY TITRES OF PREPARATIONS OBTAINED BY METHOD I, II, III AND IV

Virus	Method	HA units/ml.	P.F.U./ml.
WEE	I	1,280	$5 \times 10^4$
	II	20,480	$7 \times 10^4$
	III	20,480	$3 \times 10^4$
Sindbis	I	320	$10^4$
	II	1,280	$< 4 \times 10^4$
	III	1,280	$< 4 \times 10^4$

mentioned haemagglutinin. Another haemagglutinating component of the acetone and ether preparation was associated with infectivity and was detected at a density of 1.247, suggesting that this component represents "complete virus particles" which have not been destroyed by the acetone and ether extraction procedure.

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### "Normal" Antibodies which react with Heterogenetic Bacillary Antigen

STUDIES have been reported of a bacteriogenic transfusion reaction<sup>1</sup> in which the recipient's red cells became poly-agglutinable, apparently as a result of the presence of a species of *Bacillus* in the donor bottle which was capable of altering erythrocytes *in vitro* in a similar way. It was suggested that modification of the cells by bacterial antigen with subsequent haemolysis by "normal" anti-bacterial antibody might provide the mechanism for such reactions. In further investigations<sup>2</sup>, heterogenetic antigens from various genera and species of Gram-positive bacteria were examined, some prepared by the methods of Rantz<sup>3</sup> and Neter<sup>4</sup>, and were shown to react with anti-bacterial antibodies found in normal human sera. Crude preparations of at least two such antigens, present in bacilli and in *Staphylococcus aureus*, as well as a third, found only in *Streptococcus*, reacted with antibodies in sera of practically 100 per cent of apparently normal persons. Whether this high frequency of reactivity resulted from the cumulative effect of several antibody specificities or from a single specificity was re-examined with purified antigen.

Antigen was extracted from 72 h cultures of *Bacillus* sp. No. 372-56 by boiling, as described previously<sup>2</sup>. The organisms and debris were removed by centrifugation and the supernate was mixed with ten volumes of acetone, which was decanted after the antigen had been precipitated. Precipitates were dried to remove traces of acetone and redissolved in 0.85 per cent solution of sodium chloride, buffered at pH 6.7 with phosphate salts. These antigens were tested<sup>2</sup> for their content of hexose and their ability to modify erythrocytes. Washed human group O red cells were mixed with twenty volumes of an optimal concentration of antigen, incubated at 35° C for 30 min, washed again, and resuspended in buffered saline solution (pH 6.7) at a concentration of 2 per cent. Tests were conducted on one hundred consecutive normal blood donor sera by mixing 0.05 ml. of serum with 0.05 ml. of modified cell suspension, incubating the mixtures at 5° C for 30 min, centrifuging lightly, and reading the results microscopically. Duplicate tubes were incubated at 37° C for 1 h, the cells washed, and tested with antiglobulin serum. Each donor serum was also tested against control cells which had been treated with buffered saline instead of antigen solution. Reactive sera of known titre were included in each test protocol to control the sensitivity. Of the one hundred normal donor sera, eighty-six produced agglutination of modified cells in saline and ninety-seven yielded positive results by the antiglobulin test (Table 1). Two sera failed to react by either procedure. An attempt

to demonstrate blocking by these two sera failed, although previous work<sup>2</sup> has indicated that blocking may be produced by anti-streptococcal human sera which react with the NSS heterogenetic antigen of Rantz<sup>3</sup>, and some of the other twelve non-agglutinating sera appeared to exhibit a slight blocking effect.

Table 1. REACTIONS OF ONE HUNDRED NORMAL SERA WITH ERYTHROCYTES MODIFIED BY PURIFIED BACILLARY ANTIGEN

No. of sera	Haemagglutination	Indirect antiglobulin
85	+	+
12	—	—
1	+	—
2	—	—
Percentage reactive	86	97
Total reactive 98 per cent.		

Table 2. ABSORPTION OF NORMAL HUMAN ANTIBODY BY BACILLI (HAEMAGGLUTINATION AND INDIRECT ANTIGLOBULIN (COOMBS) TESTS)

Dilution	Human serum vs. modified cells		Human serum absorbed with bacilli vs. modified cells	
	5° C	37° C	5° C	37° C
1:1	4+	3+	4+	1+
1:2	4+	2+	4+	2+
1:4	2+	±	4+	—
1:8	—	—	4+	—
1:16	—	—	4+	—
1:32	—	—	4+	—
1:64	—	—	1+	—
1:128	—	—	—	—

Table 3. EFFECT OF HEATING (56° C) ON NORMAL HUMAN ANTIBODY (HAEMAGGLUTINATION TESTS)

Saline agglutinins	Serum dilutions					
	1/1	1/2	1/4	1/8	1/16	1/32
Unheated	4+	3+	2+	—	—	—
15 min at 56° C	4+	3+	1+	—	—	—
30 min at 56° C	4+	3+	2+	—	—	—
45 min at 56° C	3+	2+	—	—	—	—
60 min at 56° C	3+	1+	—	—	—	—
Incomplete antibodies (indirect antiglobulin)						
Unheated	4+	3+	2+	1+	—	—
15 min at 56° C	±	—	—	—	—	—
30 min at 56° C	—	—	—	—	—	—
45 min at 56° C	—	—	—	—	—	—
60 min at 56° C	—	—	—	—	—	—

The reactivity of normal human sera with bacillary carbohydrate antigen was associated with the gamma globulins when sera fractionated by certain electrophoresis were tested against modified erythrocytes. These antibodies were partially absorbed by bacillary cell suspensions (Table 2). Some of the saline-active antibodies were heat-labile at 56° C (Table 3) if heated for more than 30 min, and the reactivity of sera containing incomplete antibodies was removed even more readily. The instability of these antibodies was confirmed by demonstrating a loss of reactivity after treatment with 2-mercaptoethanol.

Although agglutinins for the purified carbohydrate antigen from bacilli were demonstrated in only 86 per cent of the sera, 98 per cent were reactive in either one or both of the saline and antiglobulin tests. The possibility that this difference was more quantitative than qualitative has not been completely ruled out, although the sensitivity of the testing procedure was at least as great as that of previous tests with crude antigen.

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## GENETICS

## Incompatibility Alleles of Cocoa

Knight and Rogers<sup>1</sup> found five incompatibility alleles in the *Theobroma cacao* clones Parinari 7 (alleles 1 and 5), Parinari 35 (3 and 5), and Nanay 32 (2 and 4). Allele 1 is dominant to the others; alleles 2 and 3 are independent but are dominant to allele 4, which in turn is dominant to allele 5. A tree is incompatible both as male and female with another which has the same dominant allele; a tree carrying two independent alleles is incompatible with trees carrying either in the absence of others dominant to them.

Cope<sup>2</sup> found that the reaction is associated with failure of male and female gametes carrying the same dominant (or independent) allele to fuse. An ovary containing "non-fusions" aborts, although normal fusion may have occurred in the majority of the ovules. The recessive alleles of Parinari 7 and Parinari 35 are distinct, but this does not alter Knight and Rogers's basic theory. Cope's results also indicate that the dominant allele of Scavina 12, which he termed 0, is independent both of Knight and Rogers's allele 1, and of the incompatibility allele I found in the Trinitario population.

Further work at Tafo<sup>3</sup> has extended the range of known alleles, and has shown that (a) an allele may be independent of two other alleles, one of which is dominant to the other, and (b) some incompatibility alleles are recessive to the allele for self-compatibility. An example of (a) is provided by an allele present in Iquitos 47 and Iquitos 60, which is independent of both allele 1 and allele 2 although allele 1 is dominant to allele 2. A further example is provided by the Trinitario allele I, which in addition to being independent of allele 0 is independent of alleles 2 and 3. An example of (b) is allele 4; thus a progeny obtained by crossing a tree known to carry alleles 1 and 4 to a self-compatible Amelonado tree consists of self-compatible and self-incompatible trees in equal proportions, and the self-incompatible trees carry allele 1. Similar evidence is provided by progenies obtained by crossing self-compatibles with trees carrying alleles 2 and 4 and alleles 3 and 4. Further, progenies obtained by crossing trees carrying allele 4 together with the recessive allele of Parinari 7 to self-compatible trees have proved to be fully self-compatible, which indicates that the recessive allele of Parinari 7 is also recessive to self-compatibility. All alleles dominant to self-compatibility are dominant to those recessive to self-compatibility, and where one allele is dominant to a second, all alleles dominant to the first are also dominant to the second.

The Trinitario incompatibility allele I usually seems absolute in its effects; thus Voelcker<sup>4</sup> reported only one set (persisting at least 14 days) from 245 incompatible pollinations in Trinidad, and I obtained only one apparent set, which did not persist to maturity, from 300 self-pollinations on the Ghana Trinitario clone E1. Some of the alleles found in Amazon cocoa, however, may not be completely effective. Low rates of setting were obtained after self-pollination of some introduced (supposedly incompatible) Nanay clones, and investigation of introduced progenies suggests that the recessive allele carried by Nanay 31 is particularly inefficient.

Many of the data on which this report is based were collected by R. Knight and H. H. Rogers, and I am indebted to them for advice.

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## PATHOLOGY

## Villous Atrophy and Coccidiosis

MEDICAL gastroenterology has advanced considerably since the introduction of a biopsy technique which permits histological examination of the intestinal mucosa<sup>1-4</sup>. The use of this procedure, and work on the determination of epithelial turnover times<sup>7,8</sup> in the intestine of mammals, has given impetus to the study of villous atrophy (maturation arrest<sup>5</sup>) in patients suffering from malabsorption syndromes.

In recent pilot experiments at Weybridge an attempt has been made to apply these findings to a study of the pathogenesis of infection with species of *Eimeria* in domestic animals. *Eimeria acervulina* and *E. crandallis* inhabit the small intestine of the fowl and sheep respectively and, apart from the first generation schizont of the latter species, all stages are confined to the epithelium<sup>9</sup>. My own observations (unpublished) have confirmed this.

Two-week-old coccidia-free chicks were infected with 80,000 sporulated oocysts of *E. acervulina*, and birds were killed by intra-cardiac barbiturate injection at 24-h intervals after infection; uninfected birds acted as controls. The intestine was injected *in situ* with Serra's fluid. Stained paraffin sections were examined under the light microscope and the tissues measured by a method similar to that used by Shiner *et al.*<sup>11</sup>. Similarly, 4-week-old cross-bred coccidia-free lambs were infected with 2,500 or 250,000 sporulated oocysts of *E. crandallis* daily for seven days. Following autopsy on the seventh day of the patent period, the tissues were examined in a manner similar to that described for the chicks.

Sections of intestinal mucosa showed that after the invasion of the epithelium the parasites "ride" in the host cells, so that "trains" of parasites can be observed proceeding up the villus. This was most marked by day 4, when maturing oocysts were seen at the tips of villi with later generations of schizonts travelling behind them, lower down on the villus.

Tissue measurements of the intestine of infected chicks showed that the ratio of villus height to total mucosal thickness gradually falls until by day 4 of the infection this parameter is most severely depressed (Fig. 1). This is the period in the infection when there is a maximum invasion of host cells by the parasitic stages.

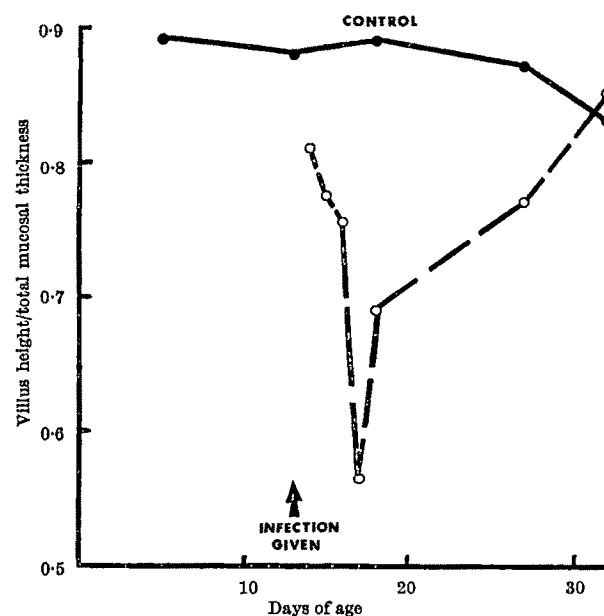


Fig. 1. Ratio of villus height to total mucosal thickness.

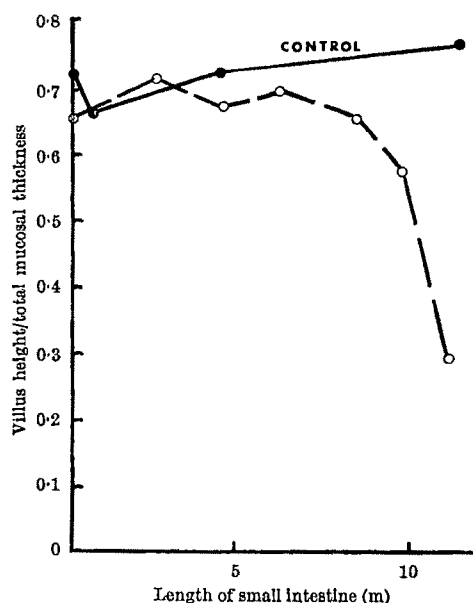


Fig. 2. Depression in the villus height/total mucosal thickness ratio in the lower ileum compared with the upper ileum and duodenum.

Infections with *E. crandallis* in lambs were confined to the lower ileum, and it was this part of the intestine which also showed villus atrophy. Fig. 2 shows the depression in the villus height/total mucosal thickness ratio in the lower ileum compared with the upper ileum and duodenum. No parasites were found when this ratio was similar to that of the uninfected control animal. Thus, seven consecutive daily doses had provided constant parasitic invasion of the epithelium for several days, and this had produced all the features of villus atrophy.

Experimental infections with *Eimeria* are commonly single dose studies, but in the field continuous acquisition of infective oocysts occurs. It is, therefore, suggested that multiple dosing will give a more accurate picture of the pathogenesis of small intestinal coccidiosis, and tissue reactions of the type mentioned here could be a useful parameter of infection. Nematodes can produce a similar effect<sup>6,12</sup> and, because most domestic animals at some time experience clinical and subclinical intestinal parasitism, veterinary interest might be profitably directed to this field of pathology.

It has been shown that the turnover time of intestinal epithelium becomes shorter as the host becomes smaller<sup>7</sup>. At the same time, studies with *Eimeria* have suggested that the pre-patent period of a species has the same relationship to the size of host<sup>8</sup>. It is interesting to speculate whether this represents a manifestation of host-parasite adaptation.

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## Milk Allergy in Infant Germ-free Rabbits

THE observations reported here were made in the course of attempts to establish a colony of germ-free rabbits for nutritional investigations. The infant rabbits were delivered by hysterectomy either into Gustafsson germ-free isolators or, as controls, into a conventional environment in which the physical conditions simulated as nearly as possible those inside the isolators. The diet was an aqueous dispersion of 12.5 g whole dried milk, 5 g calcium caseinate and 1 g vitamin supplement in glucose in 100 ml. It was sterilized by an ultra-high-temperature process, which resulted in little loss of its nutrient value. It was offered to the rabbits twice daily, in flasks fitted with rubber teats through which they readily learned to drink.

In the conventional environment 50 to 70 per cent of each litter usually survived to weaning. Losses mainly occurred during the first week and, from gross examination, were apparently due to enteritis, lung congestion and, occasionally, severe aspiration of the diet. By comparison, the germ-free rabbits seemed in better general condition but mortality was higher. Towards the end of the first week of life apparently healthy rabbits died suddenly. Death usually occurred several hours after feeding and was often preceded by convulsions. On post-mortem examination lung congestion was frequently found. The condition bears some resemblance to the so-called "cot death" in human infants<sup>1</sup>. Germ-free rabbits that survived to the twelfth day almost invariably began to exhibit signs of shock immediately after feeding. The signs were at first confined to trembling, stretching and panting, but worsened progressively to convulsions and Cheyne-Stokes respiration accompanied by cyanosis. Often the animals appeared to recover completely, but collapsed again after the next feed until ultimately a shock proved fatal.

The observed effects were suggestive of a histamine-induced reaction. For evidence on this point a histological investigation was undertaken to determine the condition of the mast cells. Specimens of gut mesentery, omentum and pleura were fixed as spreads in 80 per cent ethanol and stained with 1 per cent aqueous toluidine blue.

Tissue mast cells are not prominent in the rabbit, but there are abundant coarsely granular, strongly basophilic blood leucocytes which, it has been suggested<sup>2</sup>, are physiologically equivalent; they are commonly referred to as mast leucocytes or blood mast cells. Such cells were obvious in the blood vessels of tissue spreads from all the unshocked rabbits examined, both conventional and germ-free. When stained with toluidine blue, the granules were a dense blue-black although there was occasionally slight metachromasy.

In germ-free rabbits that had died of or been killed during a "feeding shock" spasm, and in these animals alone, the mast leucocytes appeared to be undergoing massive degranulation (Fig. 1), and it is assumed that this represents rapid release of histamine. Many of the dispersing granules, both within and outside the cell, exhibited metachromatic staining, while the cell body stained a featureless pale blue. Other cells were wholly pale blue, agranular, and without an obvious nucleus; it is believed that these were completely degranulated mast leucocytes.

A characteristic feature of rabbit omentum is the presence of small condensations of non-lymphatic tissue known as "milk spots", or "tâches laiteuses". Typical cells are irregularly oval, with a large oval nucleus, and they have been variously described as relatively undifferentiated mesenchymal cells and as fixed macrophages. The cell body is usually largely filled with strongly basophilic material, giving the cells a smudgy, mucoid appearance (Fig. 2a). Occasionally it may be seen that this material is in the form of densely crowded granules which may be stained with nuclear fast red made up in aluminium

sulphate<sup>3</sup>. In those animals where the blood mast cells were rapidly degranulating, the taches laiteuses were inconspicuous and their basophilic cells almost entirely degranulated (Fig. 2b).

The physical signs, degranulating mast leucocytes (and possibly also the changes in the taches laiteuses), in the shocked germ-free animals suggest that they were undergoing an allergic reaction to some component of the diet, and it seems likely that the bovine milk proteins, or products formed from them during processing, were responsible. It is possible that the lung congestion seen in some of the week-old conventional animals was also a manifestation of allergy but they seldom, if ever, exhibited any other signs of shock.

The markedly increased sensitivity of the germ-free animals is not readily explainable. It may perhaps be

associated with their comparatively slower response to antigenic stimuli<sup>4</sup>. It could be the result of a stronger antigenic challenge, since the thinner intestinal wall generally observed in germ-free animals might permit the passage of large molecules to a greater extent or for a longer time than is usual in conventional infant animals. These possibilities are being investigated.

We thank our colleagues, Dr. R. Fuller for sterility checks of the germ-free animals, A. Turvey for histological assistance and D. A. F. Miles for care and feeding of the rabbits.

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Fig. 1. Mast leucocytes in mesenteric venule of "shocked" germ-free rabbit. A, Normal appearance, laden with granules; B, degranulating; C, degranulated. Solid line represents 50 $\mu$ .



Fig. 2. Taches laiteuses in rabbit omentum. a, Normal appearance; heavily granule-laden cells; b, in "shocked" animal; cells partially or totally degranulated. Solid line represents 50 $\mu$ .

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### Lethal Action of Sugars on Ascites Tumour Cells *in vitro*

FARE<sup>1</sup> described a protective activity against the induction of liver tumours in rats fed with 4-dimethylaminoazobenzene by the addition to the diet of a fraction of ox liver prepared as described by Maisin and Lambert<sup>2</sup>. The protective activity of this water-soluble preparation, which was obtained by acetone extraction of liver pulp de-fatted with ether, was closely associated with its content of glucose, the only sugar detected. The reports of Nakahara and Fukuoka<sup>3</sup> concerning a bovine liver fraction lethal *in vitro* to Ehrlich carcinoma cells in ascites form, and of Tsuda *et al.*<sup>4</sup> concerning the cytostatic properties of various sugars and related compounds, were therefore of interest. Experiments with ox liver fraction and with a number of sugars lead us to conclude that the lethal action is largely attributable to the production of an acid pH resulting from breakdown of those sugars which are metabolized by the cells.

Preliminary tests using the ox liver fraction, D-glucose, D-mannose, L-rhamnose and D-ribose were carried out according to the directions of Tsuda *et al.* In the absence of definite specifications in the communication, the sugars were dissolved in phosphate buffered saline (without calcium or magnesium) pH 7.2 and the cells gently agitated during the incubation-exposure period of 1 h. We were surprised to find that our results differed materially from those of Tsuda *et al.* in that only those cells incubated with our ox liver fraction were killed; those incubated with the sugars produced tumours after the usual period when inoculated into mice. It appeared from a survey of the sugars and allied compounds cited by them that only those which are readily utilized by cells gave the positive, lethal activity and that the mechanism might be associated with the production of lactic or similar acid metabolites. Repetition of the tests with sugars in buffered (pH 7.2) saline always resulted in the cells remaining viable, but the addition of lactic acid or DL-glyceraldehyde to the medium in place of the sugar was lethal. On enquiry, Dr. Nakahara informed us that his tests were made in unbuffered 0.09 per cent sodium chloride. A further series of tests on the compounds previously examined and other related substances in buffered and unbuffered solutions was made, when, in most instances, the results from the latter were in line with those of Tsuda *et al.* (Table 1). The quantities of sugars used in all tests were 10 mg/ml. and the buffer had a pH 7.2 and was 0.1 molar. With D-galactose and dihydroxyacetone, neither of which we found to be lethal to the cells, we were unable to confirm the effect noted by Tsuda *et al.* It is possible that our strain of ascites cells is not able to utilize these compounds, or possibly essential



Table 1. EFFECT OF SUGARS AND ALLIED COMPOUNDS

	Effect on cells (Tsuda <i>et al.</i> ) Unbuffered	Effect on cells (Fare <i>et al.</i> ) Unbuffered	Effect on cells (Fare <i>et al.</i> ) Buffered
D-Glucose	+	+	— (4)
D-Galactose	+	— (2)	— (4)
D-Fructose	+	+	—
L-Arabinose	—	— (2)	—
L-Rhamnose	—	—	—
D-Ribose	—	—	— (3)
D-Arabinose	—	— (2)	—
DL-Glyceraldehyde	+	+	+
Dihydroxyacetone	+	— (2)	— (2)
Maltose	—	—	—
Sucrose	—	—	—
L-Sorbose	—	—	—
D-Sorbitol	—	—	—
D-Mannose	+	+	— (3)
Lactic acid	+	+	+
Pyruvic acid	+	+	+
Succinic acid	+	+	+
Butyric acid	+	+	+
Liver fraction	+	+	+

+ = Cells after incubation produced no tumours in five mice observed up to 15 days.

— = Cells produced tumours in five out of five mice inoculated.

. = Not tested.

Numbers in parentheses indicate tests carried out on different dates.

In every test series, control cells, washed and incubated in saline, gave tumours in all five mice inoculated.

co-enzyme factors were more thoroughly removed in our initial washing of the cells.

It should be observed (Table 2) that the pH of the final supernatant from the cells was well above pH 6 in both these instances. Accordingly, measurements of the pH of the supernatants were made after incubation of cells in presence of sugars in buffered and unbuffered saline and, as might be expected, exposure to pH below 6.0 appears to be deleterious to the cells. This would occur most readily in those tests using unbuffered media containing the metabolizable sugars. The presence of "free" lactic acid itself appears to be toxic. Its addition to the substrate to give an initial pH of 6.3 resulted in cell death (Table 3). In the final fluid the pH had risen to 6.7, presumably by extraction of buffering substances from the cells. Other organic acids, for example, pyruvic, succinic and butyric, were similarly lethal. Even when incubated in buffered saline, pH 7.2 throughout, cells to which lactic acid had been added did not produce tumours (Table 3).

Table 2. pH OF SUPERNATANT AFTER INCUBATION IN UNBUFFERED SALINE, COMPARED WITH VIABILITY OF CELLS

Sugar	Final pH	Effect on cells
D-Arabinose	7.1, 7.25*	—
L-Arabinose	7.3, 7.6*	—
D-Mannose	5.6, 5.4*	+
D-Fructose	5.6	+
Sucrose	7.25	—
D-Glucose	5.3, 5.5*	+
D-Galactose	6.8	—
Dihydroxyacetone	6.4	—
Protein extract in saline	5.4	+
Protein extract in buffered saline†	6.5	+

+ = No growth of cells inoculated into five mice.

— = Growth in five out of five mice inoculated.

\* = Tests carried out on different dates.

† = Initial pH of buffered saline = 7.2.

Table 3. LETHAL EFFECT OF "FREE" LACTIC ACID

Incubation medium	Initial pH	Final pH
Unbuffered saline + lactic acid 10 mg/ml.	6.2	7.2
Galactose + lactic acid*	6.3	6.7
Mannose + lactic acid*	6.3	6.7
Lactic acid added to buffered saline†	7.2	7.2

\* Lactic acid 10 mg/ml. in 0.09 per cent sodium chloride + 10 mg/ml. sugar.

† Lactic acid 10 mg/ml. in phosphate buffered saline, pH 7.2, 0.1 molar.

"Carcinostatic" action attributed to change in pH was also noted by Apffel<sup>6</sup> during incubation of Ehrlich ascites cells or *EL4* leukaemia cells in a medium containing a fraction derived either from liver or serum from which the protein had been removed. Loss of viability also resulted if the suspending fluid was adjusted to pH 5.5 with dilute hydrochloric acid.

We tried to demonstrate the material degradation of sugars such as glucose and mannose by analysis before and after incubation, using, for example, the "Folin-Wu" copper reagent, but this was unsuitable since comparable amounts of reducing metabolites were produced during the incubation which also reacted with the reagent. Using tetrazolium salt (2,3,5-triphenyl tetrazolium chloride, TTC) it was possible to demonstrate that such reducing products were present—an intense red formazan was produced in the cold by TTC with the supernatants only from the easily metabolizable sugars, thus correlating with the pH and viability tests (Table 4). It should be noted that no formazan reaction was observed after incubation with D-galactose.

Table 4. PRODUCTION OF REDUCING METABOLITES (TTC TEST)

Sugar	Reaction
D-Glucose	++
D-Mannose	++
D-Galactose	—
L-Arabinose	—
Saline blank	—

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## BIOLOGY

### Growth Rates in *Lolium temulentum* as influenced by Previous Regimes of Light Energy

THE relationships between the vegetative growth of single plants and the intensity of incident light have been extensively studied in various dicotyledonous species<sup>1,2</sup>, and less extensively in herbage grasses<sup>3,4</sup>. These and other investigations assessed plant growth in terms of relative growth rate (rate of increase in plant dry weight per unit of dry weight already present, RGR) and its components leaf area ratio (photosynthetic surface per unit dry weight, LAR) and net assimilation rate (rate of increase in dry weight per unit of photosynthetic surface, NAR). As in most other species, decreasing the amount of light incident on grass plants generally leads to decreased RGR for, although LAR increases in these conditions, this is more than offset by a fall in NAR.

The LAR built up at any given time depends on the previous expansion of leaf surface and rates of growth of stem and root in response to external environment, and therefore an instantaneous change in LAR, consequent on the transfer of the plant to a new light regime, is physically impossible. There is, however, a gradual adjustment of the photosynthetic surface and the distribution of weight in stem and root, and so of LAR. In contrast, NAR is in no way so dependent on a change in the physical conformation of the plant, and although the absolute rates of assimilation may be influenced by the

Table 1. EFFECTS OF CHANGE OF LIGHT INTENSITY ON DRY WEIGHT AND RGR IN *Lolium temulentum*

Light intensity Harvests	H/H/H	H/H/L	H/L/H	H/L/L	L/H/H	L/H/L	L/L/H	L/L/L	
Dry weight mg									
Preliminary									± 0.9
Spikelet initiation									± 2.3
Ear emergence									± 18.0
Seeds set									± 56.1
RGR g/g/day									
Prel.—spik. init. (11–13 days)									± 0.0064
Spik. init.—ear emerg. (15–17 days)									± 0.0045
Ear emerg.—seed set (23–45 days)									± 0.0019

previous history of the plant<sup>5</sup> the NAR usually becomes completely adjusted to a change in light intensity in a few hours. It follows that the relative rates at which these two components of RGR adjust to a new light regime could influence growth during the transition period and could temporarily result in unexpectedly high or low rates of growth until the plant is completely adapted to the new conditions. An example of these interactions has been found in recent experiments on the effect of light intensity on particular stages of inflorescence development in *Lolium temulentum*; unusually high and low growth rates were found after the transfer of plants between contrasting light regimes.

Three germinated seeds of *Lolium temulentum* L. were planted in each of a number of 3 in. pots of 'Perlite' immediately the coleoptile was visible, and the pots held in a controlled environment cabinet (temperature, 23° C day/18° C night; photoperiod, 20 h; light intensity, 3.0 cal/cm<sup>2</sup>/h). After 10 days, when the first leaf was almost expanded, the pots were distributed to one of two contrasting light intensities (6.6 and 1.6 cal/cm<sup>2</sup>/h) at the same temperatures and photoperiod as the pretreatment. Reciprocal transfers of some replicates between the two light intensities were made at spikelet initiation (onset of reproduction) and ear emergence. The time intervals between the beginning of treatment and transfer at spikelet initiation were: high light, 11 days; low light, 13 days. In all there were eight treatments—two light intensities for each of three successive stages of development designated H/H/H, H/H/L, H/L/H, H/L/L, L/H/H, L/H/L, L/L/H, and L/L/L. Plants were collected at the beginning and end of each period of development in order to determine dry weights and photosynthetic areas. The photosynthetic areas measured included one side of leaf blades and the exposed surfaces of sheaths and stems; no areas were measured after the emergence of the ears. For the purpose of calculating NAR, all photosynthetic surfaces were assumed to be equally efficient. All plants received a complete nutrient solution three times a week.

The dry weight increments per plant during the stages of development were not just a function of the incident light energy (Table 1): thus, at the emergence of the ears, plants given L/H were as heavy as, if not heavier than, equivalent plants given H/H; and plants given L/L were heavier than plants given H/L. Similarly, after further transfers at ear emergence, plants given H/L/H, L/H/H or L/L/H were heavier than plants in continuous high light (H/H/H); and plants given H/H/L, H/L/L and L/H/L were only about as heavy as those in continuous low light (L/L/L). The RGRs illustrate these differences in more detail (Table 1). The rate of gain of dry weight in a particular light intensity differed by as much as 50 per cent after the first transfer, and by as much as 100 per cent after the second transfer, depending on the previous light regimes. The efficiency of utilization of light energy per plant was thus very different; for example, plants weighing about 500 mg were produced after 28 days at 6.6 cal/cm<sup>2</sup>/h, or after 13 days at 1.6 cal/cm<sup>2</sup>/h plus 16 days at 6.6 cal/cm<sup>2</sup>/h.

The separation of RGR into its two components NAR and LAR was possible for stages until the emergence of the ears (Table 2). There appeared to be no large effect of the previous light regime on the NAR of plants transferred to contrasting light conditions, although the NAR of plants in high light, after low light (L/H), was significantly lower than that of plants in continuous high light (H/H). This difference is similar to that reported by Björkman and Holmgren<sup>5</sup> and is in the reverse direction to that needed to account for the differences in growth rate already noted. In contrast, LAR was very dependent on the previous light regime. No attempt was made to calculate the mean LAR over the periods of development, and the data given in Table 2 are the LARs calculated for the beginning and end of such periods. In addition to the ontogenetic drift common to both light intensities<sup>4,6</sup>, a much greater LAR was always developed in the low than in the high light conditions. Furthermore, on transfer to a contrasting light regime, plants started their growth with an LAR characteristic of the previous conditions. It appeared that the approximately fortnightly duration of the growth periods was about sufficient for complete adjustment of LAR to the current light conditions. Nevertheless, the initial disparity in LAR between that appropriate to current conditions and that appropriate to previous light conditions would result in unusually high or low RGRs, when considered in conjunction with an NAR characteristic only of current conditions. It is still not clear how rapidly LAR can become adjusted to new light conditions. The period of 15–17 days in this experiment is similar to the estimate given elsewhere for another perennial grass<sup>4</sup>. In this experiment, adjustment of LAR on transfer to contrasting light conditions involved changes in the percentage of dry weight invested in root and leaf, as well as changes in the leaf area to leaf weight ratio. Clearly, some time must elapse for these changes to be completed.

It appears that unusually high or low rates of growth can be achieved, at least for short periods, by suitable alternation between high and low light intensities, and that this follows from the different rates at which NAR and LAR become adjusted to new conditions. If more were known of the magnitude and duration of these interactions between past and present environmental effects on LAR, it might be possible to achieve higher efficiencies of utilization of light by exposing plants to intermittent high and low light intensities, rather than to continuous uniform

Table 2. EFFECTS OF CHANGE OF LIGHT INTENSITY ON NAR AND LAR IN *Lolium temulentum*

Harvests	Light intensity	H/H	H/L	L/H	L/L	
NAR g/dm <sup>2</sup> /day						
Prel.—spik. init.		H 0.109		L 0.088		± 0.0046
Spik. init.—ear emerg.		0.120	0.034	0.106	0.040	± 0.0084
LAR cm <sup>2</sup> /g						
Preliminary						± 7.4
Spikelet initiation		H 156		L 394		± 8.0
Ear emergence		90	283	102	250	± 10.4

light. Such knowledge is also needed in the analysis of growth in the natural environment.

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## Control of Leaf Senescence by Growth Retardants

THE degradation of RNA, protein and chlorophyll is closely associated with the development of senescence in plant leaves<sup>1,2</sup>. Senescence is delayed by treatment with various growth regulators<sup>1-6</sup> which apparently stimulate RNA turnover and protein synthesis<sup>2,4-9</sup>.

In a recent account we reported that (2-chloroethyl) trimethyl-ammonium chloride (CCC) markedly stimulated the *in vitro* synthesis of polyribonucleotides<sup>8</sup>. At the same time, the loss of protein and RNA in plants subjected to drought and previously treated with CCC and phyto-kinin was retarded and the plants survived longer under these conditions<sup>9</sup>. These findings tended to indicate that CCC, like phytohormones<sup>1-7</sup>, may interact with proteins and nucleic acids in leaves and thus possibly retard senescence.

To test this aspect, bean plants (*Phaseolus vulgaris* var. 'Brittle Wax') were grown at 25° C in a growth room. Leaf disks 0.6 cm in diameter were punched from inter-veinal areas of primary leaves (6-10 days after germination) and placed immediately on Whatman No. 1 filter paper in Petri dishes of 9 cm in diameter.

The filter papers were moistened with 4 ml. of the respective solutions and the closed Petri dishes were kept in the dark at 25° C. Each day after treatment samples of four replicates were collected, each replicate being composed of five disks.

Chlorophyll was determined by Arnon's procedure<sup>10</sup>. The decolourized tissue was homogenized with 5 per cent trichloroacetic acid. The proteins of the acid-insoluble fraction were assayed according to Lowry *et al.*<sup>11</sup>.

In case of incorporation experiments<sup>12</sup>, individual leaf disks were shaken at 30° C in a 0.5 ml. solution containing 1 µc./ml. of <sup>14</sup>C-leucine (specific activity 155 mc./ml.). The disks were then washed thoroughly and homogenized with water. All homogenizing and washing solutions contained 0.1 normal <sup>14</sup>C-leucine. A portion of the homogenate was used to estimate the total uptake of labelled leucine. The remaining homogenate was treated with trichloroacetic acid (5 per cent final concentration) and after standing in the cold for 1 h it was filtered through 'Millipore' filters of 0.65µ pore size. After successive washings with large volumes of trichloroacetic acid and perchloric acid (1 per cent) the filters were dried and counted in a three-channel liquid spectrometer. Incorporation of carbon-14 into the acid-insoluble fraction continued linearly for about 90 min.

The changes of protein and chlorophyll in senescing leaf disks are recorded in Figs. 1-4. The levels of protein and chlorophyll dropped markedly during the experimental period. The growth retardants CCC and B-995 (*N,N*-dimethylaminosuccinamic acid) preserved protein and chlorophyll as did kinetin, even giving rise to a slight initial increase. The disks on water started to disintegrate after 5-6 days. During the same period the disks treated with the growth retardants remained relatively green and turgid.

The changes in protein during senescence were followed by parallel incorporation assays (Table 1). The incorporation with time of tagged leucine into acid-insoluble

Table 1. INCORPORATION OF <sup>14</sup>C-LEUCINE INTO THE ACID-INSOLUBLE FRACTION OF 6-DAY-OLD BEAN LEAF DISKS SENESEING ON CCC (IN C.P.M./DISK)

Day	Water	Bean leaf disks from:	CCC
0		21,000	
1	11,700		11,500
2	10,400		10,000
4	7,400		7,800

Individual leaf disks, after senescing on water and CCC from 0 to 4 days, respectively, were incubated for various intervals of time in solutions containing 0.5 ml. of <sup>14</sup>C-leucine (1 µc./ml.; specific activity 155 mc./mmolar). In the case of the disks senescing on CCC the incubation solution contained the same concentration of this material. Incubation was carried out, under vigorous shaking, at 25° C. In the table, values for a 3-h incubation period are recorded. After incubation the disks were prepared for counting as described in the text.

products in the disks treated with CCC and the disks senescing on water decreased at parallel rates.

It seems from the incorporation experiments that, since CCC does not enhance the incorporation of <sup>14</sup>C-leucine into proteins, it should somehow retard the rate of protein breakdown in order to account for the higher protein level in the leaf disks senescing on CCC.

The stimulation by CCC and kinetin of protein accumulation was further analysed by including actinomycin D in the experimental solutions on which the disks were senescing. Actinomycin D was expected to prevent the increase of protein synthesis—it did so during the first day (Fig. 1). On the other hand, in contrast to expectation<sup>7</sup>, actinomycin D accentuated markedly the delaying effects of CCC and kinetin on senescence during the following 3 days. These effects of actinomycin D will be discussed in a separate account.

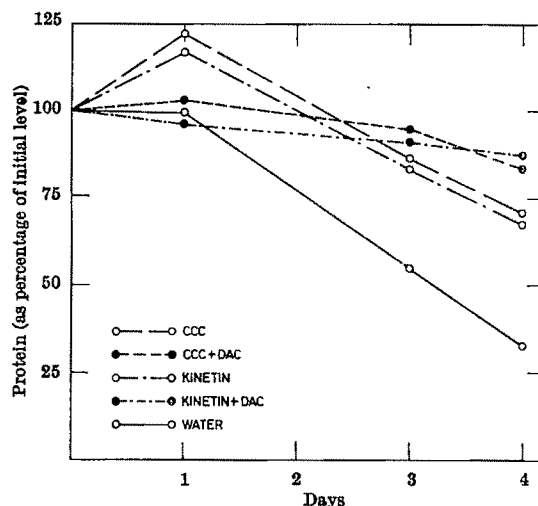


Fig. 1. Retardation of protein breakdown in 6-day-old bean leaf disks by CCC, kinetin and actinomycin D. Concentrations were 100, 20 and 40 mg/ml., respectively. Actinomycin D was included in the Petri dishes in which the leaf disks were senescing.

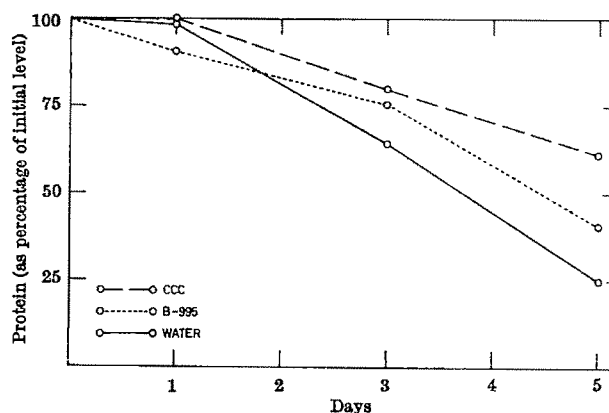


Fig. 2. Retardation of protein degradation by CCC (50 mg/ml.) and B-995 (500 mg/ml.) in 10-day-old bean leaf disks.

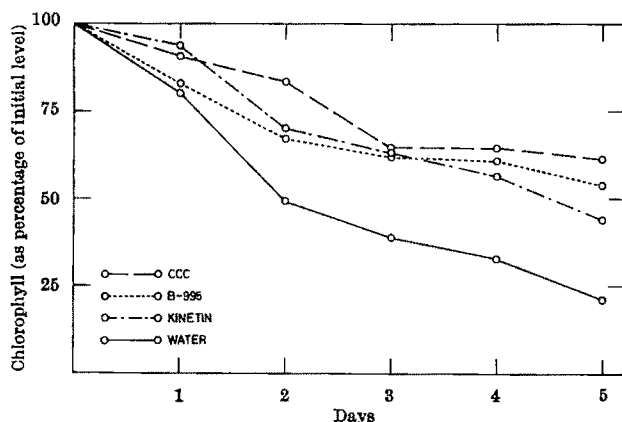


Fig. 3. Preservation of chlorophyll by CCC (50 mg/ml), B-995 (500 mg/ml.) and kinetin (20 mg/ml.) in 6-day-old bean leaf disks.

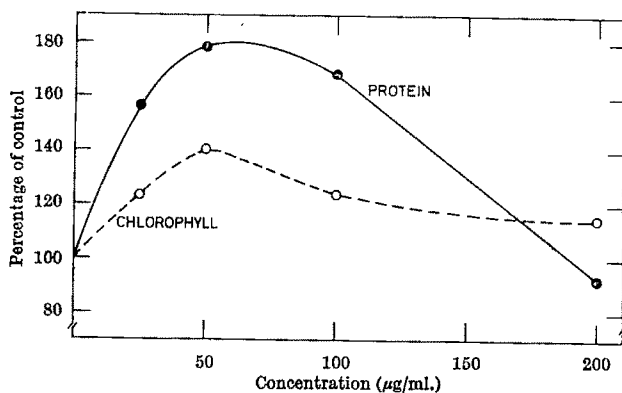


Fig. 4. Protein and chlorophyll levels in bean leaf disks following treatment with different concentrations of CCC. 3 days after start of the experiment.

The losses of RNA and protein are considered to represent key factors in the senescence of leaves<sup>2,4,5</sup>. The decline in the levels of chlorophyll and proteins is retarded in different plants by phytochemicals<sup>1-5</sup>, auxins<sup>6</sup> or gibberellins<sup>7</sup>, respectively. These findings led to suggestions that senescence is closely linked with a hormonal regulation and maintenance of protein synthesis. The present finding that growth retardants bring about senescence responses similar to those brought about by phytochemicals may suggest that, as in other aspects, the maintenance of a proper balance between the chief groups of endogenous hormones is a prerequisite for the retardation of leaf senescence.

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## Autoradiography of <sup>32</sup>P in Maize Roots

THE concentration of ions in the vascular system of a plant root may be very much higher than that in a surrounding nutrient solution. The phosphorus concentration in the transpiration stream of shaded barley seedlings has been estimated to be about  $5 \times 10^{-4}$  molar whereas that of the nutrient solution around their roots was  $3 \times 10^{-6}$  molar<sup>1</sup>. Neither the mechanism of concentration nor its location is known. It is obvious, however, that an accurate knowledge of the distribution of ions within the root is essential to an understanding of the mechanisms of accumulation and translocation. Autoradiography of frozen tissue sections has been successful in the location of low molecular weight substances in cells and tissues<sup>2,3</sup>. This communication describes the use of this technique to locate phosphorus-32 in transverse sections of maize roots.

The method used was that described previously<sup>2</sup>, but it was modified to allow the results to be analysed with an orthodox microdensitometer. This method of analysis requires a denser photographic image than the more usual method of counting grains used to evaluate autoradiographs. High concentrations of radioactivity were therefore used. It was also essential that only the photographic image should appear in the final preparation. The tissue section would interfere with densitometry. The method of film development was modified to ensure that no fragment of plant tissue remained attached to the film after development. The absence of any histological detail superimposed on the autoradiograph made it necessary to stain and mount alternate sections for comparison with the autoradiographs.

Maize seeds, *Zea mays* (L), variety 'White Horse Tooth', were germinated on moist filter paper and grown for 6 days in an aerated solution of  $10^{-5}$  molar calcium sulphate at 25° C. The seedlings were transferred to a  $3 \times 10^{-6}$  molar solution of potassium phosphate which was labelled with 1 mc./l. of phosphorus-32 (carrier free orthophosphate). After 15 min the seedlings were removed, rinsed, blotted dry and the apical 0.75 cm was embedded in a 1 cm cube of fresh swine's liver. The block was frozen on a microtome object holder by immersing the metal base in isopentane cooled with liquid nitrogen. This method froze the block and root in about 20 sec. Transverse sections, 10 µ thick, were cut from the region 5 mm from the root tip, with a cryostat microtome at -20° C.

Cover slips were prepared for coating with Kodak AR.10 stripping film by soaking in a 1 per cent solution of R.B.S. 25 detergent, immersion for 1 h in a solution of 1 per cent gelatine, 0.05 per cent chrome alum ( $\text{Cr}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ ), and drying in a stream of cool air. This careful preparation was essential if the film was to remain attached to the coverslip in the extreme conditions which are required during development to ensure the complete removal of the tissue section. The coverslips were coated with film, and the sections were transferred from the microtome knife to the emulsion, using the methods and conditions already described<sup>2</sup>. The coverslips bearing sections were placed 3 mm apart in plastic racks and left in light proof boxes at -20° C for exposure of the film. After a week boxes were removed from the deep freeze and the contents were allowed to reach room temperature before processing. The films were developed in Kodak D19b at 18° C, fixed in Johnson's 'Fixsol' at 22° C, and washed in distilled water at room temperature (17° C-24° C). These variations in temperature and the absence of any cytological fixation usually left no trace of any plant material on the film. The section following each one that was used to prepare an autoradiograph was picked from the microtome knife with a slide coated with albumin, fixed in 95 per cent alcohol, 5 per cent acetic acid, stained in Ehrlich's haematoxylin, and mounted ready for comparison with the autoradiographs.

The variation in optical density over the autoradiograph was measured using a microdensitometer, which was adjusted to measure continuously the optical density of a square, of side  $10\mu$ , as the specimen was moved in a straight line past the aperture. The instrument produced a plot of optical density against displacement along any selected  $10\mu$  wide strip across the autoradiograph. For the optical density of the photographic image to be proportional to the radioactivity of the specimen it was essential that the probability of "coincidence" is low ("coincidence" is the production of one observable silver grain by two or more disintegrations). In this investigation the optical density of fully exposed emulsion was compared with the maximum optical density observed in the autoradiograph; the results were discarded unless the former was at least three times as great as the latter<sup>4</sup>.

Fig. 1 shows a transverse section, cut 5 mm from the tip of a 6 day old maize root. The preservation of cell contents is excellent, but crushing and shattering of the material are a problem when plant organs are being cut. Section quality depends on careful control of temperature and this is facilitated by the provision of a means of direct measurement of temperatures of knife and tissue. These temperatures may be different from the temperature of the air in the cabinet after the microtome has been in use for 1 or 2 h. Fig. 2 shows an autoradiograph prepared from the section cut immediately before that shown in Fig. 1. Fig. 3 shows the change in optical density along a diameter of the autoradiograph.

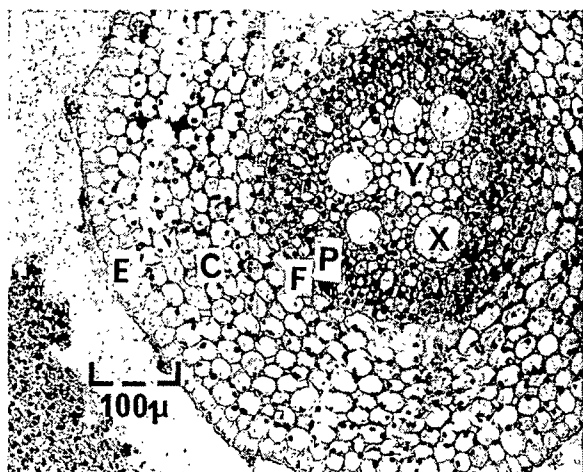


Fig. 1. Transverse section of a young maize root. The section was  $10\mu$  thick and was cut 5 mm from the root tip. E, Epidermis; F, endodermis; P, phloem; X, xylem vessels; Y, xylem parenchyma; C, cortical cells.

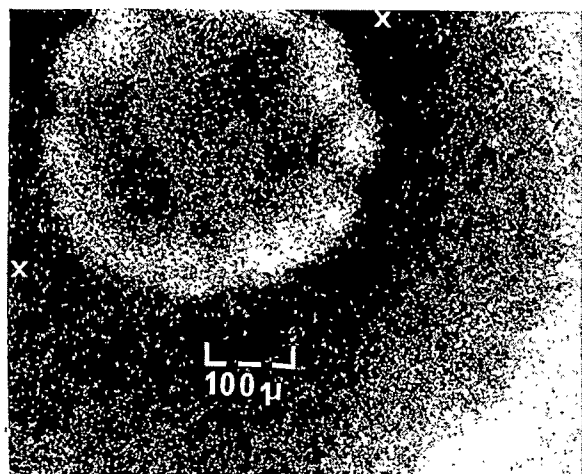


Fig. 2. Autoradiograph of a section similar to that shown in Fig. 1.

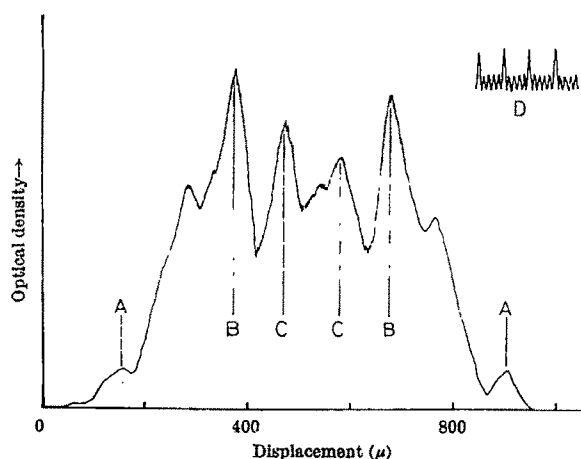


Fig. 3. Microdensitometer trace showing the variation in optical density along the diameter  $\times-\times$  of the autoradiograph shown in Fig. 2. A, Epidermis; B, endodermis; C, xylem vessels; inset D, variation in optical density along a graticule which had etchings  $10\mu$  apart.

The high energy (1.7 MeV) of the electron emitted during decay of phosphorus-32 leads to considerable image spread, due to "crossfire"; that is when electrons which originate from one structure dissipate their energy in a part of the emulsion corresponding to another. Estimates of crossfire corrections applicable to phosphorus-32 autoradiographs<sup>4</sup> indicate that it is impossible to equate image density and concentration of phosphorus-32 in structures as small as the vessel elements in maize roots. The results of densitometry must therefore be regarded as qualitative. Nevertheless three observations are valid. (a) Phosphorus-32 is more concentrated in the vessel elements than it is in the surrounding phloem and parenchyma. (b) There is a gradient of concentration of phosphorus-32 across the root cortex, towards the endodermis and there is a point of inflexion in this midway between epidermis and endodermis. The detection of this illustrates the greater sensitivity of microdensitometry when compared with visual analysis of preparations or photographs. (c) There is an area of accumulation of phosphorus-32 which corresponds to the surface of the root, bacteria on that surface, or the epidermal cells.

The interpretation of single preparations must be cautious. It is unwise to regard optical density gradients in autoradiographs as evidence of gradients of activity in pathways of phosphorus movement without further information on the chemical form of the phosphorus-32 within the root, the turnover rates of the various species, and the volumes of the compartments through which it is moving, or in which it is accumulating.

I thank the Salters' Institute of Industrial Chemistry for the award of a fellowship.

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### Peculiar Placenta in African Cucurbit, *Ruthalicia*

*Ruthalicia* (*Phyzedra* Hook. f.) has been established<sup>1</sup> out of two African species *Phyzedra longipes* Hook. f. and *P. eglandulosa* (Hook. f.) Hutch. and Dalz., and is considered a taxonomic synonym of *Coccinia* Wight and



Arn. which again is a taxonomic synonym of *Staphylosyce barteri* Hook. f. transferred to *Coccinia* by Keay<sup>2</sup>.

The species *Ruthalicia eglandulosa* (Hook. f.) Jeffrey is widely distributed all over Ghana. It is an extensive dioecious climber which has creamy yellow flowers with digital five-lobed leaves. The fruits when ripe have an attractive crimson colour with yellow patches. The ovules are arranged horizontally over the inner wall of the triplacentiferous unilocular ovary and are completely wrapped over in beaded fashion by a yellow succulent placental membrane. A second finely hyaline tissue covers the black and somewhat bluntly deltoid seeds.

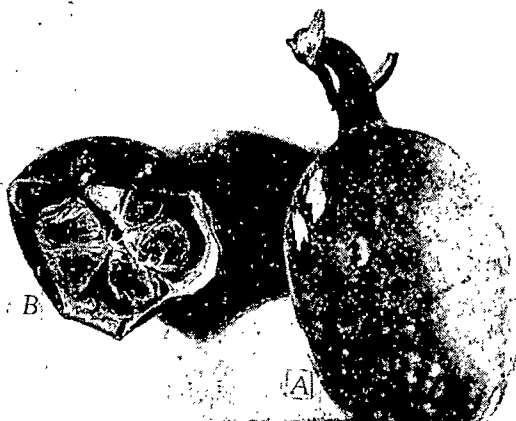


Fig. 1. A, Fruit of *Ruthalicia eglandulosa* (x c. 0.65); B, cross-section of the same (x c. 0.65).

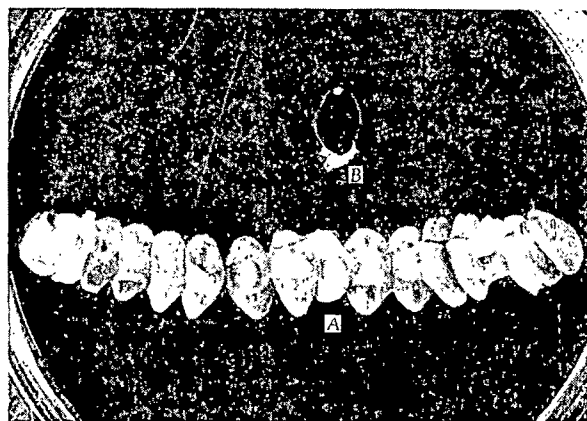


Fig. 2. A, Chain of seeds wrapped over by the placenta (x c. 0.9); B, a single seed, placenta removed (x c. 0.9).

The seeds are packed up serially in six rows bilaterally and attached to a parietal placental rod or axis and wrapped over by the cloth of placental membrane. The vertical placental axis is formed by the fusion of two contiguous vascular strands of the carpel margins. When slightly pressed, the placenta detaches itself from the vertical placental axis in the form of a perfect chain enclosing the seeds like a beaded necklace (Fig. 2A) and it spreads automatically when placed on a Petri dish filled with water. The succulent yellow placenta is partially dissolved into mucilaginous consistency when the fruit ripens and the contents are invariably eaten in the wild state by birds or other animals. Seldom is an intact ripe fruit found in nature. The empty shells of the fruits are often found hanging on the vine with pulp completely eaten away. When unripe the shell is hard and is green in colour with a few white stripes radially disposed at the

apical part. On keeping, the green area turns brilliantly crimson and the white stripes yellow within two weeks and the hard shell softens considerably. If not disturbed the fruit keeps well for over a month or more under room temperature. Morphology of this peculiar placenta is being investigated.

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### Polymorphism in the Spider, *Theridion ovatum*

REFERENCE has often been made<sup>1-3</sup> to the colour varieties of *Theridion ovatum*. According to Locket and Millidge<sup>4</sup>, the abdomen may have one of three colour patterns: Clerk's *lineatum*, abdomen creamy white; Clerk's *redimitum*, abdomen with a pair of wide longitudinal carmine stripes; Clerk's *ovatum*, abdomen dorsally covered with carmine. Field observations confirm that these varieties are polymorphic, in accordance with the accepted definition<sup>5</sup> for genetic polymorphism.

Counts of the three varieties in the field indicate that populations are panmictic, and a computation of the type used to determine the frequency of MN blood groups<sup>6</sup> suggests that the colour varieties, *lineatum* and *ovatum*, are under the control of a single pair of alleles. The heterozygote, which shows incomplete dominance with respect to abdominal colour, is *redimitum*.

The following results are derived from counts by Nielsen<sup>2</sup> and Bristowe<sup>3</sup>. Nielsen made counts in two separate habitats, while Bristowe's figures represent the sum total of many counts. The frequencies of the three forms of spider are found to vary between populations, a point which is made by Bristowe. Separate and total counts provide independent material with which to test whether the observed data differ significantly from what is expected with the Hardy-Weinberg law.

Table 1. COMPARISON OF OBSERVED AND EXPECTED FREQUENCIES OF VARIETIES OF *Theridion ovatum*

Variety	No.	Percentages		
		Observed	Expected	$\chi^2$
<i>Lineatum</i>	55	55.0	51.8	1.98
<i>Redimitum</i>	34	34.0	40.4	1.01
<i>Ovatum</i>	10	10.0	7.8	0.62
Total 3.61, $P > 0.10$				
<i>Lineatum</i>	19	33.9	37.9	0.42
<i>Redimitum</i>	31	55.4	47.3	1.39
<i>Ovatum</i>	6	10.7	14.7	1.09
Total 2.90, $P > 0.20$				
<i>Lineatum</i>	2,714	64.24	63.7	0.004
<i>Redimitum</i>	1,316	31.15	32.2	0.034
<i>Ovatum</i>	195	4.16	4.0	0.001
Total 0.039, $P > 0.98$				

I thank D. W. Mackie (secretary of the British Spider Study Group) and G. Corris for their assistance in this work.

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### Presence of *Haemoproteus* sp. in House Sparrows in England

ALTHOUGH there have been many records of *Haemoproteus* in the English sparrow (*Passer domesticus*) from America and Europe<sup>1-3</sup> there appears to be no published record of the parasite occurring in Britain.

Recent examinations of blood films taken from the peripheral blood of nineteen sparrows which had become trapped in experimental animal buildings at Weybridge showed two of the birds to be infected with *Haemoproteus*. One bird had a pure infection of *Haemoproteus*, the other a mixed infection of *Haemoproteus* and *Plasmodium relictum*. *P. relictum* was present in less than 1 per cent of the erythrocytes. Because of morphological differences between the two *Haemoproteus* parasites, those from the sparrow with the double infection were designated Type A, and those from the pure infection Type B.

In the blood parasitized by Type A, the parasitaemia was 0.69 per cent, calculated from 10,000 erythrocytes, most of the parasites being mature gametocytes similar in shape to those usually described (Fig. 1). With only a few exceptions there was only one parasite in each parasitized cell. Of the gametocytes 67 per cent were macrogametocytes and 33 per cent were microgametocytes. The microgametocytes in Type A showed a hyaline cytoplasm which stained pale blue or pinkish, with a dispersed nucleus containing fine chromatin granules. By comparison the cytoplasm of the macrogametocytes stained a much deeper blue. The nucleus was compact and contained a karyosome. The light brown pigment granules of the microgametocyte were generally aggregated into masses towards the poles, whereas those of the macrogametocyte were uniformly scattered throughout the parasite. Counts of the numbers of pigment granules showed that the

average number for microgametocytes was 9.14 (minimum 6, maximum 12), and for macrogametocytes 12 (minimum 9, maximum 15).

In the B type infection the parasitaemia was 10.06 per cent. Many cells contained two or three young gametocytes. There was a greater morphological difference between the mature gametocytes than in Type A. In particular the microgametocytes were larger and broader with their poles flat rather than rounded and some with finger-like edges. There was also slight displacement of the host cell nucleus in many of the parasitized cells. This was not observed with Type A.

Immature gametocytes were more predominant. Differential counts of mature gametocytes showed that 41 per cent were macrogametocytes and 59 per cent microgametocytes.

Pigment granules were yellow in colour, and counts showed the average number for microgametocytes to be 10.6 (minimum 6, maximum 17) and for macrogametocytes 13.12 (minimum 9, maximum 19). Although the range between minimum and maximum numbers is greater in Type B than in Type A, it is unlikely that this difference is significant.

The absence of tissue stages from these infections, and the lack of sufficient visual descriptions from existing records, makes it difficult to enable an accurate comparison to be made with specifically recorded species of the parasite.

It is not possible to be sure whether the infections in the two birds are of different species of *Haemoproteus* or whether they merely represent different morphological phases. Different species of the parasite may possibly infect the same species of bird<sup>4</sup>.

It is hoped that this record of *Haemoproteus* will stimulate further examinations of *Passer domesticus* throughout the British Isles, and that the vector will eventually be identified.

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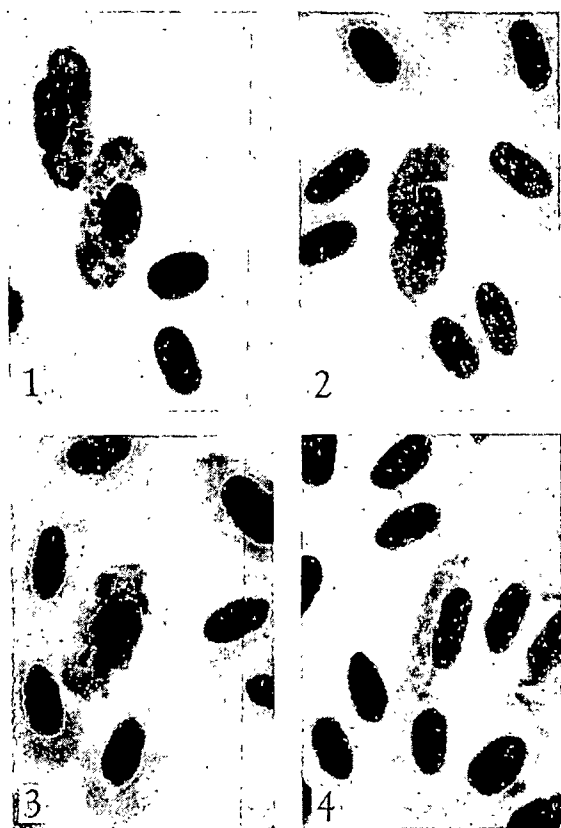


Fig. 1. Macrogametocyte Type A.

Fig. 2. Macrogametocyte Type B.

Figs. 3 and 4. Microgametocytes Type B. (Giemsa,  $\times 1,550$ .)

### Breeding Frequency in the Albatrosses *Diomedea melanophris* and *D. chrysostoma*

It is now generally accepted that the two great albatrosses *D. exulans* and *D. epomophora* take about twelve months to rear their young, and if successful breed only in alternate years<sup>1-4</sup>, but the breeding cycles of the smaller species are not so prolonged and there has been less reason to suppose that annual breeding is not the rule. Recent studies at Bird Island, South Georgia (54° 00' S., 38° 02' W.) (ref. 5), however, indicate that although the black-browed albatross *D. melanophris* breeds annually, the grey-headed albatross *D. chrysostoma* breeds less frequently.

The two species are closely related, and both belong to that group of albatrosses known as mollymauks. There is no significant difference in size or body weight between adults, and although the head plumage and bill markings are strikingly different, the similarity of their habits and behaviour on the breeding grounds has led to the observation that remarks on the nesting of one species apply equally to the other<sup>1</sup>. In fact, although they are not easy to demonstrate, differences are apparent in breeding biology, oceanic distribution<sup>6</sup> and feeding<sup>7</sup>.

Both species breed in large dense colonies on steep slopes of rock and tussock grass *Poa flabellata* at the tops of sea cliffs. Generally they are segregated, but colonies of one species sometimes merge into those of the other, and it is not uncommon to find nests of *D. melanophris* in the midst of *D. chrysostoma* colonies, although the reverse does not seem to occur<sup>8</sup>. The breeding cycle of *D. chrysostoma* is about a month longer than that of *D. melanophris*. *D. chrysostoma* first return to the island on about September 15, about ten days before *D. melanophris*, and the peak departure of *D. chrysostoma* fledglings, May 15, is about three weeks later than for *D. melanophris*. Most intact pairs of *D. melanophris* use the same nest each season; we have records of 38 pairs using the same nests for two consecutive seasons and of five pairs for three seasons.

Between January and March 1963, 97 *D. melanophris* adults were banded at 50 nests where they were feeding nestlings. The following breeding season 38 (76 per cent) of these marked pairs are known to have laid eggs in the same nests. At the other twelve nests, one of the original pair was seen in nine, and in three neither of the birds was observed. None of the missing birds bred elsewhere in the colony. In any representative sample, mortality alone will remove a proportion of the breeding population each year and thereby interrupt breeding at some nests. In *D. melanophris* the adult mortality calculated from the return of 138 marked birds over two years was 6.7 per cent per annum. On this reckoning about seven of the original 50 pairs probably failed to produce eggs in the second season owing to the death of one bird, the probability of one bird of a pair dying being greater than that of both birds dying in the same year. We have no record of the remaining five pairs (10 per cent) which failed to breed in the second season.

In the spring of 1963-64 winter conditions persisted later than usual. Because of ice and snow, both species had difficulty building nests and a large number of eggs were lost. Birds that lose eggs soon after laying frequently do not stay in the colony, and since observations generally lasted only 2-3 h each day it is reasonable to suppose that some marked birds were not seen. In these circumstances we consider a 10 per cent discrepancy to be acceptable, and the data sufficient evidence of annual breeding in *D. melanophris*.

Between March 22 and April 3, 1963, one hundred *D. chrysostoma* at 88 nests were marked while feeding well grown fledglings, most of whom departed in early May. The following season, in spite of a very close watch, only 19 of these birds were observed in the colony; none of them laid eggs and all had departed by the end of the egg laying period. Bad weather conditions prevented field workers visiting Bird Island in 1964-65, and confirmation of breeding in the third season is therefore not available. There is evidence, however, from five other nests that pairs which lose eggs or very young chicks do in fact return and breed again the next year.

The possibility of intermittent breeding in the Fulmar *Fulmarus glacialis* was pointed out by Wynne-Edwards in 1939<sup>9</sup>. A more recent study of the Laysan albatross *D. immutabilis* by Rice and Kenyon<sup>10</sup> reported 63-87 per cent of birds breeding again after one year, and led to speculation about the possibility of "... a decreasing tendency to nest the following year with an increase in the length of the portion of the breeding cycle that is successfully completed. . .". The 10 per cent of *D. melanophris* pairs unaccounted for at Bird Island could indicate similar behaviour, but since we cannot be absolutely sure that some birds which lost eggs were not missed, we are bound to adopt the simplest hypothesis consistent with the error, that is, annual breeding. Similarly, it is clear from our evidence that *D. chrysostoma* does not breed annually; and from what we know of the reproductive cycles of the great albatrosses biennial breeding might be inferred, were it not for the marked

difference between the lengths of breeding cycle in the great albatrosses and *D. chrysostoma*. Wandering albatrosses, which are still feeding advanced fledglings when other birds are arriving in full nuptial condition, would have no post-nuptial season unless one breeding season were missed. Circumstances are quite different for *D. chrysostoma*; at least four months intervene between the departure of fledglings and the arrival of the next season's breeding adults, but for some reason this is not sufficient to enable *D. chrysostoma* to breed each year. In contrast, *D. melanophris* breeds annually although absent from the breeding grounds for only five months each winter.

Among the five species of mollymauk, Buller's albatross *D. bulleri* and the yellow-nosed albatross *D. chlororhynchos* closely resemble *D. chrysostoma*. There are no data available to indicate whether there is any similarity in breeding frequency, but Rowan<sup>11</sup> makes a significant observation in her paper on *D. chlororhynchos*: "... Three marked nests at Sandy Point, Tristan da Cunha, which were all in use during the summer of 1949-50 were left untenanted the following spring although the numbers of nesting birds in the locality had definitely increased . . .", a hint at least that breeding may not have occurred each year.

The discussions of Lack<sup>12</sup>, Wynne-Edwards<sup>13</sup> and Ashmole<sup>14</sup> have all drawn attention to relevance of breeding frequencies to population control in Procellariiformes, and while it is impossible at the moment to suggest mechanisms behind different frequencies in *D. melanophris* and *D. chrysostoma* it is evident that they reflect profound differences in the niches that these species occupy in the oceanic environment. This work was undertaken while the authors were in receipt of grants from the U.S. National Science Foundation.

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### Effect of Two Diuretic Drugs on Liquid Consumption and Free Choice of Alcohol in Albino Rats

EARLIER experiments in our laboratories<sup>1</sup> with animals on a free choice between water and an ethyl alcohol solution have indicated that the amount of alcohol consumed relative to the total fluid intake, the so-called alcohol preference, is influenced by the water requirement of the animal and by its ability to metabolize alcohol. This

Table 1. ALCOHOL AND WATER CONSUMPTION BEFORE AND DURING TREATMENT WITH DIURETIC AGENTS

		Alcohol preference	Total liquids (ml.)/100 g body weight/day	Amount of alcohol consumed calculated as absolute alcohol/100 g body weight/day	Food (g)/100 g body weight/day
Group 1	Normal free choice period	51.1 ± 18.4	8.50 ± 2.8	0.44 ± 0.22	4.52 ± 1.5
	Statistical significance	0.01 < P < 0.02	0.001 < P < 0.005	P < 0.5	P < 0.5
	'Dichlotride' period	36.3 ± 12.7	11.73 ± 2.0	0.43 ± 0.18	4.49 ± 1.5
Group 2	Normal free choice period	64.8 ± 19.1	8.89 ± 1.5	0.57 ± 0.23	4.89 ± 0.4
	Statistical significance	0.001 < P < 0.005	P < 0.001	P < 0.5	P < 0.5
	'Hygroton' period	38.9 ± 14.5	12.79 ± 2.6	0.51 ± 0.21	4.83 ± 0.9

means that the absolute amount of alcohol per unit of body weight remains unchanged when the concentration of the alcohol solution is changed. On the other hand, an increasing water requirement may change the calculated alcohol preference, even if the absolute amount of alcohol remains unchanged.

In this experiment changes in liquid consumption were induced by two different diuretic drugs and their effect on the free choice of alcohol was investigated. Twenty white Wistar male rats were used in the experiment. They were 4 months old at the start and weighed  $298.6 \pm 35.5$  g. The rats were divided into two equal groups. They were placed in individual cages and freely offered tap water, 10 per cent (v/v) alcohol solution and our standard laboratory food. This normal free choice period lasted 14 days. Then the first group received in its food 30 mg/kg of hydrochlorothiazide 'Dichlotride', which is the 3,4-dihydro derivative of chlorothiazide (6-chloro-7-sulphamyl-1,2,4-benzothiadiazine-1,1-dioxide). The second group was given 40 mg/kg of 'Hygroton', 1-oxo-3-(3-sulphamyl-4-chlorophenyl)-3-hydroxy-isindolinum. The choice of liquid consumed during the drug period was recorded for 10 days.

Table 1 shows that liquid consumption during the drug period in the animals on 'Dichlotride' was 38 per cent, and in those on 'Hygroton' 44 per cent, above normal. In both groups the increase was statistically significant. As the absolute amount of alcohol per unit of body weight remained unchanged, the relative amount of alcohol decreased. This change is shown by a statistically significant decrease in the percentage of preference. There were no individual exceptions to this rule. Even those rats in which the preference percentage was originally low and absolute alcohol consumption minimal showed a decrease in percentage intake and did not change the amount of alcohol consumed. No adverse side-effects of the drugs were recorded. Food consumption remained unchanged.

From the results of this experiment it seems evident that an alcohol solution cannot replace water in a free choice situation when the alcohol concentration is high. Each individual has its fixed level of alcohol intake, which it does not surpass. The reaction to different concentrations is partly determined by this level. The observed increase in alcohol preference with increasing age<sup>2,3</sup> is probably caused by the same thing, at least in young rats. The total fluid intake decreases with age, and so the proportion of alcohol increases although the absolute alcohol consumption remains unchanged. In free choice experiments on alcohol, the absolute consumption seems to be more significant for the interpretation of the results than the relative alcohol preference.

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## APPLIED SCIENCE

### Strain Rate Effects on Low Endurance Fatigue

FATIGUE investigations in which the strain is high and the endurance low have been prominent in the past decade. Usually these have utilized testing machines that have previously been used for conventional fatigue investigations, that is for lives greater than 100,000 cycles. In consequence series of high strain tests have been performed with a constant frequency of cycling.

Using the notation where  $\Delta\epsilon$  is the total strain range,  $\Delta\epsilon_e$  the elastic strain range,  $\Delta\epsilon_p$  the plastic strain range,  $\Delta\theta_p$  the plastic torsional twist range,  $\dot{\epsilon}$  the straining rate,  $N_f$  the number of cycles to failure at a given strain range,  $n$  the number of cycles completed at a point in the fatigue life at a given strain range,  $t$  the time per cycle, and  $f$  the frequency of cycling, it follows that

$$t = 2\Delta\epsilon/\dot{\epsilon} \quad (1)$$

and

$$\Delta\epsilon = \Delta\epsilon_e + \Delta\epsilon_p \quad (2)$$

From equation (1) it can be seen that in the strain range for fatigue in which the endurance is low and which involves an appreciable amount of plastic deformation, a constant frequency of testing will necessarily cause a wide variation in straining rates because

$$2\Delta\epsilon.f = \dot{\epsilon} \quad (3)$$

For example, with data from fatigue tests in which the strain is high and the endurance low, I find that the ratio of the maximum to the minimum straining rate would have been 10:1 if tests had been performed at a constant frequency. It seems necessary therefore that, in the range of low endurance, fatigue tests should be carried out at constant straining rates if a better basic understanding of the phenomenon is to be achieved. Frequency, after all, is only the number of cycles performed within an arbitrary time interval and should not be thought of as an important parameter.

The argument for using constant straining rate machines can be furthered by considering the Miner-Palmgren linear cumulative damage law

$$\Sigma n/N_f = 1 \quad (4)$$

With reference to fatigue data that are usually presented in the now well established Coffin-Manson relationship form

$$\Delta\epsilon_p.N_f^a = \text{constant} \quad (5)$$

Fig. 1 shows results of torsional fatigue investigations in which the endurance is low carried out at constant straining rates on a 2.5 nickel-chromium-molybdenum steel ( $E_n$  25). The upper curve applies to the maximum straining rate investigated while the lower curve was deduced by extrapolation from a number of tests at various straining rates. From these results it appears that there exists a minimum endurance curve corresponding to a zero straining rate and a maximum endurance curve corresponding to a critical level of straining rate beyond which there is no strain rate effect on the endurance characteristics. Increasing the straining rate between

these limits causes a significant increase in the fatigue endurance at high strain values. The result is not unexpected when it is compared with recent results obtained by increasing the frequency of cycling<sup>1</sup>; however, the importance of such results can best be seen by investigating Fig. 2 and the Miner-Palmgren law.

Let  $a-a$  and  $b-b$  represent endurance curves for a material strained at low and high rates, respectively. Thus a curve similar to  $c-c$  will represent endurances for a constant frequency of cycling. If a two step investigation into cumulative damage is studied between levels 1 and 2 and if the low level strain range is applied initially to account for 50 per cent life, then this will correspond to distance  $2-4a'$ . When the second step and higher level of strain are applied, the 50 per cent proportion of life already used is equal to the distance  $1-3a$  at the same straining rate. If the second half of the test is carried out at the same frequency as the first half, however, it can be assumed that 50 per cent damage is equated to point  $3b$ , not  $3a$ , and an apparently extended life results. The reverse applies if the higher level strain is applied initially and the summation  $\Sigma n/N_f$  is less than 1 as a result of the non-coincident points  $4a'$  and  $4b'$ .

This argument can also be used to invalidate the hypothesis that endurance to high strain fatigue is determined by a critical level of accumulated plastic strain energy. Recent work<sup>2</sup> on this subject tends to disprove this hypothesis, but if the results are referred to constant straining rates then the curves of a plot of the logarithm of the total plastic strain energy to failure against the logarithm of the number of cycles to failure would have slopes greater than those recorded and so be further removed from a constant critical level of accumulated plastic strain energy.

In the discussion of Mackenzie and Benham's paper<sup>2</sup> cumulative damage summation terms are presented for

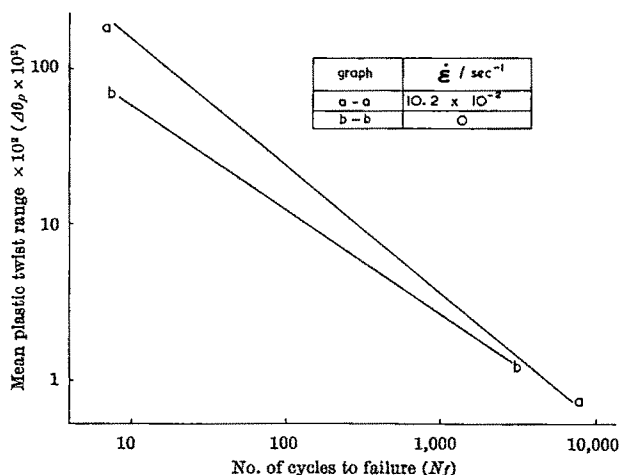


Fig. 1. Comparison of  $\log \Delta\theta_p - \log N_f$  relationships for two straining rates.

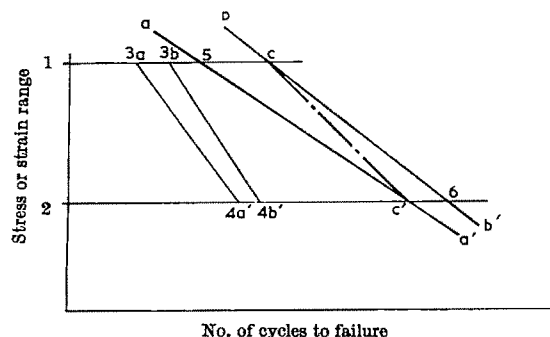


Fig. 2. Effect of strain rate on cumulative damage.

low endurance fatigue, and while the two levels of total plastic diametral strain ranges are in a ratio no greater than 1:2.25, high to low level 2 step test summation terms are always less than 1 and from low to high levels the summation is always greater than one. This is typical of the deviations from the Miner-Palmgren law recorded by numerous investigators who have used constant frequency of cycling machines.

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### Reciprocating-jet Pump

A PUMP designed to operate with corrosive liquids at extremely high temperatures will usually be constructed from refractory oxides and hence, if possible, must not rely on solid moving parts such as valves, pistons and rotors. A simple method of achieving this is to make use of some non-reciprocal hydrodynamic effect.

A conventional jet pump is valveless and uses a fluid jet separately pumped into a suitably shaped main pipe, which carries the fluid to be pumped. The main body of fluid is propelled along this pipe by a momentum transfer and mixing process. The injected fluid must, however, be suitable for pumping by conventional means, be compatible with the pumped fluid, and be acceptable as a diluent. At high temperatures these conditions will usually eliminate all fluid combinations apart from a gas or vapour to pump a liquid, and vice versa. Unfortunately there is then a large mismatch in density which causes considerable energy loss during momentum mixing.

Another possibility is to extract from the main pipe an appropriate amount of the fluid to be pumped, in readiness for re-injection. During this extraction or "suck" stroke the fluid mass removed at a sink or jet orifice contains very little momentum before entering the orifice. During the injection or "blow" stroke, however, the jet orifice acts as a source of momentum directed as desired. This is then coupled to the surrounding fluid in the main pipe, with an unavoidable but not excessive energy loss. Over a complete "suck-blow" cycle a net forward thrust is created, which may be used to generate a pressure rise or a velocity increase in the main body of fluid, depending on the shape chosen for the main pipe.

The non-reciprocal action of an alternating jet depends on the fact that the flow pattern for a steady suck stroke is fundamentally different from that for a steady blow stroke. This may be seen in the streak photograph (Fig. 1), which was taken in a two dimensional water tank using polystyrene beads as markers. The left-hand jet is sucking and the right-hand blowing. During the suck stroke irrotational potential flow occurs with a convergent "scalar" flow pattern in which  $\text{curl } \mathbf{v} = 0$ , where  $\mathbf{v}$  is the fluid velocity. At the start of the blow stroke a divergent flow pattern occurs momentarily, but because of viscous forces in the boundary layers the liquid cannot sustain the large velocity gradients around the edges of the jet orifice, and a rotational motion is started. The transient potential flow pattern therefore breaks down as inertial forces take over control. Ring vortices are generated around the periphery of the jet orifice and are shed downstream, so that in the steady state there is a gradual erosion of the jet associated with a widening of its influence as ejected fluid enters a turbulent mixing region. A steady thrust is the ultimate result. The flow pattern of the blow stroke might be regarded as a "vector" pattern in which  $\text{curl } \mathbf{v} \neq 0$ .

The problem of pumping a steady stream of fluid has now been transformed to that of cycling fluid through a



jet orifice. In a severe environment the latter problem is much more simple to solve, for example, by use of a vertically reciprocating gas-liquid interface stabilized by gravity. This forms a piston in a chamber coupled to the jet orifice by means of a suitable diffuser or nozzle. In the case of a pump for liquids, the gas pressure above the liquid surface may be cyclically varied by means of a reciprocating mechanical piston or by a valve-controlled supply of compressed gas, so as to blow and suck alternately through the jet orifice. Two alternative methods of varying the gas pressure are based on the two-stroke internal-combustion engine and the steam engine. In the latter method, it might be advantageous to use the vapour of the liquid being pumped. A more speculative technique, which is particularly suited to the pumping of some very hot liquids, would be to inject water on to or below the surface of the hot liquid within the piston chamber. This water would be converted immediately to steam, forcing liquid out through the jet. The piston chamber could then be opened to a condenser for the suck stroke. The pumping power would be derived at the expense of a small drop in liquid temperature.



Fig. 1. Reciprocating-jet pump.

Experiments have been carried out on the pumping of water in various configurations of pump, using up to four jets. Particular attention is now being given to "twin-jet" forms of the pump in which each stage comprises two jets in anti-phase directed at a venturi orifice. The streak photograph (Fig. 1) shows that there is little interference between the two jets. An experimental pump, with angled jets attached to the main pipe like the branches of a tree, has been constructed from drilled refractory bricks and will be tested with molten salts at 1,500° K.

A simple theory, based on separate consideration of steady state blowing and steady state sucking, has been found to give reasonable agreement with the results. The pump head ( $h_p$ ) in m or ft. of the liquid pumped is given for equal periods of blow and suck by:

$$h_p = [n\alpha\bar{v}_j^2/2g][1 - 2(v_p/\bar{v}_j) - \alpha/2]$$

where  $n$  is the total number of jets;  $\alpha$  is the ratio of the area of each jet to that of the pipe;  $\bar{v}_j$  is the mean jet velocity;  $\bar{v}_j^2$  is the mean square velocity;  $v_p$  is the pipe velocity just before mixing, and  $g$  is the acceleration due to gravity. It is assumed that when more than one jet is involved, the jet velocities are suitably phased to give zero net addition of fluid at any given moment.

At high frequencies, or short wavelengths, as defined by the ratio of pipe velocity to frequency, the steady state treatment is likely to be less accurate. In order to allow for transients during switch-over, a more sophisticated approach is required which involves considering the ring vortex streets.

The main drawback to the reciprocating-jet pump is that the pumping efficiency is unlikely to exceed 38 per cent at best, and a more realistic figure would probably be half this. Nevertheless there are many applications where efficiency is less important than some other consideration such as the absence of solid moving parts. The reciprocating-jet pump should be considered for application to the pumping of the following materials: molten metals, slags, glasses and salts, where high temperatures and corrosion are a problem; blood, where contamination must be avoided; solid suspensions, where blockage and erosion can occur, and liquid gases such as helium at low temperatures.

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## GENERAL

### Entropy and Information in the Universe

POPPER<sup>1</sup> denies the cosmic significance of the principle of increase of entropy, stating that "the entropy in almost all known regions (of sufficient size) of our universe either remains constant or decreases, although energy is dissipated (by escaping from the system in question)". This statement seems in error when we assume the "cosmological principle" that the distribution of energy and matter in the universe on a large scale is uniform in space. Consider a volume of space so great that it contains several thousand galaxies. Through the limits of this system there is—on the large scale—no flow of energy or entropy. In the interior of the system the total entropy at least does not decrease; it increases (among other things) to the extent that the radiation emitted by the stars is transformed (by interaction with matter, for example) into black-body radiation uniformly distributed throughout the volume.

Popper appears to assume that the total entropy of an energy conserving expanding universe should decrease because of the cooling caused by the expansion. This assumption contradicts the well established calculations of R. C. Tolman, made on the basis of general relativity<sup>2</sup>. Tolman shows that the total entropy of such a universe either remains constant or (in general) increases.

Popper criticizes the idea that a living organism would be "fed on negentropy by its environment", for "during the incubation of birds' eggs, entropy rather than negentropy is supplied to them, though they are in a period of increasing organization; and . . . when an organism dies of cold . . . its entropy certainly decreases". This criticism seems to be based on a misunderstanding of what is meant by "feeding on negentropy". To clarify things, let us consider a watchmaker putting the separate parts of a watch together to make a functioning mechanism. By

doing so the watchmaker "puts information into the watch", that is, the information of how the single parts are to be combined. The information contained in the watch can be measured in bits, as has been shown by Brillouin and others<sup>3</sup>. Whenever a mechanism containing an information of  $n$  bits is built, the thermodynamic entropy of that mechanism or its environment must increase by the amount of at least  $kn \log 2$  where  $k$  is Boltzmann's constant. The quantity  $kn \log 2$  is, in fact, the information measured not in bits but in caloric units (cal/grad). So we may consider the information to be "structural negentropy" contained in the mechanism, and state the principle: whenever structural negentropy is produced, the thermodynamic entropy must increase by at least the same amount. This principle is proved in the case of structural negentropy produced by man, but very probably it also holds for structural negentropy produced by nature in an organism. The increase of thermodynamic entropy may be found in the evolving organism itself; so the birds' eggs quoted by Popper do not constitute a rebuttal. The death of an organism means a loss of its structural negentropy, but not necessarily an increase of its thermodynamic entropy.

*Note added in proof.* When the space as a whole is considered as expanding, the part of space of which we spoke in the discussion of the entropy question must, of course, be considered as correspondingly expanding, too (see Tolman's book).

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<sup>2</sup> Tolman, R. C., *Relativity, Thermodynamics and Cosmology*, Part III (Oxford, 1934).

<sup>3</sup> Brillouin, L., *Science and Information-Theory* (Academic Press, New York, 1956); for some philosophical aspects see: Büchel, W., *Philosophische Probleme der Physik*, chap. 2 (Herder, Freiburg i.B., 1965).

### Time's Arrow and Feeding on Negentropy

My article, "Time's Arrow and Entropy"<sup>1</sup>, has produced so far only one voice of dissent, Professor Büchel's letter<sup>2</sup>. This letter consists of three paragraphs, to which I shall reply in order.

(1) I cannot find any argument in Professor Büchel's first paragraph which is even an attempt to come to grips with my arguments. Professor Büchel writes: "Consider a volume of space so great that it contains several thousand galaxies. Through the limits of this system there is—on the large scale—no flow of energy or entropy". This simply repeats the current view, which I criticized, without making any attempt to counter my arguments; for I quoted arguments to show that there always is a flow of energy in the form of radiation, and, if we assume an expanding Universe, also of matter, from any "well-defined geometrical volume" which is sufficiently large.

(2) In his second paragraph, Professor Büchel speculates on what I "seem to assume"; however, I did not assume that "the total entropy of an energy conserving expanding universe should decrease because of the cooling caused by the expansion". Rather, I tried to explain the fact that "[in almost all sufficiently large systems [that is, spatial regions] known to us, entropy production seems to be equalled, or even exceeded, by entropy loss through heat radiation", supporting this by "the conjecture that every entropy producing region is open towards some large (perhaps infinite) sinks of energy"<sup>3</sup>.

I stated explicitly that these considerations would not apply if we assume "a finite and non-expanding universe with non-zero energy density"; or, to put it more precisely, my arguments would not apply, according to Einstein<sup>4</sup>, to a closed universe which expands, if at all, to a temporary maximum, and then contracts; that is,

to a universe with positive spatial curvature, or to an Einstein universe the average mass density  $\rho$  of which exceeds  $3H^2/\kappa$ , where  $\kappa$  is Einstein's gravitational constant and  $H$  is Hubble's expansion constant. (Although Einstein sometimes showed support for such a universe, he was, of course, perfectly clear that this view involved very great difficulties<sup>4</sup>; moreover, he regarded the conjecture  $\rho > 3H^2/\kappa$  as untestable, and the opposite conjecture as testable or refutable<sup>5</sup>, which might make it preferable to some.) I did, therefore, admit that there might be relativistic universes with increasing entropy; but I tried to direct attention to the fact that we have reason to believe that there are others also, since every spatial region known to us, however large, loses heat, and thereby entropy.

(3) In the last paragraph of Professor Büchel's letter he refers to the last paragraph of my article, in which I criticized Schrödinger's suggestion<sup>6</sup> that organisms characteristically "feed on negentropy". Obviously, all heat engines "feed on negentropy" in some sense, because their intake contains less entropy than their total output; in fact, they are producers of entropy. Thus, there does not seem to be anything here which is characteristic of organisms. On the contrary, it seems that at least some organisms are extremely efficient and produce less entropy than most heat engines; they "feed entropy" to a lesser extent than non-organisms.

Professor Büchel claims that my criticism is based on a misunderstanding of the passage criticized, and that the passage should be interpreted in the sense of information theory. First, Schrödinger's book was published four years before Shannon's paper and even longer before "Information Theory" in the sense of "Brillouin and others", to whom Professor Büchel appeals. Second, all that Professor Büchel says about information theory and "structural entropy" is now well known (though neither "proved" nor necessarily accepted by all critics of the theory). And third, Professor Büchel again fails to counter my arguments. These are in two parts: (1) that in saying that organisms feed on negentropy, Schrödinger fails to say anything that distinguishes organisms from heat engines; and (2) that if, to use Professor Büchel's words, "the increase of thermodynamic entropy may be found in the evolutionary organism itself" (which simply means that the organism is an entropy producer), it is particularly interesting and strange that while birds' eggs appear to produce structural negentropy by increasing their structural organization, they are, as far as we know, "fed", in Schrödinger's sense, exclusively on heat (that is, on entropy). Professor Büchel does not attempt to explain why this does "not constitute a counter example" to Schrödinger's views.

Yet it would seem to force us, if we accept the usual views on entropy, to assume that all the negentropy needed by the developing bird was contained in the egg and spermatozoon, in the form of information. Though this may be so, it obviously speaks against Schrödinger's remark on the need of organisms to feed on negentropy.

I feel I should add in conclusion that I always was, and still am, a great admirer of Schrödinger; but I know that he would have abhorred the idea that anybody should be prevented by admiration or friendship from criticizing him.

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<sup>2</sup> Büchel, W., preceding communication.

<sup>3</sup> Einstein, A., *The Meaning of Relativity*, 116 (Methuen, London, 1946 and 1956).

<sup>4</sup> Einstein, A., *Ibid.*, 126.

<sup>5</sup> Einstein, A., *Ibid.*, 124.

<sup>6</sup> Schrödinger, E., *What is Life?* 72 (Cambridge University Press, 1944).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, January 23

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Mr. M. G. Hall and Mr. D. Weaver: "The Evaluation of Ordinary Shares Using a Computer".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Bryan Robertson, O.B.E.: "The Arts" (second of four Cantor Lectures on "Some Aspects of the U.S.A. To-day").

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 6.15 p.m.—Dr. M. C. Joseph and Dr. G. A. Miller: "The Special Problems of Paediatric Cardiology".\*

INSTITUT FRANCAIS DE ROYAUME-UNI (at Queensberry Place, London, S.W.7), at 8.15 p.m.—Programme of French Scientific Films.

## Monday, January 23—Thursday, January 26

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2)—Conference on "Acoustic Noise and Its Control".

## Tuesday, January 24

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. J. W. Lloyd, Mr. D. S. H. Drennan and Mr. B. M. U. Bennell: "A Groundwater Recharge Study in North Eastern Jordan".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. I. C. Whitfield: "Coding in the Auditory Nervous System".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. G. Porter, F.R.S.: "Electrons in Molecules" (lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on January 25, 31 and February 1).

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. J. R. A. Mitchell: "Platelets, Thrombosis and Arterial Disease". (Fourth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31-34 Gordon Square, London, W.C.1), at 5.45 p.m.—Mr. Richard E. Linton: "Recent Archaeological Research by the Lerici Foundation".\*

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (Joint meeting with the Plastics and Polymer Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. N. Uri: "New Aspects of the Autoxidation of Polymers and Polymer Model Substances".

INSTITUTION OF THE RUBBER INDUSTRY (at the Eccleston Hotel, Victoria, London, S.W.1), at 7 p.m.—Mr. P. Whitaker: "Comparison of Press and Injection Moulding".

## Wednesday, January 25

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. K. J. R. Wilkinson: "Prospect of Employing Conductors at Low Temperatures in Power Cables and in Power Transformers".

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—227th Scientific Meeting. Dr. Reuben Lasker (Bureau of Commercial Fisheries, La Jolla): "The Biology of an Euphausiid Shrimp".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Dr. J. Pendered: "Fragility".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. J. F. Hearne: "Changes in Quality in Processed Foods During Long Storage".

## Thursday, January 26

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.20 p.m.—Dr. J. W. Mullin: "Crystallization—Art, Science and Technology".\*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. T. L. Altbuler and Mr. J. W. Christian: "The Mechanical Properties of Pure Iron, Tested in Compression over the Temperature Range 2 to 293°K". Mr. J. Harding: "The Yield and Fracture Behaviour of High-Purity Iron Single Crystals at High Rates of Strain".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Weir Lecture Hall 10 Upper Belgrave Street, London, S.W.1), at 5 p.m.—Dr. R. L. Townsin: "The Frictional and Pressure Resistance of Two Lucy Ashton Geosims". Mr. A. Emerson: "The Calculation of Ship Resistance; Application of Guillotons' Method".

UNIVERSITY OF LONDON (at Guy's Hospital Medical School, London Bridge, London, S.E.1), at 5 p.m.—Prof. R. Amprino (Bari): "Histophysiology of Bone".\*

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W.C.2), at 5 p.m.—Prof. Maurice Freedman: "Rites and Duties".\*

ASSOCIATION FOR SCIENCE EDUCATION (at the Institute of Education, Malet Street, London, W.C.1), at 5.30 p.m.—Mr. F. C. Brown and Mr. R. C. Champeney: "Electrons".

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. W. J. Newby: "Operations Research".

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Shopping Centres—Traffic and Economic Aspects" introduced by Mr. H. D. Peake.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.1), at 5.30 p.m.—Mr. D. Gignoux and Mr. J. Lazar: "Ion Propulsion".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. G. B. Ansell: "The Metabolism of Brain Phospholipids". (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (at the Institute of Laryngology and Otolaryngology, Royal National Throat, Nose and Ear Hospital, Gray's Inn Road, London, W.C.1), at 5.30 p.m.—Prof. G. J. Cunningham: "On Cancer".\*

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (Joint meeting with the Polarographic Society, in the Chemistry Department, Imperial College, London, S.W.7), at 6.30 p.m.—Dr. A. B. Hart: "Fuel Cells".

## Thursday, January 26—Friday, January 27

CHALLENGER SOCIETY and REPRESENTATIVES FROM THE MARINE LABORATORIES (Natural Environment Research Council Scheme) (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10 a.m. daily—Papers on Climatology and Geology; Fisheries; Biology; Instrumentation and Physical Oceanography.

## Friday, January 27

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Prof. G. Porter, F.R.S.: Introductory Lecture.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 6.30 p.m.—Discussion meeting on "Use of Electric Optic Effect for Light Modulation" opened by Mr. J. M. Ley.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. S. G. Hooker, C.B.E., F.R.S.: "The World of the Jet Engine Designer".

## Saturday, January 28

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. Andrew Strathern: "A People of the New Guinea Highlands and their Mythology of Wealth".\*

## Monday, January 30

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "Ion Implantation" opened by Dr. J. Dearneley and Mr. L. Large.

UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 5.30 p.m.—Prof. S. J. Pitt: "The Cultivation of Microbes and Cells" (Inaugural Lecture).\*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Gordon Sutherland, F.R.S.: "Science" (third of four Cantor Lectures on "Some Aspects of the U.S.A. Today").

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN MATHEMATICS—The Secretary of the Council, Girton College, Cambridge (February 1).

RESEARCH ASSISTANT to collaborate with other Imperial College physicists in a group working at CERN with a large magnet spark chamber, and to be resident principally in Geneva—Prof. C. C. Butler, F.R.S., Physics Department, Imperial College of Science and Technology, London, S.W.7 (February 1).

LABORATORY SUPERINTENDENT (with technical and administrative ability) IN THE SCHOOL OF BIOLOGICAL SCIENCES to be responsible for the organization and general technical services in the School—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C (February 4).

CHAIR OF APPLIED MATHEMATICS at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 6).

LECTURER or ASSISTANT LECTURER IN THE HISTORY AND PHILOSOPHY OF SCIENCE—The Registrar, The University, Leeds, 2 (February 6).

CHAIR OF TELECOMMUNICATION SYSTEMS WITHIN THE DEPARTMENT OF ELECTRICAL ENGINEERING SCIENCE—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (February 7).

LECTURER IN STATISTICS—The Registrar, University of Kent at Canterbury, Canterbury, Kent, quoting Ref. A/32 (February 8).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Liverpool, quoting Ref. 312 (February 9).

LECTURER or ASSISTANT LECTURER IN MORAL PHILOSOPHY—The Secretary of the University Court, The University, Glasgow (February 10).

LECTURERS or ASSISTANT LECTURERS IN STATISTICS—The Acting Registrar, University College of Wales, Aberystwyth (February 10).

ASSISTANT LECTURER or LECTURER (with a medical qualification and preferably a dental qualification) in ORAL PATHOLOGY—The Registrar, The University, Sheffield (February 11).

LECTURER (preferably with a special interest in some aspect of electronics, for example microwaves or electromagnetic theory, together with some relevant research experience) in the DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING—The Registrar, The University, Sheffield (February 11).

SENIOR LECTURER and a LECTURER IN METALLURGY—The Registrar, The University, Sheffield (February 11).

ASSISTANT LECTURER or LECTURER (with special interests in the field of climatology and meteorology) IN GEOGRAPHY—The Acting Registrar, University College of Wales, Aberystwyth (February 13).

LECTURER (with a good honours degree in science or technology, and preferably research experience in building or civil engineering materials research) IN BUILDING SCIENCE—The Registrar, The University, Sheffield (February 13).

LECTURER or ASSISTANT LECTURER in (a) PHYSICAL GEOGRAPHY and in (b) ECONOMIC GEOGRAPHY at the University of the West Indies, Jamaica—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 16).

LECTURER or ASSISTANT LECTURER (with an honours degree in pharmacology, physiology or pharmacy, and for the lecturer a higher degree or research experience is desirable) IN PHARMACOLOGY—The Clerk to the Council, School of Pharmacy, University of London, 29/39 Brunswick Square, London, W.C.1 (February 17).

SECOND CHAIR IN THE DEPARTMENT OF ENGINEERING—The Registrar, The University, Leicester (February 18).

SECOND CHAIR IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, The University, Newcastle upon Tyne 1 (February 18).

HAYWARD RESEARCH FELLOW (medically qualified candidate) in the DEPARTMENT OF EXPERIMENTAL OPHTHALMOLOGY to undertake research on the early diagnosis of glaucoma—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1 (February 20).

SENIOR LECTURERS or LECTURERS in MATHEMATICS at the University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 21).

PROFESSOR OF AGRICULTURAL EXTENSION at the University of the West Indies, Trinidad—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 22).

UNIVERSITY LECTURER (prepared to carry out research, supervise research students and co-operate in the teaching of one or more of the following fields: sedimentology, stratigraphy, geomorphology) IN GEOLOGY—The Secretary of Faculties, University Registry, Oxford (February 23).

ASSOCIATE PROFESSOR, SENIOR LECTURER or LECTURER GRADE I in the Division of Microbiology, Department of Pathology and Microbiology, University of Lagos—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 24).

ASSISTANT LECTURER IN PLANT PATHOLOGY—The Acting Registrar, University College of Wales, Aberystwyth (February 25).

CHAIR OF ANTHROPOLOGY and SOCIOLOGY at Monash University, Melbourne—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or the Academic Registrar, Monash University, Clayton, Victoria, Australia (Australia and London, March 1).

LECTURER IN GENERAL PHYSIOLOGY in the School of Biology—The Registrar, The University, Leicester (March 1).

LECTURER IN PHILOSOPHY at Fourah Bay College, The University College of Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 1).

PROFESSOR OF ZOOLOGY and CHAIRMAN OF THE DEPARTMENT OF ZOOLOGY AND COMPARATIVE PHYSIOLOGY, Monash University, Victoria, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 1).

DEPUTY HEAD OF THE MATHEMATICS DEPARTMENT to teach mathematics and statistics to scholarship level—The Headmaster, Monmouth School, Monmouth.

GOLDSMITHS' FELLOW (graduate, normally under 30 years of age, with a research degree (normally Ph.D.) in chemistry from a British university) IN CHEMISTRY at University College, Dar es Salaam, for research in the general field of the chemistry of the natural products—The Inter-University Council, 33 Bedford Place, London, W.C.1.

GRADUATE (with an honours degree in chemistry and zoology from a British university) to work on a 3-year programme of lipid research—The Reader in Chemical Pathology, Westminster Medical School, Research Laboratory, Udall Street, London, S.W.1.

LECTURER (well qualified in electrical engineering or physics and research experience to at least the doctorate level) in the DEPARTMENT OF ELECTRICAL ENGINEERING—Prof. J. H. Calderwood, Royal College of Advanced Technology, Salford, Lancs.

PHYSICAL CHEMIST (preferably Ph.D. and experience in the fields of spectroscopy, photochemistry or polymer characterization) for basic research—The Director, Arthur D. Little Research Institute, Inveresk, Musselburgh, Midlothian.

PRINCIPAL LECTURER in SOCIOLOGY—The Registrar (RS), Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire.

PROFESSOR OF MICROBIOLOGY—The Registrar, The University, Reading.

SENIOR LECTURER or LECTURER in BIOCHEMISTRY in the School of Pharmacy—The Registrar (RS), Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Building Research Station Digest No. 75 (Second Series): Cracking in Buildings. Pp. 8. (London: H.M. Stationery Office, 1966.) 4d. [3110]

The Newsletter of the U.K. Panel on Gamma and Electron Irradiation, No. 1 (October, 1966). Pp. 7. (Oxford: Dr. E. J. Berry, Technical Secretary of Research Committee, U.K. Panel on Gamma and Electron Irradiation, c/o Radiobiology Laboratory, The Churchill Hospital, 1966.) [3111]

Meteorological Office. Geophysical Memoirs, No. 110 (Fifth number, Vol. 14): Secular Variations of the Atmospheric Circulation Since 1750. By H. H. Lamb and A. I. Johnson. Pp. 125. (Met. 0.711e.) (London: H.M. Stationery Office, 1966.) 27s. 6d. net. [3112]

The Real Flavour of Physics. By Prof. C. A. Taylor. (An Inaugural Lecture delivered at University College, Cardiff, 23rd March, 1966.) Pp. 24. (Cardiff: University of Wales Press, 1966.) [3113]

Productivity Bargaining. By Ken Jones and John Golding. (Fabian Research Series, No. 257). Pp. 39. (London: Fabian Society, 1966.) 4s. 6d. [3114]

General Register Office. The Registrar General's Quarterly Return for England and Wales, Quarter ended 30th June 1966, No. 470 (2nd Quarter 1966). Pp. 31. (London: H.M. Stationery Office, 1966.) 2s. 6d. net. [3115]

The Boundary Layer. By Prof. K. Stewartson. (An Inaugural Lecture delivered at University College London, 1 March 1965.) Pp. 26. (London: H. K. Lewis and Co., Ltd., 1966.) 5s. net. [3116]

Political and Economic Planning. Annual Report 1965-66. Pp. 16. Planning, Vol. 32, No. 498 (November 1966): Problems Facing the Teaching Profession. Pp. 193-236. 6s. (London: Political and Economic Planning, 1966.) [3117]

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Volume XXV, No. 3: The Fishery for Cockles (*Cardium edule* L.) in the Barry Inlet, South Wales. By D. A. Hancock and A. B. Urquhart. Pp. iv + 32 + 16 plates. (London: H.M. Stationery Office, 1966.) 20s. net. [411] Agricultural Research Council. Memoirs of the Soil Survey of Great Britain—England and Wales. The Soils of the Church Stretton District of Shropshire. By D. Mackney and C. P. Burnham. (Sheet 166.) Pp. vii + 247 + 9 plates. (Harpenden: Rothamsted Experimental Station, 1966.) 35s. net. [3118]

Report of the Commonwealth Economic Committee. Forty-First Report: Fish. Pp. xiv + 212. (London: H.M. Stationery Office, 1966. Published for The Commonwealth Economic Committee.) 17s. 6d. net. [3119]

University of Oxford. Annual Reports 1964-1965. Pp. 27. (Supplement No. 10 to the *University Gazette*, Vol. 96, August 1966.) (Oxford: The University, 1966.) 2s. 6d. [911]

British Antarctic Survey. Scientific Reports. No. 44: The Geology of the South Shetland Islands. 3: The Stratigraphy of King George Island. By Dr. C. M. Barton. Pp. 33 + 2 plates. 18s. net. No. 52: The Petrology of Stonington and Trepassey Islands, Marguerite Bay. By Dr. A. G. Fraser. Pp. 51 + 6 plates. 30s. net. (London: British Antarctic Survey, 1966.) [1011]

### Other Countries

Centro de Cooperación Científica de la Unesco para América Latina. Monografías 1: Progresos en Biología del Suelo: Actas del Primer Coloquio Latinoamericano de Biología del Suelo. (Realizado en la Universidad Nacional del Sur Bahía Blanca (Argentina), 13-17 Octubre 1965.) Pp. xvii + 715. (Montevideo, Uruguay: Unesco, Latin American Science Co-operation Office, 1966.) [111]

Forest Research Institute, Dehra Dun. Indian Forest Leaflet No. 176 (Entomology): Termite Control Service at the Forest Research Institute and College, New Forest Estate, Dehra Dun. By P. N. Chatterjee and R. S. Thapa. Pp. 4 + 1 plate. (Delhi: Manager of Publications, 1966.) Rs. 0-80; 1s.; \$0.20. [111]

Publications de l'Université Officielle du Congo, à Lubumbashi. Vol. 13: A la Recherche de Racine Chez Marcel Proust. Albertine, Ma Soeur, de Quel Amour Blessée... Par James LeB. Boyle. Pp. 18. (Lubumbashi: Université Officielle du Congo, 1966.) [111]

American Museum Novitates. No. 2237 (December 17, 1965): Systematic Notes on the Bird Family Cracidae. No. 4: *Ortalis garrula* and *Ortalis ruficauda*. By Charles Vaurie. Pp. 16. No. 2250 (July 8, 1966): Systematic Notes on the Bird Family Cracidae. No. 5: *Penelope purpurascens*, *Penelope jacquacu*, and *Penelope obscura*. By Charles Vaurie. Pp. 23. No. 2251 (July 8, 1966): Systematic Notes on the Bird Family Cracidae. No. 6: Reviews of Nine Species of *Penelope*. By Charles Vaurie. Pp. 30. (New York, N.Y.: The American Museum of Natural History, 1965, and 1966.) [111]

Food and Agriculture Organization of the United Nations. Report of the Joint FAO/WHO Technical Meeting on Methods of Planning and Evaluation in Applied Nutrition Programs, Rome, Italy, 11-16 January 1965. Pp. vi + 77. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1966.) 4s.; \$0-80. [111]

South Australia. 27th Annual Report of the Council of the Institute of Medical and Veterinary Science, July 1964-June 1965. Pp. 76. (Adelaide: Institute of Medical and Veterinary Science, 1966.) [111]

Svenska Linné-Sällskapet's Arsskrift, Årg. XLVIII, 1965. Pp. 89. (Uppsala: Svenska Linné-Sällskapet, 1966.) [111]

Australia: Commonwealth Scientific and Industrial Research Organization. Bulletin No. 285: Scientific and Common Names of Insects and Allied Forms Occurring in Australia. Edited by F. J. Gay. Pp. 52. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1966.) [311]

United States Department of Commerce: National Bureau of Standards. Technical Note 403: Microchemical Analysis Section—Summary of Activities July 1965 to June 1966. Edited by John K. Taylor. Pp. x + 77. (Washington, D.C.: Government Printing Office, 1966.) \$0-50. [311]

Pan American Health Organization. Pan American Sanitary Bureau, Regional Office of the World Health Organization. Scientific Publication No. 142: Migration of Health Personnel, Scientists, and Engineers from Latin America. (Report prepared by the PAHO Subcommittee on Migration for the PAHO Advisory Committee on Medical Research.) Pp. ix + 118. (Washington, D.C.: Pan American Health Organization, 1966.) [411]

Annals of the New York Academy of Sciences. Vol. 115, Article 3: Alcohol and Food in Health and Disease. By Giorgio Lolli and 14 other authors. Pp. 787-882. (New York: New York Academy of Sciences, 1966.) \$4. [411]

United States Department of the Interior. Fish and Wildlife Service: Bureau of Commercial Fisheries. Statistical Digest No. 58: Fishery Statistics of the United States 1964. By Charles H. Lyles. Pp. ii + 541. (Washington, D.C.: Bureau of Commercial Fisheries, 1966. Available from U.S. Government Printing Office.) \$2.50. [411]

National Science Foundation. NSF 66-21: Current Projects on Economic and Social Implications of Science and Technology, 1965. Pp. v + 187. (Washington, D.C.: National Science Foundation, 1966. Available from U.S. Government Printing Office.) \$0-65. [411]

New Zealand Meteorological Service, Department of Civil Aviation. Misc. Pub. 109: Meteorological Observations for 1963—Stations in New Zealand and Outlying Islands, including the Cook Group. Pp. 100. (Wellington, New Zealand: Government Printer, 1966.) [411]

United States Department of the Interior: Geological Survey. Bulletin 1198-F: Phytocology of a Greenstone Habitat at Eagle, Alaska. By Hansford T. Shacklette. Pp. iii + 36. Water-Supply Paper 1535-K: Chemical Composition of Rainfall, Eastern North Carolina and Southeastern Virginia. By Arlo W. Gambell and Donald W. Fisher. Pp. v + 41 + plate 1. \$0.65. Water-Supply Paper 1949: Quality of Surface Waters of the United States 1963. Parts 5 and 6: Hudson Bay and Upper Mississippi River Basins, and Missouri River Basin. Prepared under the direction of S. K. Love. Pp. x + 411. \$1.50. Professional Paper 422-I: An Approach to the Sediment Transport Problem from General Physics. By R. A. Bagnold. Pp. v + 37. \$0.85. (Washington, D.C.: Government Printing Office, 1966.) [411]

National Science Foundation. NSF 66-15: Basic Research, Applied Research, and Development in Industry, 1963. (A Final Report on a Survey of R and D Funds, 1963, and R and D Scientists and Engineers, Jan. 1964.) Pp. vii + 185. (Washington, D.C.: National Science Foundation, 1966. Available from U.S. Government Printing Office.) \$1. [411]

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## GOVERNOR AND PRESIDENT

MR. RONALD REAGAN seems bent on validating the predictions of those among his political opponents who were saying, only a few weeks ago, that he could not fail to be a bad governor of California. In retrospect, possibly when it comes time to stand again for election in 1970, he may reflect that it was foolish to have brought the differences between himself and others on the administration of the University of California so crudely to a head. It may now seem smart to have organized the dismissal of the president of the state university within three weeks of taking office, but a few years from now, when the governor will be a little more used to the exhilaration of power, it will not seem particularly remarkable to him that a man with the right to appoint to the Board of Regents should also have power over those the regents appoint. With luck, he may by then appreciate that it is a good deal easier to do serious damage to intellectual institutions such as universities than to work improvements on them. But Mr. Reagan has not lacked good advice, only the sense to listen to it.

It is, of course, too soon to know what will be the effects of Mr. Reagan's brashness on the welfare of the great university over which political chance has given him authority, but it is important to be clear why his doings in the past few days are reprehensible. The dispute about tuition fees at the University of California, important though it may be, is not the central issue. Whether the time has come in California for the state university to follow the precedent of most others in the United States (*Nature*, 213, 222; 1967) and to ask its students to pay tuition fees is necessarily a difficult question, with a case to be made on each side. The hard core of Mr. Reagan's offence is that he has forced his own view on the University of California in such a way as to make it plain that he is insensitive to the need that universities—wherever they are—should enjoy a real measure of independence in the running of their affairs. To scorn this principle is an assault on civil liberty as real as to suppose that newspapers can be censored with impunity, or judges bribed. And if Dr. Kerr has been sacked because he was stubborn about tuition fees, who can be sure that his successor will not be sent packing on some issue bearing even more directly on the right of the university to determine what to teach, and how.

The next few weeks will be important. The first rumblings from the campus at Berkeley suggest that the sacking of Dr. Kerr may canalize the formidable energies of the student body there away from the exotic causes which have in the past caused the new governor such pain into a protest against this affront to the independence of the university. Rough justice, some will say. But in the long-term interests of the

university, an early return to near normality would be best. The governor himself could do a lot to help, chiefly by providing some tangible assurance that the sacking of Dr. Kerr is not—as some speeches during his election would suggest—the first move in a serious assault on the university. Much, of course, will depend on the new president, whoever he will be. If Mr. Reagan has learned anything from his first blunder, it should be that the new man should be known to be at least as staunch a defender of academic freedom as Dr. Kerr. To appoint a yesman may seem politically expedient, but it would be disastrous for the university. Before the staff and students go cheerfully back to work, they have every reason to ask for assurance on this point. It would also be reasonable to ask for a clearer definition of the way in which the Board of Regents is empowered to act. If, as it should, the board can hire and fire the president of the university, it cannot also be empowered to initiate and to implement new policies without making each difference of opinion with the president, however legitimate, seem an occasion for dismissal. The ideal would be some real separation between the academic administration of the university and the making of political decisions about the scale on which it should be supported from public funds. Unfortunately, to judge from what Mr. Reagan has been saying, there is a danger that the politicians will seek an even firmer grip on what happens at the university.

And what is at stake? Mr. Reagan's previous career as a film star may not have equipped him to appreciate how critically the intellectual well-being of a university depends on the sense of freedom which people—students as well as staff—enjoy. Certainly he cannot have realized that the capacity of his university to attract talented recruits from all over the world—not just the United States—is a simple proof of the esteem in which the university is held. His first duty is naturally to the taxpayers of California, but it is unthinkable that they would wish him to cause irreparable damage to an institution which is even more widely admired than their climate. And it is also, of course, unthinkable that all of them can be as indifferent as Mr. Reagan seems to be to the benefits which society derives from independent universities.

## ALSO IN BRITAIN

THERE is an ironic echo of what has been happening in California in the special report on *Parliament and Control of University Expenditure* by the Public Accounts Committee of the House of Commons



(H.M.S.O., £1 10s.). Not for the first time, but with more persistence than is their custom, members of Parliament are seeking closer control of the details of university administration in Britain. Their present interest, the nub of the new report, is that the Comptroller and Auditor-General should be given regular access to the books of British universities. The committee insists that it is concerned only with seeing that public money is spent without impropriety, and it is understandable that anxiety on this score should have grown in the past two decades, during which the scale of public support for universities has multiplied fifty-fold to £211 million in the present academic year. But the fallacy is that even the candle-counting operation which the parliamentarians hanker for is inseparable from unwarrantable and necessarily ill-informed interference with academic matters.

The difficulty of separating accountability for detailed expenditure from policy issues should by now be thoroughly familiar. Under the British system, accountability to Parliament is the responsibility of the Permanent Secretary of the department to which money is voted. But no Permanent Secretary worth his salt would shoulder the task of asking universities how they spend their money without asking at the same time for their reasons. To attempt to behave differently would be at once futile and an assault on the view that, in the control of public expenditure, old-fashioned book-keeping should be replaced by more modern techniques of management. In the process, the Department of Education and Science would be caught up in enquiries into the uses made of particular equipments and buildings, and a great deal of detailed policy would have to be decided in Curzon Street, not in the universities. But this is not what Parliament wants. The public interest requires a continuing assurance that public money is well spent, but the University Grants Committee exists to provide just that. Parliament and the Department of Education and Science have a right to press for closer and more professional study of the ways in which universities make policy decisions and use the facilities with which they are equipped. They should also be encouraged by progress in the past eighteen months. But there is no case for more direct control of university administration. The example of the University of California should be a salutary example of the trouble that can bring.

## READY TO GO

THE Zuckerman committee (see page 325) has emerged a more healthy looking creature than seemed likely and even possible only a few weeks ago. By most tests, it is a strong committee. Its members are able, experienced and influential. It is particularly pleasing that the committee now formed includes several members of the Council for Scientific Policy, for that is at once an assurance that the expertise which Sir

Harrie Massey's committee has built up in the past two years will not be overlooked, and at the same time an assurance that the new committee will not be tempted to spend its time ferreting out generalizations about the research councils and the administration of civil science as a whole, for this is the part of the spectrum in which there is least to be unhappy about. Whether, in the long run, the advisory council will be able to become the particular kind of representative body which the Prime Minister seemed to have in mind right at the beginning is another matter. Because it is a Cabinet committee, its members must be appointed and not elected by the societies which they might be held to represent. It follows, of course, that the Zuckerman committee can claim to represent the scientific community as a whole only to the extent that its members command respect—which they do. This is, of course, the best arrangement, because both the committee-men and the learned societies are then free to say what they think. Since it is by no means beyond the bounds of possibility that the advisory council and the learned societies may one day find themselves at odds, this is a point well worth remembering.

But what will the advisory council advise on? And what kind of advice will it be tempted to give? As yet there are only the most fleeting hints of what may turn out to be possible. Much will depend on how quickly the committee can win for itself, within the government machine, the right to have a say about the important decisions which are being made by the Ministries of Defence and Technology. As luck will have it, the past week or so has provided several examples of matters which affect the pattern of scientific effort in the United Kingdom, and which seem to have been determined—not necessarily wrongly—without a detailed appreciation of all the consequences. The Anglo-French project for building a variable geometry military aircraft for the mid-seventies is a case in point. Mr. Denis Healey, the Minister of Defence, arrived in London from Paris with the assertion that the decision to commit £250 million to this project "will be a tonic to the aircraft industry", and so it seemed. But is it really wise to spend this sum of money, and a similar amount in France, on an option to keep in being aircraft manufacturing capacity which may never be profitable? If there is a case for thinking that Britain, or Europe, may one day make money by selling aircraft, might it not have been better to build something even more advanced than a Mach 3 aircraft? Or would it be better to invest in computers or hovercraft instead? Perhaps the most important need is to inform decisions about technical matters by government departments with an appreciation of the economics of innovation. There is also valuable work to be done in redeploying the skilled manpower now employed and often under-employed in government establishments. By chance, the advisory council may be helped in its work by the refreshing realism which the Ministry of Technology seems to have brought to technical decision making in the past few weeks.

## NEWS AND VIEWS

### Ructions in California

DR. CLARK KERR, the President of the University of California dismissed by the Board of Regents on January 19, has been associated with the university for 22 years and president since 1958. His dismissal by a majority of 14 to 8 came at the end of a week of discussions within the Board of Regents of the proposals put forward by the new Governor, Mr. Ronald Reagan, for a contribution by students to tuition fees at the nine campuses of the university. The dismissal seems to have been something of a surprise, for there had earlier been rumours that a compromise would be reached between those in favour of a tuition levy and those resisting the proposal at least for the academic year beginning in 1967.

The crisis at the university has come at the end of a decade in which the university has multiplied its size by a factor of nearly four. In the current year, the running cost of its operation will be \$730 million, while new plant and equipment will cost \$145 million. (In strictly financial terms, therefore, the University of California is bigger than all the universities of the United Kingdom put together.) In practice, the State of California contributes just under a half of the cost of keeping the university going. The federal government is almost equally important as a source of funds, largely through research contracts with university departments. Altogether the university has 87,000 students, with nearly 30,000 of them on the campus at Berkeley which has been the recipient of a great deal of Mr. Reagan's vituperation against the university both in the month before his election in November last year and in the weeks since his coming to office at the beginning of January this year. Mr. Reagan has frequently referred to the events in 1964 when students complained against the university administration of lack of freedom and when outside critics of the university complained that students were given too much licence. The present dispute has come to a head, however, because of Mr. Reagan's determination to reduce the contribution of the State of California to the running of the university by an amount reported to be equivalent to ten per cent.

The immediate reaction at the university has been to suggest to some heads of departments that uncertainty about the future of the university may make it easier for other universities to tempt away members of the faculties of the nine campuses at California. As yet there is no news of a successor to Dr. Kerr, and at this stage no assurance that this appointment will be made from among the nine chancellors who serve as heads of the individual campuses of the university.

### Committee at Last

NEARLY three months after his first announcement on October 25, the Prime Minister was able to tell the

House of Commons on January 17 of the composition of the Central Advisory Council for Science and Technology under Sir Solly Zuckerman. The Prime Minister said that the members would be: Sir Eric Ashby, Master of Clare College, Cambridge; Sir Harrie Massey, Professor of Physics at University College London and Chairman of the Council for Scientific Policy; Sir Hugh Tait, managing director of Esso Petroleum Co. Ltd.; Professor P. M. S. Blackett, Deputy Chairman of the Advisory Council on Technology and Chief Scientific Adviser to the Ministry of Technology; Professor A. B. Pippard, Cavendish Laboratory, Cambridge; Professor B. R. Williams, Professor of Economics at the University of Lancaster and an adviser to the Ministry of Technology; Dr. A. H. Cottrell, Chief Scientific Adviser (Studies) to the Ministry of Technology; Dr. F. S. Dainton, Vice-Chancellor of the University of Nottingham; Dr. F. E. Jones, managing director of Mullard Limited; Mr. F. Cousins, General Secretary, Transport and General Workers Union and Minister of Technology 1964-66; and Mr. R. D. Young, Alfred Herbert Limited. The Prime Minister said that the terms of reference of the council are to advise the government "on the most effective national strategy for the use and development of our scientific and technological resources."

The strength of the new committee is widely acknowledged and held to be an assurance that it will be on the side of the angels. The Council for Scientific Policy, which at one point seemed to be in danger of being overshadowed by the new council, is strongly represented, principally through Sir Harrie Massey. However, Dr. Dainton, Dr. F. E. Jones and Professor Blackett are all members of the Council for Scientific Policy. The presence of Mr. Frank Cousins on the committee is at first sight a surprise, although Mr. Cousins was a member of the council of the Department of Scientific and Industrial Research between 1962 and 1964. The interests of the Ministry of Defence are represented by Dr. Cottrell. In the formal announcement of the committee, no reference is made to the way in which the Royal Society was originally asked to appoint a representative although, of course, Professor Blackett is also President of the Royal Society. Apparently the original intention that the council should function on a confidential basis has not been changed, although it is considered that the council will be accessible to the Select Committee on Science and Technology being formed in the House of Commons. It is considered that the new committee will begin work on attempts to secure a more effective distribution of skilled manpower within government and other public laboratories. Its hardest task is bound to be the better articulation of civil and defence research.

### Reservoirs in the Sea

THE United Kingdom is likely to be short of water within one or two decades. One possible solution to the problem is to build barrages across estuaries and bays, turning them into reservoirs. The Ministry of Housing and Local Government appointed consultants to investigate the feasibility of two such schemes, across Morecambe Bay and Solway, and the consultants' reports have now been published (H.M.S.O., *Morecambe Bay*, £1 2s. 6d.; *Solway*, 16s.; *Report*, 10s. 6d.).

So far the studies have been desk bound, and have not involved detailed engineering or geological work. Moreover, the consultants were asked to consider only the problems of water and not those of communications, power generation and amenity. Their conclusions are therefore tentative, but they suggest that both schemes are feasible. Certainly some action will have to be taken, for the river authorities foresee a shortage of about 425 m.g.d. (million gallons per day) by 1981, and 1,200 m.g.d. by 2001.

The Morecambe Bay barrage should have priority, the report concludes, because the need for water is greatest in the North West, and because the barrage would free the Lake District water for use in other areas. The storage capacity of Morecambe Bay would be 55,000 m.g., and if the barrage were built it could be supplying 500 m.g.d. by 1977-79. Inland schemes will therefore have to meet the demand until the late seventies, but a decision on barrages must be made in the early seventies if not before. The total cost of the Morecambe Bay scheme would be between £54 and £69 m, including a dual two-way highway across the barrage and full treatment works, and it is estimated that the cost of fully treated water would be between 6.7 and 8.4 pence per 1,000 g.

The water initially enclosed would of course be saline, but the consultants believe that this could be removed by filling and emptying the reservoir while the road was being built. The draining and re-filling cycle, repeated four times, would reduce the chloride content to about 100 p.p.m., and ordinary filtration equipment could be used for final purification.

## European Exchanges

THE most tangible result so far of the expressions of interest by the Royal Society in European collaboration is the announcement of a new programme of grants for financing visits by working scientists between European institutions. In a statement published a week ago, the Royal Society said that it has received "substantial financial support for the programme from several donors", and that the intention is "not only to benefit science but also to strengthen the European community as a whole". Although it is expected that a "large fraction of the funds" will be used to help British workers to travel abroad, it is also intended that they shall be used to enable scientists from the mainland of Europe to visit Britain.

The funds so far available will be spent in three distinct ways. First, there will be postgraduate fellowships, ideally for people wishing to stay a year or more at a laboratory in some other country, and with a financial basis sufficiently generous to enable recipients to live without discomfort in the places at which they settle, and even to buy modest amounts of equipment if their host laboratories cannot provide them. The Royal Society says that it has funds to enable seven or eight British scientists to go abroad under this part of the scheme, and for roughly a third as many people to stay in Britain. The programme also includes provision for what are called study visits, on which junior and senior people will spend between a week and six months at laboratories abroad, and it is thought likely that there will be money enough for 50 British scientists and 20 from abroad. In addition, there is a plan to sponsor between six and ten research confer-

ences a year, with between 25 and 100 participants and with some provision for inviting a small number of participants from outside Western Europe.

## Chrysler in Britain

THE Ministry of Technology announced on January 17 its approval of an agreement by which the Chrysler Corporation gain control of Rootes, the British motor manufacturer. In a Europe increasingly sensitive to charges of technological domination by the United States, the British Government seemed surprisingly cheerful about the takeover. There are two reasons for this; first, the Government has probably decided that only those industries using the newest technologies are worth protecting, and second, there was nobody else who would touch Rootes with a barge-pole. Chrysler was committed to Rootes by its partial takeover in 1964; at present it owns 45 per cent of the ordinary voting shares, and 66 per cent of the non-voting "A" shares. A rights issue, underwritten by Chrysler, will now be made, giving shareholders the opportunity of obtaining five shares for each four held at present. This will raise £10 m, and an additional £10 m will be raised by a 15 year loan from Chrysler.

The agreement is hedged around with some splendid sounding but somewhat symbolic safeguards. The most significant is probably the involvement of the new Industrial Reorganization Corporation, which will purchase a seat on the Board by investing £3 m. Since the I.R.C. is also arranging an export consortium arrangement for the whole of the British motor industry, it will have to be very careful to ensure that participation in Rootes does not interfere with industrial security. The other safeguards agreed to by Chrysler are that the corporation will maintain a majority of British directors on the board, continue expansion at Linwood in Scotland, put no obstruction in the way of the export of Rootes products to all "practicable" markets, and exchange directors between Chrysler International, Simca and Rootes. These safeguards are unlikely to interfere with the way Chrysler runs Rootes. Markets already controlled by Chrysler or Simca may well be considered "impracticable", but similar limitations do not seem to have affected Ford, one of Britain's best exporters, while Simca is the leading French motor exporter.

Alternative solutions, in any case, were singularly unappealing. Outright takeover by the British Government was one possibility, but Rootes has been losing money for some time—£3 m before tax in the year to last July, and nearly £5 m since then. Inevitably Chrysler had to step in to protect its earlier investment; any attempt to stop it would have been futile—and costly as well.

The British market is now divided almost equally between companies under British and American control. The British Motor Corporation and Standard Triumph Leyland make up about 48 per cent of the market, and Vauxhall, Rootes and Ford split between them an almost exactly equal share. The remainder is made up of imported vehicles, largely from Europe.

## Survey for History

THE Indian Census of 1961 included a special count of scientific manpower which has recently been published

as a monograph by K. Ray of the Council of Scientific and Industrial Research at New Delhi. While it is certainly difficult to publish census details quickly, there is a strong case for rapid publication of specific surveys such as this, which are important for planning. By now the survey is interesting only as history. In 1961 the survey showed that there were 250,000 qualified graduates in science, technology and medicine in India, of whom 6.5 per cent were women. For every 100 scientists there were 75 engineers (the United Kingdom has 130 engineers for every 100 scientists, and the United States has 250). The deployment of these graduates comes as something of a surprise; 18.6 per cent had found non-technical work, and more than 10 per cent were unemployed, although the census was carried out at the time of year when graduate unemployment in India is at a minimum. Of those who said how long they had been unemployed, more than one half had been out of work for more than a year. As the census points out, it is difficult to reconcile the high rate of unemployment among doctors (7.3 per cent) with the fact that in India there are 6,000 people for each doctor. The unemployed could best be offered the opportunity of becoming teachers, of which India has a particular need; but perhaps by now they have all found jobs.

## Doubt and Mistrust

STIRRINGS of discontent within the National Trust have come to a head with the dismissal of Commander Conrad Rawnsley from his post as Appeals Director and Director of Enterprise Neptune. Enterprise Neptune was conceived as a means of saving Britain's coastline from indiscriminate development, and has raised a large sum of money for this purpose—£900,000 by the trust's reckoning, and more by Commander Rawnsley's. In recent months, costs of the appeal have amounted to 20 per cent of receipts, which the trust considers to be too high a price to pay, and which is why the trust decided to absorb the appeal into its general administration. Commander Rawnsley disagrees: "It could have gone on to raise by the end of May 1968 £3 m at an overall cost of 7 per cent—an all time record low for an appeal of such a nature".

Certainly the trust is ill-equipped to cast itself in the role of a pauper. Since its foundation in 1907 as a means of preserving places of historic interest or natural beauty, it has grown into the third largest landowner in Britain, possessing 350,000 acres of land, investments worth £7 m and 200 historic houses. On the face of it, this looks like good management, but the Reform Movement headed by Commander Rawnsley disagrees. Under the banner of Enterprise Neptune they have mobilized a small army of supporters. Nobody quite knows how many.

On the one hand, the objectors say that the trust is undemocratic, and that its committees are self-perpetuating cliques appointed by head office. Even the local committees, they say, are unrepresentative, and there is no devolution of power. The trust already concedes that "an increasing decentralization is called for". On the other hand, the objectors claim that the trust is an inefficient manager, landowner and employer, and that the annual accounts are deliberately obscure. Only where the great houses are concerned do the

reformers feel that the trust has done a good job; they feel, however, that preservation as in *aspic* may be appropriate to great houses, but is quite inappropriate to areas of land. They want to see more development of the land, with freer access for the public, and the creation of proper camping sites.

These objections go far beyond the pique of a dismissed employee, and in fact reflect a difference of attitude as to how the trust should be run. They will be put in the form of motions to the extraordinary general meeting which the reform movement has called on February 11. Whatever the result, the controversy will do the trust no harm, and may lead to a situation in which ordinary members feel more involved in its work. In any case, the fuss is likely to have a healthy effect on recruitment.

## Mathematics and the Mantle

SPHERICAL harmonic analysis has been used in the past by the proponents of continental drift to try to demonstrate that the figure of the Earth is related to convection cells in the mantle. This approach—which considers the Earth's topography as a whole—has not been entirely satisfactory so far, because the contribution of the principal tectonic features to the analysis is obscured by the steep slopes of the continental margins. In an attempt to overcome this difficulty A. M. Coode (*Geophys. J. Roy. Astro. Soc.*, **12**, 55; 1966) has ignored the continental features and has analysed only recent tectonic features—oceanic ridges and mountain-trench systems. Because the features chosen for analysis are roughly of the same age, any confusion which arises from a temporal change in the mechanism which generates these features, and therefore a change in the features themselves, is avoided. Assuming that the ridges are tensional and that the mountain trench systems are compressional features, Coode demonstrates that the principal tectonic features of the Earth are related to a global generating mechanism of the fifth degree. Following Runcorn, he interprets the mechanism as one of a convecting mantle and also supports the hypothesis that the pattern of convection has recently changed from a dominantly four-cell to a five-cell pattern.

## Mind and Gene

SOME of the problems of the links between psychiatry and genetics are discussed in *Research on Genetics in Psychiatry*, the report of a scientific group of WHO. There is clear evidence that genetic factors are involved in the aetiology of a number of mental diseases, although the genetic factors are more obvious in some than in others. Huntington's chorea is the best example of a hereditary mental disease—family trees showing very clearly the inheritance of a single dominant gene have been compiled for a number of families in which the disease is current. In most of the more common mental diseases, schizophrenia, epilepsy and manic-depression, for example, the genetic component is far less clear cut, although it probably plays a considerable part in most of these states. The lack of a standard international nomenclature only adds to the intrinsic difficulties of these studies.

Understanding of the two general categories of mental disease is at very different levels. Mental

defect has been studied by a number of fruitful techniques and its genetic aspect is much better understood than that of mental disorder. A number of syndromes arising from chromosome defects, such as Klinefelter's syndrome (the presence of an excess sex chromosome) and Down's disease (21-trisomy), are well characterized. Not surprisingly, what the report calls "mental retardation of unknown aetiology" is poorly understood, although here the statistical methods of population genetics can be of great value. These methods have made it possible to divide the cases of retardation into two main groups—one in which it seems that there is a strong genetic involvement and one with a low risk. Genetic factors are responsible for many cases in the group at high risk. These include at least one chromosome abnormality and a number of single recessive genes with high penetrance.

The WHO group puts forward a number of proposals for topics on which work should be concentrated. It calls for research into the frequency of chromosome abnormalities and the relationship of these abnormalities with mental disorder, particularly from the biochemical point of view. It would like to see more work on twins, preferably on an international scale, including studies of twins separated in early life, and on adopted children and their families. It also thinks workers should hurry to examine inbred populations before they are submerged by admixture and the spread of urbanization.

## New Ghost Town

THE new town promised by the Minister of Housing and Local Government in North Buckinghamshire will not only be the largest in the United Kingdom but probably the most diffuse as well. By the end of the century it will hold 250,000 people from London at an overall density of about 11 to the acre. Most of the new town, to be called Milton Keynes, will be between the trunk roads M1 and A5, and it will absorb the existing towns of Bletchley, Wolverton, and Stony Stratford. The original plans specified an area of 25,200 acres, but the report of the public inquiry conducted by Mr. G. C. Godber, an independent inspector appointed by the ministry, has persuaded Mr. Greenwood to reduce this by 3,300 acres. Observing that the site is roughly as large as Bristol (population 434,000) and substantially larger than Coventry (327,000), the inspector reported that "The objectors were not convinced and I am certainly not convinced that it can be right to take so low a density as a target . . . One thing is certain—the plan will fill whatever land is designated, and it is not right to sacrifice good farms and good farmers needlessly to make a planners picture."

Not all the planners are cock-a-hoop at the opportunity offered by the minister, however. There are two schools of thought—at least—on town density. One, in which architects are conspicuous, holds that high densities are necessary both as a discipline and to create what is known as urbanism (or occasionally urbanity, although that is usually taken to mean something quite different). This point of view is coloured by sociological studies such as that of Michael Young and Peter Wilmott—*Family and Kinship in East London*—who showed that when people are moved from a close-knit community (Bethnal Green) to a

suburban housing estate (Woodford), their strong sense of community is left behind. Among the greater population densities used, with the support of many local authorities and the Ministry of Housing, is that at Cumbernauld new town in Scotland, where 85 people are housed on each acre.

The other point of view is probably put most forcefully, and certainly most often, by the Town and Country Planning Association, whose attack is directed chiefly at flats, which are said to be unpopular with tenants, and more expensive to build and maintain than houses. Certainly the association is convinced that only about 5 per cent of British people want flats, and that even this minority taste is probably declining. Bungalows, semi-detached and detached houses vie for popularity. To those for whom this presents a doleful vision of mile upon mile of suburban houses and bungalows, combining the worst features of American urban sprawl and British design, the association says that urban sprawl is not necessarily the result of low density housing but simply of bad design.

The aesthetic and sociological argument is a fascinating one, but may become an irrelevance, if it has not already done so. The South-East Study (H.M.S.O., 15s., 1964) estimates that the population increase of South-East England is likely to be 3.5 million by 1981. Housing this increase in towns as thinly spread as Milton Keynes would need an area of about 310,000 acres—greater than that of Bedfordshire. Nobody dares to think about A.D. 2000, when the new town will have reached its leisurely target.

## Cinnabar with Everything

THE production of gold from base metals has long been known as the ambition of the alchemists, but they had another preoccupation—the lengthening of life. Chinese alchemy in particular was almost totally dedicated to this end, as can be told from *A History of Ideas about the Prolongation of Life* (Trans. Amer. Phil. Soc., N.S., 56, part 9; 1966).

One of the principles of the Chinese alchemists was a kind of vitalism, which implied that certain substances contain the essence of life and can be used to produce medicines which help to prolong it. Certain characteristics were considered necessary for a substance to be a vitalizer; these included being shining, fluid, strong to the taste and blood red in colour. The alchemists had a list of materials which possessed the vitalizing property, and there was a hierarchy with minerals at the top and herbs at the bottom. Drugs of vegetable origin were inefficient vitalizers; they can only decompose and decay, but the minerals had many of the necessary characteristics. The most valuable were the rare minerals. Silver, gold and cinnabar were at the top of the list; mercuric sulphide occupied a supreme position in Chinese alchemy, although it was only of minor importance in other cultures. Cinnabar had the blood-red colouring which bestowed life giving qualities, which were already recognized, for neolithic people in China had deposited vermilion pigment with their dead. The change of cinnabar, on heating, to mercury, and the conversion of this to mercuric oxide which sublimes back to mercury, showed the alchemists a seemingly endless cycle involving "living" red cinnabar and "living" fluid mercury. This represented longevity, which



man could increase by taking medicines prepared from cinnabar.

## Isolation of a Repressor Molecule

from a Correspondent in Biochemical Genetics

In 1961 Jacob and Monod proposed a scheme to explain how the activity of genes might be controlled. Their theory was constructed from observations on the lactose operon of *E. coli*, a cluster of three genes which code for three enzymes, two of which are necessary for the utilization of the sugar lactose and other  $\beta$ -galactosides. When wild type *E. coli* is grown with glycerol as a carbon source, all three enzymes are present only in very small amounts. Addition of  $\beta$ -galactosides and, in particular, a non-utilizable  $\beta$ -thiogalactoside, induces a high rate of synthesis of these enzymes. There are at least two regions on the *E. coli* chromosome concerned with the regulation of the lactose operon—a regulator gene and an operator region. Jacob and Monod proposed that the regulator gene codes for a regulator molecule, or repressor, and that this combines with the operator region adjacent to the three structural genes so as to prevent the reading of the operon. The repressor molecules were also supposed to recognize  $\beta$ -galactosides with the result that they were removed from the operator region by the formation of a  $\beta$ -galactoside—repressor complex. This sequence of events would now allow the expression of the lactose operon, probably by permitting the synthesis of a messenger RNA.

The regulator gene has recently been shown to have properties which show that the repressor is a protein molecule. Insight into the molecular mechanism of regulation has, however, rested only on indirect experiments because it has not been possible to detect and assay repressor molecules in the test-tube. The whole status of the field has now been altered by the recent work of Professor Walter Gilbert and Dr. Benno Müller-Hill of Harvard University, who have detected the repressor molecule for the lactose operon (*Proc. U.S. Nat. Acad. Sci.*, **56**, 1891; 1966). Their assay depends on the ability of the repressor to bind an inducer  $\beta$ -galactoside. Using a radioactive inducer, they showed by equilibrium dialysis that more inducer was found inside the dialysis bag containing a cell extract than outside the bag. Control experiments on extracts from cells which contain a deletion of the regulator gene show that these do not bind radioactive inducer. They have shown that this repressor is a protein molecule with a probable molecular weight of about 150,000–200,000, and that there are about ten molecules of repressor per gene copy in *E. coli*.

This is the first and crucial step towards the biochemical study of the control of gene expression.

## Enzyme Mechanisms

from a Correspondent in Molecular Biology

THE power of relaxation methods for the measurement of fast chemical processes is nowhere more spectacularly revealed than in the temperature-jump studies of enzyme kinetics which have been emerging from a number of laboratories. This month a further study on ribonuclease throws new light on the mechanism of

the enzyme, and an important article from the laboratory of Eigen goes some way towards defining the characteristics of an allosteric system of interacting sites.

Erman and Hammes (*J. Amer. Chem. Soc.*, **88**, 5607 and 5614; 1966) have used a stopped flow temperature-jump technique to study the interaction of both cytidine 2':3'-cyclic phosphate and cytidylyl-3':5'-cytidine with ribonuclease. In this system the usual temperature-jump method fails because the equilibrium is overwhelmingly biased in favour of the product of hydrolysis—cytidine 3'-phosphate—and its complexes with the enzyme. By introduction of the stopped flow, the temperature jump can be applied 16 msec after the mixing of enzyme and substrate. The system is found to exhibit two relaxation processes, one independent of concentration at high excess of substrate, the other dependent on it. These are attributed respectively to an isomerization in the enzyme-substrate complex and to the binding of the substrate to the enzyme. Taken together with earlier work on the interaction with cytidine 3'-phosphate, a mechanism for the hydrolysis of the substrate has been evolved which appears to account for all relaxation, equilibrium and steady-state data. Moreover, the pH-dependence of the relaxation processes is interpreted as indicating the existence of five states of the enzyme-substrate complex, involving three or more ionizing groups in the enzyme, with specified  $pK$ 's. Parallel reaction paths are also inferred.

In the accompanying article, the same authors consider the ribonuclease-cytidylyl-3':5'-cytidine system, which is shown to have similar characteristics. It is recognized that there is at present no means whereby the formalism relating the states of the system can be interpreted structurally. To the extent that certain groups, among them two histidines, are believed to be present in the active centre, a number of speculations are possible, but this is another case in which the results of crystallographic analysis of the protein are eagerly awaited.

An article by Kirschner *et al.* (*Proc. U.S. Nat. Acad. Sci.*, **56**, 1661; 1966) examines the binding of NAD to glyceraldehyde-3-phosphate dehydrogenase, an enzyme known to contain four identical sub-units. The system was selected because it was believed to represent a tractable case of "homotropic" ligand binding. Three relaxation processes in substantially different time ranges were observed, so that the dependence of each step on concentration could be determined. If it is assumed that conformational transitions are slow in comparison with bimolecular binding processes, the three relaxation times can be used to derive rate constants. The kinetics can be very satisfactorily accounted for in terms of the postulates of the appropriate allosteric hypothesis—that the apoenzyme exists only in two tetrameric modifications and that the intrinsic dissociation constants of the binding sites are identical in both forms. It is also shown that there is a high degree of co-operative (all-or-none) behaviour between the sub-units. These results can evidently be most simply explained in terms of the allosteric theory, for which they may be regarded as providing support. As the authors point out, it will be of considerable interest to obtain similar information on a range of other sub-unit enzymes, and to see which generalizations appear to survive.

## Parliament in Britain

IN a written answer in the House of Commons on January 17 the Joint Parliamentary Secretary to the Ministry of Technology, Dr. J. Bray, said that the ministry was supporting the development of fluid logic devices for computer peripherals by two contracts under the Advanced Computer Techniques Project. One was with British Telecommunications Research, Ltd., and the other with International Computers and Tabulators, Ltd.; both were for 18 months and the ministry was paying half the total cost of each, £52,000 and £50,000, respectively. The aim of the contracts was the development of cheap individual fluid logic elements and of complex integrated arrays of fluid logic elements to perform particular logical functions.

IN a written answer in the House of Commons on January 19, the Secretary of State for Education and Science, Mr. A. Crosland, stated that Parliament would be asked to vote funds to meet the additional cost for all overseas students financed from British official sources (including the British Council), mostly from developing countries. A fund would also be provided to reimburse the additional £50 payable by students already embarked on courses and who were financed by the governments of developing countries. No part of the fees of overseas students was met directly by his Department, but even after the recently announced increase in fees, public funds would still be providing on average some two-thirds of the recurrent costs of overseas students at universities and colleges of further education.

REPLYING to a question in the House of Lords on January 19 regarding university fees of overseas students, the Parliamentary Under-Secretary of State for Commonwealth Affairs, Lord Beswick, said that while the Government did not lay down fees to be charged by the universities, it had decided that the next quinquennial settlement of recurrent grant to universities should assume a free income of £250 a year from overseas students starting on courses in the next academic year. To avoid hardship, the increase in the fee assumed for those who had already started on courses extending into the next academic year would be limited to £50 a year for the remainder of their present course. The decision was taken in the light of the recommendations of the Robbins Committee and the Select Committee on Estimates that the general level of fees should be increased to meet at least 20 per cent of expenditure. For most United Kingdom students the general effect would be to transfer expenditure from central to local government. The existing fee structure resulted in a concealed subsidy to overseas students at United Kingdom universities which had grown from £3-4 million in 1955-56 to about £12 million in 1965-66. Even with the increased fee, this element of subsidy would be higher than it was 5 years ago. Some 30 per cent of overseas students came from countries such as the United States, Canada and the Scandinavian countries.

MR. GORONWY ROBERTS, replying to a question on January 19, said that during 1963-64 a total of 605 graduates of United Kingdom universities emigrated to the United States—298 had first degrees and 307 higher degrees.

## University News: California Institute of Technology

THE California Institute of Technology has announced that the new Robert Andrews Millikan chair in physics will be held by Professor Murray Gell-Mann. Dr. Gell-Mann has been associated with the California Institute of Technology since 1955 and is distinguished for his contributions to the theory of fundamental particles and in particular for his introduction of the concepts of "strangeness" and the "eight-fold way", the second of which has provided a framework within which it may be possible to classify the particles. Dr. Gell-Mann also pointed to the value of "quarks" as a means of rationalizing relations between different particles.

London

DR. K. A. PORTER, reader in morbid anatomy at St. Mary's Hospital Medical School, has been appointed to the chair of pathology, tenable at that school.

Manchester

DR. T. S. L. BESWICK, at present reader in virology, has been appointed to the newly established chair of virology as from February 1. Dr. Beswick is known for his work on poliomyelitis vaccines and the experimental pathology of virus diseases.

University College of Swansea

DR. P. J. SYRETT, reader in botany in University College, London, has been appointed professor of botany in succession to Professor H. E. Street.

## Appointments

DR. R. H. SIMPSON, associate director of the United States Weather Bureau, has been appointed director of the National Hurricane Centre in Miami, in succession to Dr. G. E. Dunn. Dr. Simpson will be succeeded by K. R. Johannessen, at present director of ESSA-Weather Bureau's Eastern Region.

## Announcements

SIR ROBERT WYNNE-EDWARDS has retired as chairman of the Council of Engineering Institutions, a post which he has held since the council was established. A graduate of Christ Church, Oxford, he spent many years in Canada before returning to Britain in the 1930's, and during the Second World War he worked in the Ministry of Works. After the War he returned to private industry, and was managing director of Costain-John Brown (later Constructors John Brown Ltd.) from 1948 until 1961; he has also served as member and chairman of the Building Research Board and the Coal Research Board, and became president of the Institution of Civil Engineers in 1964. He will be succeeded as chairman of the C.E.I. by Mr. H. N. Pemberton, who has represented the Institute of Marine Engineers on the board of the C.E.I. since 1964. Mr. Pemberton has been a member of the Ministry of Power nuclear safety advisory committee and of the marine engineering and atomic energy committees of the British Ship Research Association, as well as vice-president of the Institute of Marine Engineers.

MR. H. J. BUNKER has been elected president of the Institute of Biology. Dr. T. G. Onions has been elected honorary secretary and Dr. J. A. Freeman honorary treasurer of the institute.

THE gold medals of the Royal Astronomical Society have been awarded to the following: PROFESSOR H. ALFVÉN, Royal Institute of Technology, Division of Plasma Physics, Stockholm, for his fundamental work on cosmical electrodynamics; DR. A. R. SANDAGE, Mount Wilson and Palomar Observatories, for his fundamental work on stellar evolution and the history of the galaxy, and in providing optical data basic to modern cosmology. The

Eddington medal has been awarded to PROFESSOR R. F. CHRISTY, California Institute of Technology, for his work on the non-linear theory of pulsating stars.

THE Steacie Prize for 1966, which consists of a cash award of \$1,200, has been awarded to Professor G. H. Dixon of the University of Columbia for his important work on the synthesis of insulin and on the structure of haptoglobins.

THE Royal Society of Arts has awarded the Benjamin Franklin Medal for 1967 to Dr. Detlev Wulf Bronk, president of the Rockefeller University in New York.

DR. EDWARD M. PURCELL, professor of physics at Harvard University, has been awarded the Oersted Medal by the American Association of Physics Teachers.

THE American Microchemical Society has created a fund to commemorate the work of the late Dr. A. A. Benedetti-Pichler. The award will be made to an individual who has made outstanding contributions to the practice or teaching of microtechniques. The recipient will give a lecture at a gathering of analytical chemists. Nominations must be in the hands of the committee by April 1, 1967. A letter of 300 words or fewer, giving the name and address of the nominee and citing, in general, his work, may be sent to the chairman of the 1967 Committee, Dr. Leo K. Yanowski, Department of Chemistry, Fordham University, New York, N.Y., 10458.

PROFESSOR P. M. S. BLACKETT will give the 1967 Chelsea Lecture on the subject "Continental Drift" at Chelsea College of Science and Technology on February 7. This is the first of a series of lectures established to commemorate the admission of the college as a school of the University of London.

THE fourth symposium on "African Geology" will be held in the University of Sheffield during April 19-22. Further information can be obtained from Mr. P. Wilkinson, Department of Geology, University of Sheffield, St. George's Square, Sheffield, 1.

A CONFERENCE on "Inorganic Reaction Mechanisms", sponsored by the Irish National Committee for Chemistry and Professor F. L. Scott, of University College, Cork, will be held in Kinsale, Co. Cork, during April 10-14. Further information can be obtained from Dr. N. Mulcahy, Chemistry Department, University College, Cork.

THE second United Kingdom Automation Council control convention on "Advances in Computer Control" is to be held in the University of Bristol during April 11-14. Further information can be obtained from the Convention Secretariat, The Institution of Electrical Engineers, Savoy Place, London, W.C.2.

A SYMPOSIUM on "Liquid-Liquid Extraction", organized by the Northern Branch of the Institution of Chemical Engineers, will be held at the University of Newcastle upon Tyne during April 20-21. Further information can be obtained from the Institution of Chemical Engineers, 16 Belgrave Square, London, S.W.1.

A CONFERENCE on "New Developments in Optics and their Applications in Industry", organized by the British Scientific Instrument Research Association, will be held in Eastbourne during April 11-12. Further information can be obtained from Mr. P. J. Geary, Publicity and Literature Services Department, SIRA, South Hill, Chislehurst, Kent.

A SYMPOSIUM on "Mean Sea Level", organized by the International Association of Physical Oceanography with the support of the United Nations Educational, Scientific and Cultural Organization, will be held in Washington during April 13-15. Further information can be obtained from Mr. S. D. Hicks, Coast and Geodetic Survey, U.S. ESSA, Washington Science Center, Rockville, Maryland.

A CONFERENCE on "Image Detection and Processing", organized by the Optical Group of the Institute of Physics

and the Physical Society, will be held at the Royal Radar Establishment, Great Malvern, during April 24-26. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A CONFERENCE on "The Teaching of Mathematics to Physicists", arranged by the Education Group of the Institute of Physics and the Physical Society in collaboration with the Institute of Mathematics and its Applications, will be held in the University of Exeter during April 12-14. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "The Use of Isotopes and Radiation in Plant Pathology Studies", organized by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency, is to be held in Vienna during April 17-21. Further information can be obtained from Mr. Nils Lund, Division of Public Information, International Atomic Energy Agency, Karntner Ring 11, A-1010 Vienna.

(Continued on page 336)

## CORRESPONDENCE

### Doomsday 1967

SIR,—As one of the participants in the BBC programme "Challenge" broadcast on January 5, I would like to express agreement with all of the comments in your leading article of January 14. My own contribution, as recorded, consisted of three parts. The first showed what might be unpleasant effects of our elimination of all previously effective limits on world population if we fail to develop new ones. The second started with the comment that naturally I do not believe for a moment that any such catastrophe will be allowed to occur and went on to point out that there have already been developed new methods, the most important being practical means of birth control. The Japanese have already demonstrated the effectiveness of these by cutting their net reproduction rate from 2 to 1 in less than 20 years. The third part pointed out that we have now for the first time a choice as to whether we limit the population, and if we do, a wide range of choices of how to do it. The whole was consistent with my belief that the fundamental value to the world of science and technology is that it gives us greater freedom of choice both of action and in thought.

The first alone of these three parts was broadcast.

I consider it perfectly fair for the BBC to stress the disadvantages of wrong choices, and hence the danger of carelessness in making them. But to omit altogether the advantages of the choices which have been made and the huge range of alternatives which we could make seems to me, as to you, a serious distortion. I am not complaining that the producer of the programme deceived me in any way; rather my lack of experience may have misled him. I had not seen any of the other contributions when I made my recording and it did not occur to me to ask that, if reduction of my allotted time were necessary, my share should be cut down in a balanced way.

Nevertheless, if I had known that there would be time for the first aspect only of my contribution, I would have preferred not to take part at all.

Yours faithfully,

J. H. FREMLIN

Department of Physics,  
University of Birmingham.



# Organization for Science

by our Special Correspondent

Mr. Anthony Crosland, Secretary of State for Education and Science, is responsible for the administration in Britain of public support for scientific research and for the universities. The following is the outcome of an interview on January 18—the day after the membership of the Zuckerman committee was announced in the House of Commons.

THE Zuckerman committee will be able to do a useful job without disturbing the existing machinery for the administration of civil science in Britain. This is the view of Mr. Anthony Crosland, Secretary of State for Education and Science. He said that there was a "case for a committee looking at the whole field" of civil science, technology and defence.

In Mr. Crosland's view, it is probable that the committee will not wish to concern itself directly with the administration of the research councils—the field in which the Council for Scientific Policy under Sir Harrie Massey operates. The Minister seems to be happy with the way in which his own parish is being regulated, and clearly does not consider that there is much that outsiders would find to criticize in his own machinery for dealing with the research councils. That said, however, he is as ready as anybody to confess that it would be a great help if somebody could design rules to indicate the optimum level of spending on science and technology, as a whole and as separate parts.

Mr. Crosland considers that the membership of the Zuckerman committee is an assurance that some hypothetical dangers will not materialize. In particular, he does not consider that the existence of a strong central committee will give the Government too much control. The "guarantees against that danger" are the men who belong to the committee, many of whom have had several years of experience as independent advisers to the Government.

The new committee, being a Cabinet committee, will function in private, and Mr. Crosland does not consider that it will be much scrutinized by the Select Committee on Science and Technology which is to be set up in the House of Commons in the months ahead. He considers that the parliamentary committee will be valuable because its field is one of "those grey areas in which you never get parliamentary debates" which provide opportunities for Members of Parliament fully to satisfy their curiosity. Mr. Crosland emphasized the educational role of the committee, especially to begin with. It "won't start off with the research councils" and, while recognizing that it might be unfortunate if the parliamentary committee were used as a platform for sensation, he hoped that it would exercise its prerogative to open its meetings to the public.

Given the rumours—or the hopes—there have recently been of massive public support for some schemes for European collaboration in academic science, Mr. Crosland had a moderately optimistic tale to tell. In the field of fundamental research the Government—prompted by the Council for Scientific Policy and supported by the Royal Society—has taken an initiative in O.E.C.D. to work out a system of international fellowships. The creation of what the Prime Minister has called a European technological community is, of course, the responsibility of the Ministry of Technology and not of the Department of Education and Science.

On the administration of the universities, Mr. Crosland is anxious to make it clear that the universities have in the past decade "obtained a rising share of an educational budget that has itself been taking a rising share" of public expenditure and of the national income as well. But he



has his eyes on the cost of the operation, and is anxious to encourage current studies of such questions as the cost of educating students in universities of different kinds and sizes and the productivity of staff. He seems to be moderately cheered by the studies recently embarked on under the Committee of Vice-Chancellors and Principals of the use of plant and facilities at universities. On the pattern for rationalizing the distribution of effort of the universities, he considers it right that, taking the nation as a whole, the University Grants Committee should work through the subject panels which it has established. There may also be a case for looking at the availability of resources in higher education (in all its forms) on a regional basis as well. Several of the regional planning councils, which have strong representation from higher education, are already doing this.

The balance between the universities and the other institutions at which higher education is provided has been a bone of contention for some time, and particularly since the British Government took steps to formalize the development of the polytechnics. Mr. Crosland points out that this is not an innovation, but an attempt to make the best of a plural system which already exists. He argues that there is a "separate social demand" for a plurality of institutions of higher education. For one thing, polytechnics can provide higher education, on a part-time basis, for people already in employment. For another, they can provide degree courses oriented towards industry which more orthodox universities might be hard-pressed to provide. Mr. Crosland does not fear that the parallel development of universities and polytechnics will create an excess capacity for higher education. Rather, he seems fatalistically convinced that the rising demand for higher education will use up whatever capacity there may be created.



# Volcanic Rings on the Moon

by  
G. FIELDER

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Are the low ring structures on the Moon older than the craters? A study of the Flamsteed *P* ring structure revealed by photographs taken by the U.S. lunar probes *Orbiter I* and *Surveyor I* throws new light on this question.

It is generally assumed that lunar rings such as Stadium (diameter 65 km) and Daguerre (44 km) are old features because, it is argued, they have been eroded to mere remnants of former grand ring structures like Copernicus. Elsewhere I have listed evidence<sup>1</sup> which points strongly to such "elementary rings" being among the most youthful of volcanic forms on the Moon's surface. I shall not repeat the evidence here: it led to an evolutionary concept of the origin and anagenesis of elementary rings which can be summarized as follows:

Ring fracturing of the lunar crust is followed by the intrusion of lava sheets. Lava is extruded at those points around the circumference of a ring structure where the

local hydrostatic conditions are favourable and the lava flows across the surface for distances of several kilometres. Some volcanic cratering (collapse pits, especially) also occurs mainly in annuli defined by the sub-parallel ring fractures. The floor of the principal ring structure subsides and forces lava to issue from fissures and rim volcanoes (cones and calderas), thus building up the low ringwalls by successive flows. In general, the inner rings of a given ring structure develop later than the outer ones.

At the time this theory was published, I knew of no support for the idea. Now, however, *Orbiter I* has produced exceptionally clear photographs of the large elementary ring Flamsteed *P*; and Dr. J. A. O'Keefe, R. G.

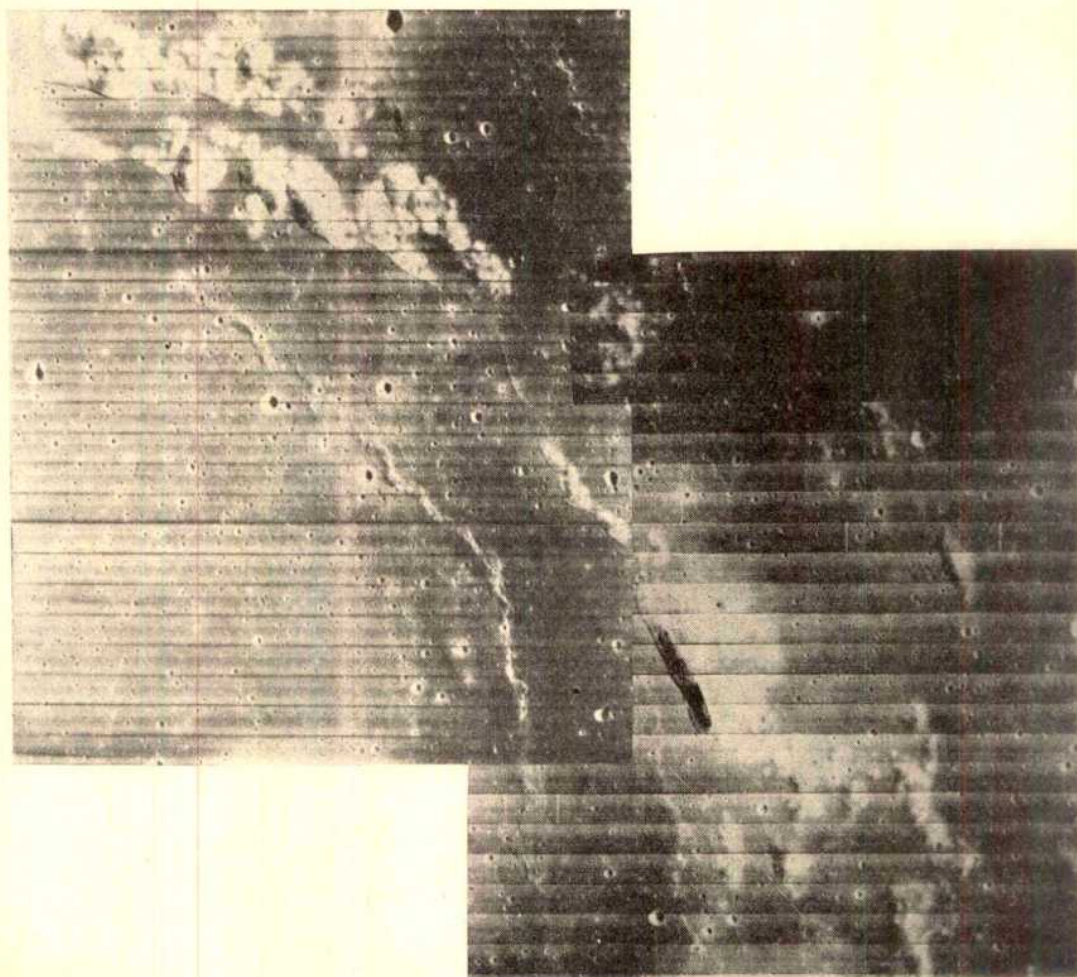


Fig. 1. The north-east rim of Flamsteed *P* as recorded by *Orbiter I*. Lava flows have formed mountains and the lower wrinkle ridges. Total width about 56 km. (N.A.S.A. photographs.)



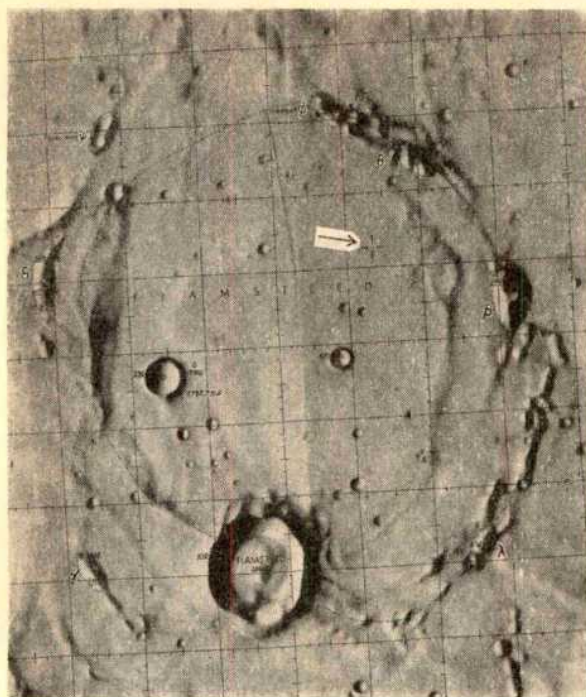


Fig. 2. Chart prepared by the Aeronautical Chart and Information Center, U.S. Air Force, showing the whole of Flamsteed P. The Surveyor I landing site is indicated (arrow); it is near to the mountains Flamsteed  $\theta$  and  $\beta$  shown in greater detail in Fig. 1. The squares have a side of 15 km.

Strom and E. A. Whitaker have independently drawn my attention to certain features of the photographs which appear to uphold the theory. The aim of this article is to examine and interpret some of the more revealing details on two recent N.A.S.A. *Orbiter* photographs.

The *Orbiter* I photographs L-66-7828 and 7843 each cover an area of about 32 by 39 km in Oceanus Procellarum. The areas overlap, and the photographs are reproduced in Fig. 1 as a mosaic. It is seen that the bright mountains in Fig. 1 form a broken arc, and that a prominent wrinkle ridge parallels the arc. The arc is in fact the north-east (astronautical convention) portion of the rim of a subcircular ring known as Flamsteed P

(Fig. 2). The highest of the prominent group of mountains in the upper part of Fig. 1 has been designated Flamsteed  $\theta$  and the most massive mountain in the lower part of Fig. 1 has been named Flamsteed  $\beta$  (Fig. 2). The profile of the upper parts of Flamsteed  $\theta$  (Fig. 3) was recorded earlier by Surveyor I, some 20 km away. Flamsteed P is 100 km in diameter; thus Fig. 1, which has a resolution in some places better than 10 m, shows part of the rim of a very large lunar ring formation.

The rim consists of discontinuous mountains and hills which have mean slopes of  $10^{\circ}$ – $25^{\circ}$  and altitudes of up to about 600 m (Fig. 2). Although there is a tendency for individual mountains to assume the form of rounded, dome shaped units characteristic of fluids which were once viscous, the overriding linear form of each block of eminences suggests that they are controlled by fractures.

Of paramount importance is the fact that details on the flanks of these eminences may be interpreted in terms of features essentially similar to those known in volcanic regions of the Earth. Special reference should be made to Figs. 4 and 5 in which the following details of Flamsteed  $\theta$  and  $\beta$  can be observed. First, several of the mountains terminate with a steep pedal slope, 50–100 m in horizontal extent, measuring  $\lesssim 25^{\circ}$ . At the top of this slope the terrain levels out somewhat to form an apron 200 m wide before hummocky material is encountered, when the general slope steepens again for 500–800 m. Here, however, the terrain is so crenulated that slope reversals occur with a wavelength of about 100 m. Most of the raised crenations are sub-parallel to the steep border that abuts on the lunabase. They assume the form of lenticles some 200 m long or define lobes that are reminiscent of the flow ridges in a lava field. For example, this distinctive appearance may be seen in a flow of intermediate lava from a Chilean volcano described by Guest<sup>2</sup> (Fig. 6).

The apron material is smoother than the hummocky material, but the former does carry crenations even though they are of smaller amplitude and wavelength than in the hummocky material. Individual hummocks, or blocks of rock, as small as 10 m across can be clearly identified on the hummocky flows under conditions of very low lighting. The two materials may be structurally or chemically different, but both are characteristic of lava.

The lava front is perhaps 20–40 m high where the apron terminates and the frontal profile is such that it cannot

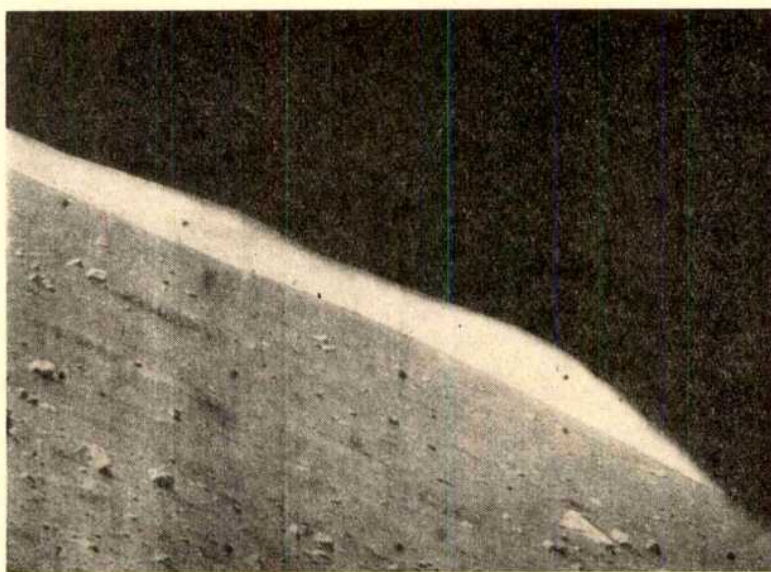


Fig. 3. The upper part of the mountain Flamsteed  $\theta$  as recorded by Surveyor I. (U.S. Information Service.)



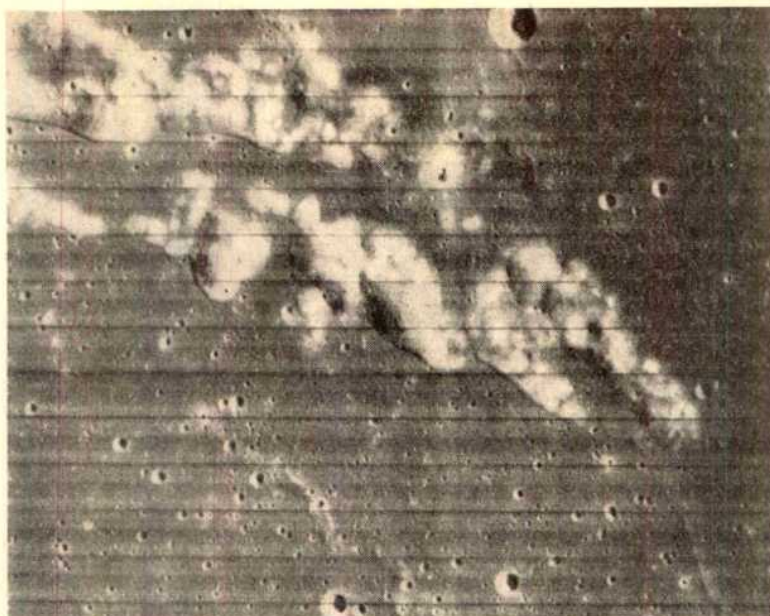


Fig. 4. Flamsteed  $\theta$  and environs, enlarged from Fig. 1, showing lobate termini of several lava flows. Total area shown about 28 km  $\times$  20 km. (N.A.S.A. photograph.)

be composed of erosional detritus, which would wedge or sliver to an unresolvable thickness. The individual fronts in the hummocky material are perhaps 15 m high. It is clear that viscous materials were involved in both cases.

It would appear that the apron was produced by a lava flow which was followed by several superincumbent flows of highly viscous lava. The later lavas issued from fissures or vents, some of which may be seen in the *Orbiter* I pictures. A lunar lava flow, however, would

de-gas rapidly and produce a very porous, indurated rock froth close to the surface. This frothy layer would be an excellent thermal insulator, and hot liquid lava would continue to flow underneath it and pucker or heap it into flow ridges. Thus the steepness of the lava fronts would be an indication of the viscosity and strength of the outgassed materials rather than of the original lavas.

Because the mechanism of coacervation of lava takes an extreme form, on the Moon, the observed shortness of the flows and the slopes of the lava fronts are to be expected even if the original lavas were of a type that would have produced longer flows with lava channels under terrestrial conditions.

Some craters can be observed in both the apron materials and the hummocky flows, but these craters are few in comparison with the numbers observed in equal areas of the surrounding terrain. Both the apron and the hummocky flows are of relatively bright, lunaritic material. These flows can be grouped together and referred to as the lunaritic lava flows. The adjacent terrain is of darker mare-type material and will be referred to as lunabase.

At least four of the largest craters in the lunaritic flows are conical or dimple craters, which suggests collapse following the withdrawal of lava. Other craters tend to collect along the saddles formed by the adjacent mountains, so it is improbable that these craters are impact craters.

Along a total of 180 km of lunaritic lava fronts, and over 60 km of wrinkle-ridge lava fronts (Figs. 4 and 5) that are clearly associated with the ring-fracturing of Flamsteed  $P$ , there are sixteen probable cases of craters which have been overlapped by the lava flows, one certain case of a 1 km ghost ring which has been overlapped, and two cases of craters which have apparently been distorted by the flow of lava. On the other hand, seven craters bite into the lava fronts, and a few partial rings also intrude into the lunaritic flows. One partial ring, 1,100 m in diameter, intrudes into a well defined hummocky flow and downfaults it. This is evident from the mutual relation of the flow lines or ridges and the wall of the ring. The ring itself is probably a caldera because its level floor is bordered by an arcuate chain of craters which may well be the locus of one of the fractures along which subsidence proceeded and from which gas-charged lavas were extruded.

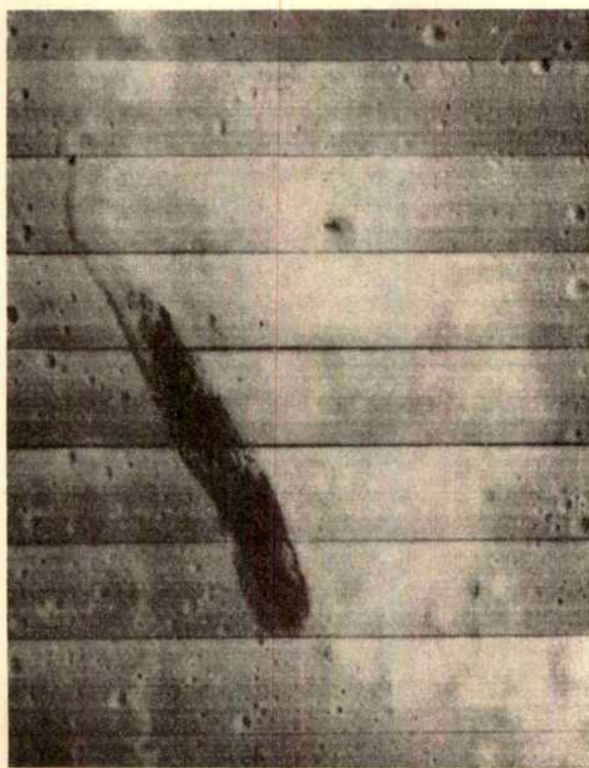


Fig. 5. Flamsteed  $\beta$ , enlarged from Fig. 1, showing flow ridging and steep terminal slope of lavas. Total area shown about 9 km  $\times$  12 km. (N.A.S.A. photograph.)



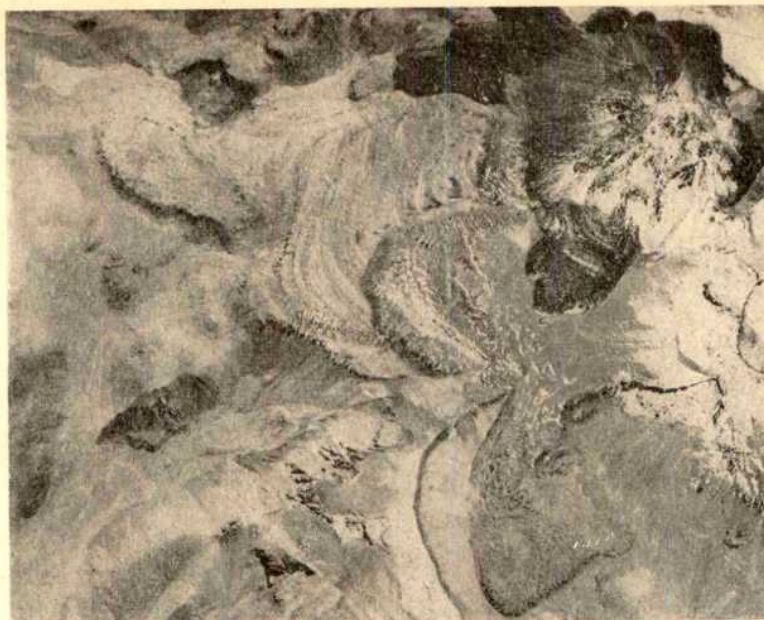


Fig. 6. Flow ridging of a viscous, intermediate lava in Chile. Characteristics of this flow are the same as those applying to the lunar lavas. Total area shown about 13 km  $\times$  10 km. (After J. E. Guest.)

Many of the plentiful eumorphic craters in the lunabase around the lunaritic lava flows are without raised rims; stereoscopic views indicate that they are pits in the ground. It is also possible that they are small calderas of collapse. Others, with rims, may be impact craters.

The relation between the lunaritic lavas, the materials of the lunabase, and the craters and rings shows that some craters and rings were formed before the peripheral lava flows of Flamsteed *P* and other craters and rings were formed later. The counts may be too few to be of great significance, but it appears that twice as many craters and rings were formed in the lunabase before the apron lavas were extruded as after they had solidified. The relative paucity of craters in the lunaritic lavas adds to the foregoing observations to suggest that the lunaritic lavas are in general more recent than the surrounding lunabase. The steep terminal slope and the apron lavas, however, are not always present; in places where these features are absent, the hummocky lavas extend down to, and seem to dip under, the lunabase. Thus it is plausible that lavas of both the lunarite and the lunabase were coeval, one sometimes flowing over the other; it therefore appears that counts of craters do not offer sufficiently reliable data on which to base the dating of the different lava flows. Indeed, it seems that the lunarite carried fewer craters than an equal area of contemporaneous

lunabase and, thus, that the majority of craters are not of impact origin.

This premise about the small craters must await confirmation, but the conclusion that the ringwall of Flamsteed *P* is a youthful volcanic feature is inescapable. Similar evidence that the walls of lunar ring structures and isolated ridges and domes are volcanic extrusives can be found on many of the *Orbiter* photographs. For the first time, the slopes of dome shaped hills are seen to preserve concentric ridging characteristic of flow ridging.

These results demolish the long held hypothesis that all low "elementary rings" on the Moon are old worn-down impact craters. They answer Marcus's<sup>3</sup> criticism of an argument I used to support the volcanic hypothesis. They imply a source of heat for comparatively recent lunar volcanism.

I am most grateful to Dr. J. A. O'Keefe, R. G. Strom, and E. A. Whitaker, all of whom drew my attention to the presence of lava flows around Flamsteed *P* before I had seen the pictures, and I thank the U.S. National Aeronautics and Space Administration for providing the *Orbiter* photographs. I also thank J. E. Guest for useful discussions on lava flows.

Received January 16, 1967.

<sup>1</sup> Fielder, G., *Lunar Geology*, 146 (Lutterworth Press, London, 1965).

<sup>2</sup> Guest, J. E., thesis, Univ. Lond. (1964).

<sup>3</sup> Marcus, A., *Mon. Not. Roy. Astro. Soc.*, **134**, 269 (1966).

(Continued from page 331)

THE Carbohydrate Discussion Group (a Chemical Society Subject Group) is to hold a meeting in the University of Bristol during April 7-8. Further information can be obtained from Dr. R. D. Guthrie, The Chemical Laboratory, The University of Sussex, Falmer, Brighton, Sussex.

A CONFERENCE on "Molecular Sieves", organized by the Society of Chemical Industry, is to be held in London during April 4-6. Further information can be obtained from the Honorary Secretary, Conference on Molecular Sieves, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

THE ninth international conference of the Society for Biological Rhythm is to be held in Wiesbaden during April 6-8. Further information can be obtained from Professor Werner Menzel, Amalie-Sieveling-Krankenhaus,

Farmsener Landstrasse 73, Hamburg-Volksdorf, West Germany.

A SYMPOSIUM on "Biomechanics", sponsored jointly by the Rock Island Arsenal, Army Weapons Command, Army Research Office-Durham and Augustana College, is to be held on the Augustana College campus during April 5-6. Further information can be obtained from RIA Biomechanics Symposium 1967, c/o Professor J. E. Ekblad, Augustana College, Rock Island, Illinois.

A THREE day symposium on "The Ocean from Space", sponsored by the American Society for Oceanography and the Gulf Universities Research Corporation, is to be held in Houston, Texas, during April 5-7. Further information can be obtained from "The Ocean in Space", American Society for Oceanography, P.O. Box 53600, Houston, Texas.



## BOOK REVIEWS

## SCIENCE AND CRAFT

## Les Mécaniques de Galilée

Traduites de l'Italien par le P. Marin Mersenne. Edition critique par Bernard Rochot. (Le Mouvement des Idées au XVII<sup>e</sup> Siècle, 4.) Pp. 80. (Paris: Presses Universitaires de France, 1966.) 10 francs.

La Vie Domestique dans le Mâconnais Rural Préindustriel Par Susanne Tardieu. (Université de Paris. Travaux et Mémoires de l'Institut d'Ethnologie, Tome 69.) Pp. 525. (Paris: Institut d'Ethnologie, 1964.) 120 francs.

ENGLISH readers have a modern translation of this work of Galileo's published by Stillman Drake in 1960. *Le Meccaniche*, composed about 1600 for the benefit of Galileo's pupils at Padua, was only printed during his lifetime in this French version (it is not a straight translation) by Mersenne. Several manuscript copies of the original are known; one had come into Mersenne's hands by 1629. This little book is not to be numbered among Galileo's major creations, for it is after all no more than his version of the "treatise on the five simple machines" that had descended from the pseudo-Aristotelian *Mechanica*. Like everything Galileo wrote, it contained interesting touches; but it does not contain his discoveries in mechanics.

In 1629, although Galileo was world famous as an astronomer and controversialist, his fruitful studies in mechanics were known only to few intimate friends, although they were (probably) virtually completed by 1609. By 1634, when this little book of Mersenne's appeared, the position had changed doubly: since his trial and abjuration in the previous year Galileo had become a hero to some, a heresiarch to others, while the cause of this trouble, the *Dialogue on the Two Chief Systems of the World*, had also revealed his new mechanical notions for the first time.

It seems to have been the intention of Marin Mersenne, a great admirer of Galileo despite his holy orders, both to add distinction to the Italian's name and to make his work in mechanics better known in France, though indeed, in the present book, he could do little enough in the latter respect. Hence he was to take up this same task again, in *Les Nouvelles Pensées de Galilée* (1639).

The main historical interest of the little book here re-issued revolves around the "translator" rather than the original author. It was significant in introducing Galileo to a wider circle of Frenchmen, and for its clear expression, by Mersenne himself, of important mechanical principles. Nevertheless, it must be said that this role was both limited and local.

In her capacious volume, Mlle Tardieu, *chef de service* at the Musée des Arts et Traditions Populaires in Paris, gives the full scholarly treatment to her subject: the household equipment, and the daily life in which it figured, of the Mâconnais region in south central France—a vine-growing region the best known growth of which is Pouilly Fuissé, verging on the Beaujolais country to the south—before it was much affected by modern industrialization, that is roughly before 1900. Mlle Tardieu's scholarship does nothing to detract from the fascination of her material, compiled partly from objects in museums or still in use, partly from the recollections of aged villagers, and partly from notarial inventories of household goods. The point of the enquiry is, of course, that life

in this quiet, poor region (of whose place-names the *Oxford Atlas* lists only the towns of Mâcon and Cluny) changed little from the seventeenth century to within living memory; changes in detail occurred—the replacement of resin tapers by tin lamps fed first with rape-oil, then kerosene—and perhaps there was some slight secular increase in comfort, but basically the same harsh structure of peasant village life continued. The domestic interior remained barely furnished, even though earthenware partially supplanted pewter, unremitting toil in the fields went on with the same beasts and the same tools. The Mâconnais has declined in population since the end of the eighteenth century; if any one still wonders why all people at all ages have fled from country to town when they could, they might reflect on the winter "veillées" of the Mâconnais, where the fuelless families gathered turn by turn in each other's cowsheds, each family in turn providing the glimmer by which the women span. The reason for sitting together in the cowshed was to join the warmth of human bodies as closely packed as might be with that of the animals, and that of their dung left to heat by putrefaction. The same people were sometimes forced to set the bread-dough to rise in their beds, employing the same resource to keep the noon soup hot for supper.

In Britain we are historically more familiar with the social problems of industrialization: the factory, mine and slum. This book describes, with great wealth of solid information but without either romance or pathos, the reality of the "old world" which must be measured against the horrors of progress: a world in which, for the village, conscription might be a family tragedy, phylloxera a demographic disaster.

A. RUPERT HALL

## ABBE AND AFTER

## The Theory of the Microscope

By L. C. Martin. Pp. xiv+488. (London and Glasgow: Blackie and Son, Ltd., 1966.) 100s. net.

It is very nearly a hundred years since Abbe formulated his diffraction theory of image formation. That hundred years has seen some tremendous advances in practical microscopy and other branches of optics. The best microscope objectives are not far short of practical perfection; new methods such as phase contrast and interference microscopy are commonplace and the development of lasers and holography offers exciting possibilities of further progress. It would be wrong to say that theory has lagged behind, but it is some measure of the complexity of the subject that this is the first comprehensive attempt to deal with the problem from the standpoint of modern wave optics.

The microscope is only an optical instrument, after all, but it is exceptional in that it uses lenses of unusually high aperture and is very much concerned with achieving the ultimate in resolving power. These peculiarities lead to problems not often encountered in other optical instruments, some of which are still unsolved. The first three chapters of this book are, in fact, a very useful introduction to diffraction theory and wave optics. The remaining five chapters deal with image formation with incoherent illumination, the theory of relative coherence, image formation with coherent illumination, the microscopy of phase objects and image formation in cases of partial coherence. There are several mathematical appendixes, including a useful one on Fourier series, integrals and transforms.

Abbe's work served to emphasize the importance of diffraction in image formation, but the narrow conservatism of some of his followers led to a long period of acrimonious discussion, much of it recorded in the *Journal of the Royal Microscopical Society*. Today, the development of partial coherence theory and the better

appreciation of the physical processes underlying image formation render such arguments sterile. Unfortunately, this does not seem to have penetrated to all writers of text-books on microscopy, and one often finds a good deal of heat generated on the relative merits of Köhler and "critical" illumination, on the reasons for using highly corrected condensers and on the exact limits of resolution. Professor Martin's book discusses such matters and many more. It is a considerable achievement to have covered so much ground while striking a nice balance between mathematics and physical principles. To have done so after official retirement is little short of amazing.

R. BARER

## ELECTRON MICROSCOPY

### Introduction to Electron Microscopy

By Cecil E. Hall. Second edition. (International Series in Pure and Applied Physics.) Pp. ix + 397. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1966.) 140s.

It is unusual to find a second edition of a book concerned with applied physics which is shorter than the original. When the subject is electron microscopy, it is little short of astonishing. As the author says in the opening sentence of his preface, the rate of advance has been rapid since the first edition appeared in 1953. Yet he has been able to cut the text by more than 50 pages, while including a certain amount of new material.

It is questionable whether his efforts have been altogether successful. Some of the matter excluded, chiefly descriptions of commercial models of electron microscopes and of particular applications, could well have been retained if only to give the reader a clearer view of what the finished instrument is like and of its power as a research tool. On the other hand, the positive revision of the text has been much less thorough than is suggested by the blurb. The main body of the text, in fact, remains unchanged although a certain amount of re-arrangement has been done, such as the transfer of the historical survey from Chapter 8 to the opening chapter where it properly belongs. The "considerable revision" of Chapters 8 and 9 comes down to the addition of a few paragraphs on electron scattering, on measurements of Fresnel fringes and on television display systems. Chapter 10, on "Techniques and Applications", has necessarily received greater revision.

From the point of view of microscopy as at present practised, there is inadequate treatment of anti-contamination devices, photographic emulsions for electrons and the examination of metal films. In respect of current research with a bearing on the future of electron microscopy, there is only a bare mention of quadrupole lenses and of energy analysis of the imaging beam, and none of Riecke and Ruska's new type of objective lens. The section on high voltage microscopes has now been omitted, in spite of the striking work going on in France and Japan. In general, recent work in France and Germany is little mentioned; the names of Castaing and Möllenstedt do not appear in the author index.

That said, it remains true that this is the best text-book on the subject we have. It was originally, and still is, based on a one-term course given in the Biophysics Department of M.I.T. As such, it is rightly concerned with fundamentals and not with a complete presentation of the state of the art. To do the latter now calls for the combined efforts of a team of specialists in its various branches, as for the recent French work in two volumes, *Traité de Microscopie Électronique*. Taken together with the books listed in his appendix, Professor Hall's text will continue to serve as the recommended introduction for research students and others new to electron microscopy.

V. E. COSSLETT

## REINVASION OF CANADA

### Evolution of Canada's Flora

Edited by Roy L. Taylor and R. A. Ludwig. Pp. viii + 137. (Toronto: University of Toronto Press; London: Oxford University Press, 1966.) 44s. net.

THE Founding Meeting of the Canadian Botanical Association held in Ottawa in 1965 developed the scientific theme of the evolution of the flora of Canada, and this volume is a symposium of the chief papers presented. Since the Tertiary uplift of the Rocky Mountains system, Canada has undergone a sequence of glaciations of great extent and severity. Very little trace remains of the effect of pre-glacial or even interglacial conditions and the whole of the existing fauna and flora is the consequence of migration and re-establishment since the maximum of the Wisconsin glaciation about 20,000 years ago. Canadian botanists have begun to exploit the essential tools for reaching objective conclusions on the Quaternary history of their vegetation—radiocarbon dating, palynology, the identification of macroscopic plant remains, the applications of genetic analysis and the detailed study of patterns of distribution. This symposium reflects the progress made by the use of these methods since the pioneering research into biogeography by Marie-Victorin and Fernald early in this century. Contributions on algal zonation on the Pacific coast and on the nature of vegetational propagation have only a marginal link with the main theme of the volume, but are very interesting in themselves. Those contributions which lie in fields of current scientific activity are of a high standard and great interest. One or two others concern themselves less rewardingly with consideration of general concepts. The general unevenness is a consequence of the sparsity of botanical scientists in so vast a territory as Canada, but the symposium affords very welcome evidence of activity and advance towards biogeographic studies which will be of great importance not only to botany but to associated disciplines of archaeology, geology and climatology.

H. GODWIN

## ADVANCING BOTANY

### Advances in Botanical Research

Vol. 2. Edited by R. D. Preston. Pp. xii + 382. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 75s.

THIS second volume of *Advances in Botanical Research* contains six articles by leading authorities in their respective fields, which include the phyletic implications of flagellar structure (I. Manton), numerical taxonomy (W. T. Williams and M. M. Dale), the ultrastructure of the wall in growing cells (P. A. Roelofsen), cell wall protein (D. T. A. Lamport), embryology (P. Maheshwari and N. S. Rangaswamy) and soft rot fungi (J. Levy).

In his introduction to the first volume, the editor explained that the authors had been invited to express opinions freely and "to speculate as widely as they dare". The articles, therefore, differ in character from the somewhat colourless catalogues so frequently encountered in review journals, and we have lively and stimulating accounts, in which the personalities and viewpoints of the authors come through clearly.

With so wide a range of specialisms, it would be presumptuous to comment in detail on the particular articles. But, judged from the standpoint of the non-specialist reader who wishes to bring his reading up to date in fields outside his own specialism, the articles differ considerably in intelligibility and in interest to the general reader; some, such as the articles on cell wall ultrastructure, embryology and soft rot fungi, can be followed easily and profitably by the non-specialist, whereas others are too difficult or too specialized and are primarily of value to



the research worker in the particular areas covered. Thus, one is led to ask, first, for whom is this type of book intended and, second, does it fill a special need not already met by existing journals? The answer to the second question would seem to be that there are certain areas of botanical research not covered by specialist review journals, and that there is therefore a place for this series, with its very wide coverage. But the very breadth of the series creates its own problem, since it is unlikely that the reader will find more than one article in each volume lying within his own field, as compared with a review journal devoted to a particular branch of botany. Consequently, if this series is to serve more than a small band of specialists, most of the articles will also have to be intelligible to the non-specialist reader who wishes to read further within, or outside, his own branch of botany, and the subjects will have to be chosen for their appeal to a reasonably wide audience.

This plea for a little more consideration for the non-specialist reader should not obscure the fact that all the papers in this volume are of a very high quality and that it can confidently be recommended as a valuable contribution to current botanical thought.

P. F. WAREING

## MOLECULAR PARASITOLOGY

### Biochemistry of Parasites

By Theodor von Brand. Pp. ix+429. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 128s.

THIS book is not simply a revision of the previous edition (1952), entitled *Chemical Physiology of Endoparasitic Animals*, for it has been rewritten with the shift of emphasis indicated by the new title. By combining all aspects of biochemical structure and function of each chemical class into a single chapter, the number of chapters has been reduced from twenty to nine. As well as three chapters devoted to carbohydrates, there are separate chapters on inorganic substances, lipids, proteins, nucleic acids, vitamins and respiration. The inclusion of nucleic acids indicates the advances in this field since 1952, while the chapter in the first edition entitled "Miscellaneous Physiologically Active Substances" becomes the basis of the present chapter on "Vitamins". Much information of a physiological nature, such as osmotic relationships and endocrinology, has been omitted from the present volume. The dispersal of information previously contained in the section "The Biochemical Basis of Chemotherapy" may be a mistake, for such a chapter would be more in line with the compact style of the current edition.

Inevitably, because of the rapid accumulation of knowledge in recent years, the size of the book has increased by almost ninety pages, even though much early work has been omitted where superseded by more recent investigations. The biochemistry of the host-parasite relationship is well dealt with in the new volume, but each topic is included in the appropriate chapter and does not form a separate section as before.

Dr. von Brand's personal experience in much of the work has enabled him to extend the usefulness of the biochemical evidence by pertinent references to related work in the fields of histochemistry, cytochemistry and electron microscopy. There are also many practical tips, such as the use of enzymes for glucose estimation (p. 46) and criteria for the inhibition of enzymes by drug action (p. 177). The book is almost overflowing with well documented facts, and relevant data have usually been condensed into useful tables. Even so, the addition of a short section at the end of each chapter, or even of a separate chapter, discussing biochemical adaptations to a parasitic existence would have provided a better insight

into these important problems of parasitology. The strict separation of chapters sometimes prevents a continuous discussion of biochemical specialization in one parasitic group—oxidative metabolism in the trypanosomes, for example. In its present form the book is therefore of greater use to the experienced research worker than the young student.

A survey as extensive as this also serves as a comment on the present state of biochemical parasitology. It indicates that most of the work has been carried out on a small number of organisms, chiefly malaria parasites, trypanosomes, trichomonads, cestodes and nematodes. Three chapters devoted to carbohydrate biochemistry also indicate the unevenness of the development of metabolic studies on parasites. Dr. von Brand can report little advanced work on the biosynthesis of macromolecules, which occupies the attention of biochemists in other fields. Such studies have been hampered by the difficulties of parasite isolation and maintenance, but the problems are not insurmountable.

In conclusion one can say that this is the only recent book which deals adequately with the biochemistry of all groups of parasitic animals. No errors of fact are to be found in the text, and the index, although not especially exhaustive, is generally adequate.

C. D. GINGER

## MICRO-ORGANISMS TO ORDER

### Theoretical and Methodological Basis of Continuous Culture of Micro-organisms

Edited by Ivan Málek and Z. Fencel. Translated by J. Liebster. Pp. 655. (Prague: Publishing House of the Czechoslovak Academy of Sciences; New York and London: Academic Press, 1966.) 208s.

THIS book is really a series of critical essays on many aspects of the continuous culture of micro-organisms. Inevitably there is some overlap between them. The range covered is wide and, indeed, in the introduction Academician Málek writes: "It is the aim of this monograph to lay firm foundations for the wide application of the continuous cultivation method in microbiological, biochemical, genetic and cytological research and a basis for progress of the fermentation industries". This it does admirably.

Málek prepares the way for his colleagues and also gives a historical account of the development of continuous processes including multi-stage systems. He then looks to the future. J. Řířica deals with the various techniques in considerable detail and K. Beran discusses their application to many problems in pure and applied research. Z. Fencel analyses fully the underlying mathematical theory, while M. Burger focuses his attention on the occurrence of mutations in chemostat systems. Pathogenic organisms are also dealt with and the advantages of continuous cultivation, not only for the production of vaccines, etc., but also in fundamental studies, are pointed out (A. Stejskal). Techniques suitable for the growth of algae and animal cells and for use in soil microbiology are next examined (R. Řetovský, M. Pospíšil, J. Macura). Finally, J. Hospodka considers industrial applications in detail.

The book contains a wealth of practical details pertinent to the construction and operation of suitable apparatus and a very comprehensive bibliography. The translation is adequate, but the legends for Figs. 5.2-1 and 5.2-3 have been interchanged and there are some typographical errors in Fencel's equations. (A list of corrections has been issued but is not, unfortunately, distributed with the book.) Although the price is high, this work should prove invaluable to anyone engaged in the study and the application of continuous processes either in the laboratory or in industry.

A. C. R. DEAN

## ECOLOGY OF MICRO-ORGANISMS

### Principles of Microbial Ecology

By Thomas D. Brock. (Prentice-Hall Biological Science Series.) Pp. xiv + 306. (Englewood Cliffs, N.J.: Prentice-Hall, Inc.; London: Prentice-Hall International, 1966.) 62s.

NOT long ago, inclusion of the word "ecology" in the title of a microbiological research project was a sure way of diverting prospective research students elsewhere. Today the situation is more cheerful and Professor Brock's book should help to dispel the notion that the subject is a dull one. As the first book of its kind, it will be informative for the younger graduate not yet committed to a particular field of research as well as for the larger audience of microbiologists, sanitary engineers, pedologists, petroleum engineers, geochemists, physicians, food sanitarians and limnologists for which it is intended.

In the first three chapters, amounting to about one-third of the text proper, Professor Brock lays the basis for the physiological and biochemical approach to the examination of the behaviour of micro-organisms in natural situations. The experienced microbial physiologist will find much that is familiar to him here. Fragmentation of some sections seems excessive—Chapter 3, on "The Ecology of the Cell", has no fewer than 43 subheadings in 57 pages.

In the remainder of the book Professor Brock sets about the integration of a diversity of information from a variety of fields and the enunciation of principles. He deals with dispersal, population ecology, interactions between microbial populations, microbial ecosystems, interactions of micro-organisms with macro-organisms and microbes in macro-ecology. Sometimes the principles remain elusive and the text becomes reminiscent of a textbook in soil microbiology or chemistry, plant pathology or medical microbiology among others. In my opinion the coverage is too wide but, to be fair, Professor Brock states clearly in his preface that he is attempting to provide a picture of the place of micro-organisms in nature and human society. He has collected and documented much information in a compact and not too expensive space. All credit is due to him for providing a welcome first text on microbial ecology. J. W. HORTON

**Biographical Memoirs of Fellows of the Royal Society**  
Vol. 12. Pp. 564 + 28 plates. (London: The Royal Society, 1966.) 100s.; \$15.

THERE is much interest and useful information in the twenty-eight short biographies contained in this volume. The more famous fellows include Sir Winston Churchill, E. V. Appleton, J. B. S. Haldane and Lord Nuffield, but perhaps the more interesting are the other less well known scientists. Theodore von Kármán was a mathematical prodigy who became a brilliant mechanical and aeronautical engineer. During the First World War he helped to develop the first stable hovering helicopter. He was later a member of the committee appointed to investigate the Akron and Macon dirigible disasters, and was a consultant on the Grand Coulee Dam Project. He was an early supporter of the science of astronautics, when other scientists still classed it as science fiction; von Kármán preferred enthusiasts with imagination to serious scientists without imagination. Hans Pettersson, another foreign member, was the Swedish oceanographer who organized the voyage of the research ship *Albatross*, during which measurements of light absorption and radioactivity as well as biological investigations of the sea were carried out.

Some of these fellows of the Royal Society had strong non-scientific interests. T. G. Brown was a neurophysiologist who climbed Mont Blanc; the Canadian physicist, J. S. Foster, who was involved in the development of radar, drew up plans for a concert hall at McGill University, and R. G. Hatton, who was for thirty years the director of the East Malling Research Station, founded the Kentish branch of the Workers' Educational Association. This volume is full of such fascinating facts; it makes interesting reading and a useful reference book.

MARY LINDLEY

### Early Public Libraries

**A History of Public Libraries in Great Britain Before 1850.** By Thomas Kelly. Pp. 281. (London: The Library Association, 1966.) 56s. (L.A. members, 42s.)

ONCE again Dr. Kelly has demonstrated the compatibility of erudition and lucidity of exposition. Though thoroughly documented, this book will be read with ease by the general reader as well as the scholar, and it has some features of particular interest to a scientist. It notes the relation between the growth of libraries and public interest in science, and the book is welcome for its emphasis on the feature which made Chetham's Library, Manchester, of outstanding importance—provision of adequate income for the purchase of books. Even today, the University Grants Committee, much less the Department of Education and Science, has failed to appreciate the significance of this simple truth for either the new or the old university libraries. Dr. Kelly's book is also of importance as a corrective to the report of the Select Committee on Public Libraries of 1849, which has attracted some attention in recent years, following the centenary of the Public Libraries Act. There is a pertinent reference, in connexion with the origins of the British Museum Library, to Bacon's "Solomon's House" and the utterly inadequate provision for libraries in London in the seventeenth century is duly noted. Primarily a book for the scholar, it will be read with care by anyone interested in social history and particularly in education, whether scientific or technical or general. R. BRIGHTMAN

### Flora of the U.S.S.R.

Vol. 12: Leguminosae: Astragalus. Chief editor V. L. Komarov. Compiled by A. G. Borisova, N. F. Goncharov, S. G. Gorshkova, M. G. Popov and I. T. Vasil'chenko. Edited by B. K. Shishkin. Translated from the Russian by N. Landau. Pp. xxviii + 681. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 182s.

THERE are numerous important revisions in the monumental *Flora of the U.S.S.R.*, edited by Academician V. L. Komarov, but the fact that they were originally written in the Russian language has often been a barrier to non-Russian botanists who wished to consult them. The present English translation is of Volume 12 of this great flora, devoted entirely to the single genus *Astragalus* (Leguminosae).

The great size and complexity of *Astragalus*, which has not been generally revised for the old world since Bunge's work, *Generis Astragali Species Gerontogaeae*, was published in 1868–70, make the present account of great taxonomic importance to any serious student of the genus. The degree of importance is indicated by the fact that, although many of the 103 sections here admitted were delimited by Bunge, no less than twenty-two were established in recent years by N. F. Goncharov, who wrote much of the present work but died before it was quite complete; and also that no less than 302 of the 849 species included were described as new by Russian botanists since 1930. As the preface states, this very large number "does not nearly exhaust the wealth of species of this genus, the greatest in our flora". It is noteworthy that very few infraspecific taxa are recognized and,

presumably as a matter of deliberate taxonomic policy, no sub-species.

The greater part of the genus was revised by Goncharov, a lesser but nevertheless important part by A. G. Borisova, and other smaller portions by other authors.

The translation by Dr. N. Landau appears to be efficient and clear, and his undaunted perseverance in ploughing through so many specific descriptions, mostly not written in a particularly enlivening style, is praiseworthy.

J. P. M. BRENNAN

**The Biochemical Genetics of Vertebrates Except Man**  
By I. E. Lush. (North-Holland Research Monographs. *Frontiers of Biology*, Vol. 3.) Pp. viii + 118. (Amsterdam: North-Holland Publishing Company, 1966.) 36s.

THIS monograph, the third in the series "Frontiers of Biology", is devoted to a tabulation of the biochemical variations uncovered so far in mammals, birds and fishes. The author has assembled a large quantity of material, and made interesting reading out of a text which, from its necessary form of presentation, might have been tedious. The subjects discussed in some detail include genetic variations in transferrin, haemoglobin,  $\gamma$ -globulin, esterase, amylase, lactate dehydrogenase and pyrimidine catabolism, to name only a few. This is the first time that an attempt has been made to collect and summarize this material from a widely scattered literature. The concluding chapter includes a clear concise treatise on deletion and duplication in vertebrate biochemical genetics, and the quaternary structure of proteins.

The treatment on the whole is descriptive rather than analytical. One of the most dynamic sciences at the moment is the investigation of the chemical nature and behaviour of the hereditary unit. This publication will serve as a convenient source of reference, not only to biochemists and geneticists interested in this field, but to all researchers who are just awakening to the realization of the powerful research tool and the potentialities afforded by biochemical variants.

H. M. MURPHY

### Radioactive Pharmaceuticals

Edited by Gould A. Andrews, Ralph M. Kniseley, and Henry N. Wagner, jun. (Proceedings of a Symposium held at the Oak Ridge Institute of Nuclear Studies, November 1-4, 1965.) (U.S. Atomic Energy Commission/Division of Technical Information.) Pp. viii + 728. (Springfield, Virginia: Clearinghouse for Federal Scientific and Technical Information, N.B.S., U.S. Department of Commerce, 1966.) \$5.

THE publication of these proceedings comes at a time of particularly rapid growth of the use of radioactive materials in medicine. The purpose of the symposium was to summarize and correlate the recent advances in the development of radioactive pharmaceuticals for use in clinical medicine and biological research. It succeeds admirably. The symposium title *Radioactive Pharmaceuticals* itself acknowledges the technical advances and the change of status which have occurred in the 28 years since thyroid function was first demonstrated with radioactive iodine. To some extent it may also mislead, since a radioactive pharmaceutical is usually administered to provide information, commonly of organ function, rather than to elicit a specific pharmacological response. The papers in these proceedings are accordingly largely devoted to the discussion of radioactive materials used in diagnosis: their production, their characteristics and the techniques for using them. Their applications in radiotherapy are not included.

Recently developed radiopharmaceuticals figure prominently and provide a convincing illustration of the more sophisticated diagnostic techniques now being achieved. The instrumental aspects are not reported in the proceed-

ings though the consequences of concurrent developments in equipment for external body scanning are everywhere apparent.

The forty papers range widely through biochemistry, pharmacology, radionuclide production, nuclear medicine and the radiopharmaceutical industry. The more critical outlook of both suppliers and users of radioactive pharmaceuticals is reflected in the number of papers concerned with quality control and licensing regulations.

The proceedings provide not only a record of what was a well timed and eminently successful symposium, but a valuable reference for all whose work brings them into contact with nuclear medicine.

C. C. EVANS

### Strong Solids

By A. Kelly. (Monographs on the Physics and Chemistry of Materials.) Pp. xv + 212. (Oxford: Clarendon Press; London: Oxford University Press, 1966.) 42s. net.

DR. KELLY has written a book quite unlike any other at present available. He has undertaken a single-minded treatment of a central question: what determines the ideal strength of solids, and what steps can be taken to approach this in practice as nearly as possible.

The first chapter shows how the ideal fracture and shear strengths can be calculated, and what physical parameters must be controlled to maximize them. Next, Dr. Kelly summarizes the fracture promoting properties of cracks, and goes on to expound very selectively some relevant properties of dislocations, in particular the forces binding them to the lattice. An illuminating chapter on presently available strong alloys, including recently developed forms of high strength steel, is followed by a long chapter on the dynamics of fibre reinforced composites. A final chapter is concerned with the practical methods of making these.

The last two chapters include much very recent material, a good deal of it arising from the work of the author and his associates. They constitute the most readable and up to date survey of the reinforcement of plastics and metals by strong fibres. The early chapters deal with older subject matter, yet make stimulating reading, because the author has firmly resisted the temptation to spread his subject matter; he has not hesitated to use without proof formulae in the theory of elasticity or in dislocation geometry, for instance, but has incorporated them in the flow of his argument in such a way that its physical basis is always clear. Also he has been unusually careful to map out his plan of campaign at the start of each chapter, and at each stage to explain exactly what he is aiming at.

The book is up to the very high standards of the Clarendon Press. Apart from a slip near the foot of page 118 and a mysterious footnote on page 150, I found no errors. The book is recommended without hesitation to students of metallurgy, materials science and mechanical engineering, and to their teachers.

R. W. CAHN

### Nonlinear Electron-Wave Interaction Phenomena

By Joseph E. Rowe. (Electrical Science: a Series of Monographs and Texts.) Pp. xiv + 591. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 144s.

THE subject of this book is the non-linear analysis of the interaction between streams of charged particles and propagating electromagnetic waves. The theoretical foundations are laid with mathematical rigour in the first part of the book, while the remainder is devoted to the detailed application of the theories to various  $O$  and  $M$  type microwave tubes, and to some related techniques and problems. It is essentially a very full and systematic theoretical analysis of microwave tubes in which the theory has been developed with sufficient generality to be of

interest and use in other areas. One such area—beam plasma interactions—is dealt with in Chapter 12.

Particularly satisfying features of the book are its depth and the way in which problems are given alternative treatments together with critical discussions. These qualities are important in view of the fact that the book is written as a research monograph at a level appropriate to advanced graduate students. Knowledge of linear theory is assumed. The treatment is almost entirely theoretical, and one seldom finds experimental evidence in support of the theories developed. A consequence of dealing with non-linear equations is the difficulty of obtaining analytic solutions. There are therefore, as one would hope and expect, a large number of figures presenting computer results. The problem of gaining physical insight from such results is recognized, and is tackled with some success.

This book, which is well referenced and adequately indexed, is written with authority derived from wide research and teaching experience, and constitutes a valuable and readable contribution to the literature on microwave tubes.

I. B. BORT

### Thermal Neutron Scattering

Edited by P. A. Egelstaff. Pp. xv + 523. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 115s.

THE investigation of molecules, of liquids and of solids by thermal neutron scattering rests on the idea that the intensities of scattered particles which have lost energy and momentum are a measure of the density and corresponding spatial and time Fourier components of scattering power. The measurement of these intensities involves refined techniques of mechanical or diffractive velocity determination, and requires reactor sources, moderators, and scintillator detectors or proportional counters. This volume surveys the development of the subject since the great advances in technique during the fifties made it a productive field of study. Although there are fourteen different authors, the unity of presentation is unusually good and as a consequence the book serves as an introduction to the subject that will be of value to the non-specialist molecular or solid state physicist. For the specialist, the volume will also be valuable, as the field is not over-endowed with review literature.

J. B. HASTED

### Beginning Geology

By H. H. Read and Janet Watson. Pp. 246. (London: Macmillan and Co., Ltd., and George Allen and Unwin, Ltd.; New York: St. Martin's Press, 1966.) 30s. net.

ALTHOUGH the layman can probably appreciate geology more easily than almost any other science, geologists (with a few notable exceptions) are notoriously bad at transmitting the essence of their subject to the uninitiated. Read and Watson's writings admirably fill this gap in communication. Unfortunately, their book is aimed primarily at schoolchildren, so that the style is sometimes reminiscent of a patronizing matey teacher. Otherwise, I have nothing but praise for this book. The scope of the book is the field of geology; this is comprehensively covered, while the treatment, though simple, is deep, and fairly complex concepts (often not dealt with adequately in supposedly more advanced text-books) are presented. The choice of examples—so important in any phenomenological science—is judicious, as is the choice of diagrams and photographs, while the layout both pleases and aids assimilation of the contents. Illustrations are drawn to a large extent from the British Isles, but the book is not nearly as chauvinistic as many others in this respect. Even if it were not so reasonably priced, this book would still be a worthwhile buy for those who are "beginning geology".

A. M. MARSHALL

## OBITUARIES

### Sir Denis Browne

SIR DENIS BROWNE, who died on January 9, can be called the father of paediatric surgery.

In his native Australia he was educated at King's School, Paramatta, New South Wales, and at the University of Sydney, where he obtained his M.B. He served during the First World War with the Australian Army Medical Corps. Afterwards he came to England and obtained his F.R.C.S. Most of his working life was spent at the Hospital for Sick Children, Great Ormond Street, where he was houseman, registrar and consultant. During the Second World War he was the leader of a surgical team in a casualty clearing station in the basement of the hospital. Browne's surgical interests ranged from genito-urinary surgery to orthopaedics, for which he developed and modified instruments, appliances and operations. He developed many instruments, including a needleholder, dissectors, plastic shears and tonsil forceps, and appliances which reflect his interest in the treatment of congenital deformities. There are splints for the treatment of club foot and harnesses for the correction of spinal deformities and congenital dislocation of the hip. He led the treatment of this last condition by means of closed reduction and manipulation. He perfected the technique for the manipulative treatment of talipes equinus—a form of club foot—and repaired hare lip and cleft palate. In other fields of child surgery his inventions included a transthoracic approach and incision to correct congenital heart deformities, and an operation for undescended testicle.

Among the awards which Browne received for his services to paediatrics was the Dawson Williams Prize, and he was an honorary member of the French Society of Urology. His other activities included his four terms as president of the British Association of Paediatric Surgeons and membership of the B.B.C. General Advisory Council. He was also an expert shot; he preferred to ride in taxis, and would never own a car.

### Dr. William Meggers

WILLIAM FREDERICK MEGGERS died of a heart attack on November 19, 1966, aged 78. In 1958 he had retired as chief of the spectroscopy section of the U.S. National Bureau of Standards, which he joined in 1914 as a laboratory assistant, with a B.A. from Ripon College, an M.A. from Wisconsin and a Ph.D. from Johns Hopkins University, all in physics.

His early work, carried out with C. G. Peters, produced measurements of the refractive index of air which were used for more than 25 years. Later he worked on the determination of atomic energy levels for the analysis of spectra, which he did very well and in very great detail. Meggers also utilized the neutrons from atomic reactors to transmute gold to a single isotope of mercury, which he used to produce his mercury 198 lamp. This lamp gives off a green spectral line so pure that its wavelength can be determined to one part in one billion. The wavelength of this light has served as a working standard of measurement in spectroscopy and metrology throughout the world. Meggers was also among the first to observe atomic spectra in the infra-red region.

Meggers was active on a number of committees concerned with spectroscopy, and in 1949–51 he was president of the Optical Society of America, which gave him its two highest awards. He also received medals from the Franklin Institute of Pennsylvania, the Department of Commerce and the University of Liège. After his retirement, Meggers remained as a guest worker at the National Bureau of Standards, and continued to contribute to research in atomic spectroscopy.

# The Solar Wind outside the Plane of the Ecliptic

by

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The scintillation of radio sources caused by the interplanetary medium offers a means of studying the motion of the solar wind well away from the plane of the ecliptic, where direct measurements have so far been confined. Observations from a triangular arrangement of radio receivers (on 81.5 Mc/s) suggest that the solar wind is faster over the solar poles than in the plane of the ecliptic.

DIRECT measurements of the solar wind carried out from space vehicles have, so far, been confined to regions close to the plane of the ecliptic at distances of the order of one astronomical unit from the Sun. Interplanetary scintillation<sup>1</sup> provides a new technique for studying the solar wind over a much wider range of heliocentric latitude and radial distance, and some preliminary measurements of the drift motion of scintillation diffraction patterns across the surface of the Earth using two spaced observing sites have already been reported<sup>2</sup>. In this note an account is given of more extensive observations made between February and July 1966, using three observing sites. Both the magnitude and direction of the solar wind have been measured and evidence has been found that the wind velocity from the polar regions of the solar

atmosphere is significantly greater than that in the plane of the ecliptic.

A triangular arrangement of observing sites was used as shown in Fig. 1. The antennas at each site were transit instruments with similar collecting areas of about 800 m<sup>2</sup> and operated at a frequency of 81.5 Mc/s. Identical receivers having a time constant of 0.2 sec were used and data from the outstations were returned to Cambridge using G.P.O. telephone lines. Before April 10, a three-track pen recorder was used and the records were analysed as described before<sup>2</sup>. After this date the output of each receiver, sampled at intervals of 0.12 sec, was digitized and punched on a single paper tape. For the latter period, which included more than half the observations, data reduction was carried out entirely automatic-

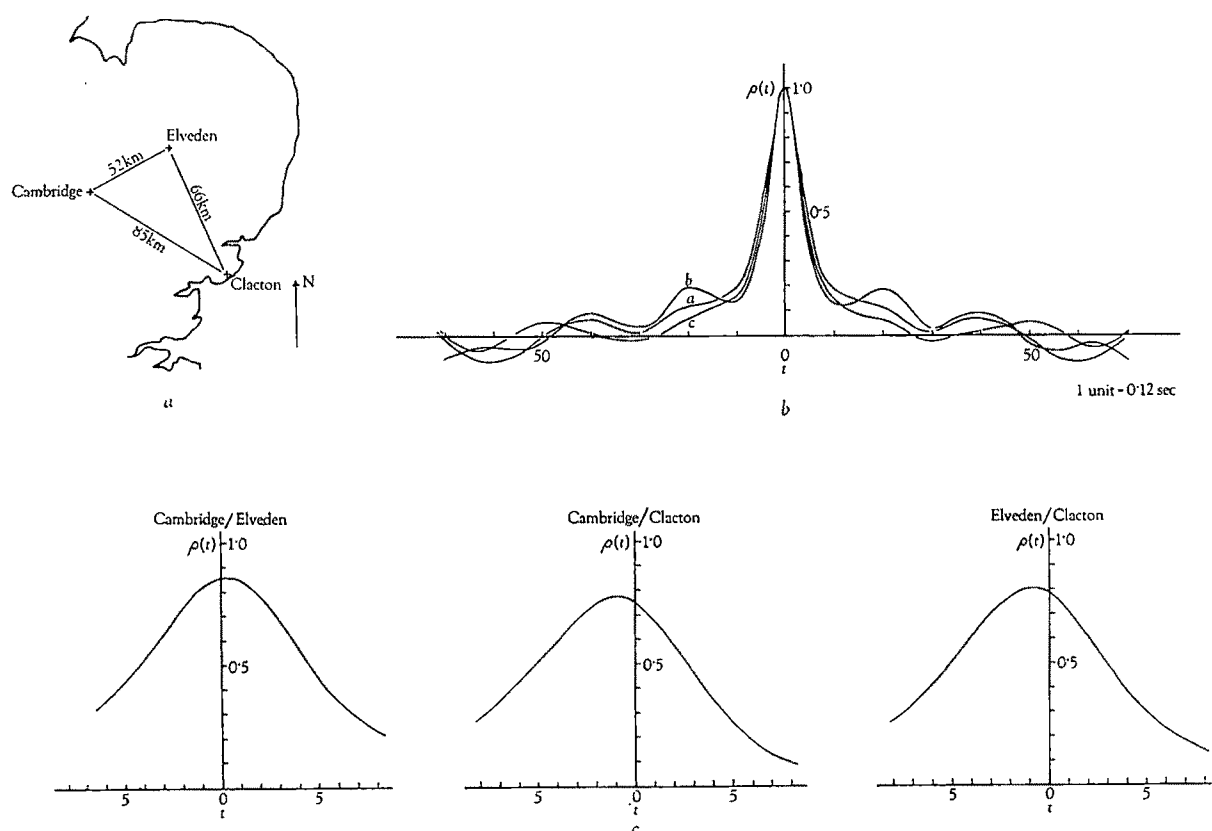


Fig. 1. (a) Location of the observing sites. (b) Typical auto-correlograms and (c) cross-correlograms derived from observations of May 9, 1966.



ally. Most of the measurements were confined to the radio source 3C 48; a few observations were also made using the small diameter component<sup>3</sup> of the Crab Nebula.

The analysis was confined to an interval of one or two minutes each day from which the auto-correlogram of each receiver output and the three cross-correlograms were derived. The results for a typical day are shown in Fig. 1. The fluctuations of intensity, the auto-correlogram of which usually decayed to  $e^{-1}$  in about 0.6 sec, were found to be highly correlated at the three sites, while the cross-correlograms exhibited systematic displacements of the order 0.1–0.2 sec.

The derivation of the drift velocity of a diffraction pattern using data of this kind is straightforward if the pattern is isotropic, while a slightly more complex analysis is necessary for the anisotropic case<sup>4</sup>. The present results were obtained by assuming that the pattern is isotropic; possible corrections arising from the errors in this assumption are believed to be small for reasons which will be discussed later. Noise fluctuations on the records led to a probable error of  $\pm 0.03$  sec in the time displacement derived from the observations on any one day, and this corresponds to an error of about  $\pm 10$  per cent in the derived velocity.

The magnitude and direction of the velocity of the diffraction pattern derived from the cross-correlogram data are shown in Fig. 2(a). It is seen that the direction of motion across the ground varied systematically from a position angle of about  $+50^\circ$  in February to about  $-100^\circ$  in June. The broken curve in Fig. 2(a) shows the expected direction calculated for an assumed radial outflow from the Sun, after making a small correction for aberration caused by the orbital velocity of the Earth, and it is seen to fit the observations within the experimental uncertainty of  $\pm 15^\circ$ .

The average magnitude of the velocity, which varied from about 300 km/sec when the line of sight passed the Sun at a distance of 0.8 A.U. to 490 km/sec at a distance of 0.36 A.U. where the line of sight crossed the polar region of the solar atmosphere, is shown in Fig. 2(b). In relating the velocity of the diffraction pattern to the solar wind the effect of integration along an extended line of sight must, however, be taken into account. The observed velocity represents a weighted average the value of which is dominated by the regions of greatest scattering which occur where the line of sight is closest to the Sun. On either side of this region apparent motion perpendicular to the line of sight corresponds to a resolved component of the

true radial velocity. Estimates based on the observed radial dependence of interplanetary scintillation<sup>1,5</sup> suggest that the true velocity may be 5–10 per cent greater than the values shown in Fig. 2(b). The random day to day changes of velocity are frequently greater than the experimental error and are believed to be real.

During the period of observation the heliocentric co-ordinates ( $p, \phi$ ) of the point of closest approach of the line of sight varied as shown in Fig. 3(a), and in Fig. 3(b) the observed velocities have been plotted as a function of these co-ordinates. There was a significant increase of velocity as 3C 48 approached the Sun, which might be associated either with decreasing radial distance or with increasing heliocentric latitude. The possibility that this increase was caused by temporal changes in the solar atmosphere, rather than by a systematic variation of heliocentric co-ordinates, does not seem likely, because the increase was maintained for a period of at least two weeks during which there was no outstanding solar activity.

Further measurements on other radio sources are needed to show that the increase of velocity is primarily a function of heliocentric latitude, although it seems unreasonable that the velocity should increase with decreasing radial distance since gravitational deceleration is negligible. Some limited data have been obtained from observations of the scintillating component of the Crab Nebula during July 1966, and the results are shown in Fig. 3(d). At this time the line of sight lay close to the plane of the ecliptic, but observations at distances less than 0.4 A.U. were unfortunately not possible because of the decrease of scintillation at small angular separation which has been noted before<sup>3</sup>. For  $p \approx 0.4$  A.U. an average velocity of 295 km/sec was obtained as compared with 420 km/sec derived from observation of 3C 48 at the same radial distance. This result strengthens our earlier supposition that the solar wind has a greater magnitude at high heliocentric latitude. Further evidence is provided by the rate of scintillation on other sources<sup>1</sup> which has been shown to increase with heliocentric latitude in a manner consistent with the same picture.

In addition to measurements of velocity it is also possible to draw conclusions about the scale and life-time of the diffraction pattern. Precise results require careful consideration of the effects of random noise in the separate records which become important when, as in the present case, the pattern is highly correlated at the three observing sites. Analysis of the cross-correlograms following the

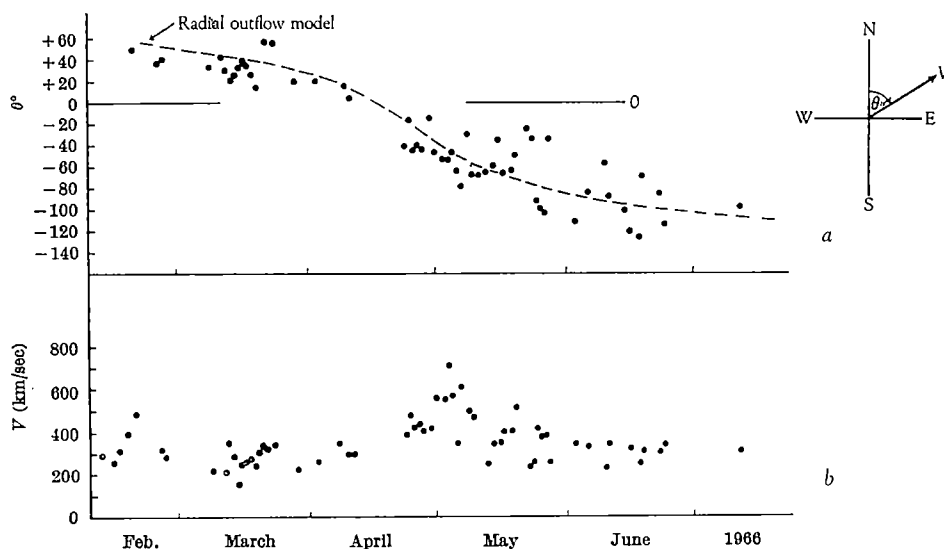


Fig. 2. (a) Observed direction of motion of the diffraction pattern across the ground. The broken curve shows the direction expected for a strictly radial outflow from the Sun. (b) The magnitude of the drift velocity.

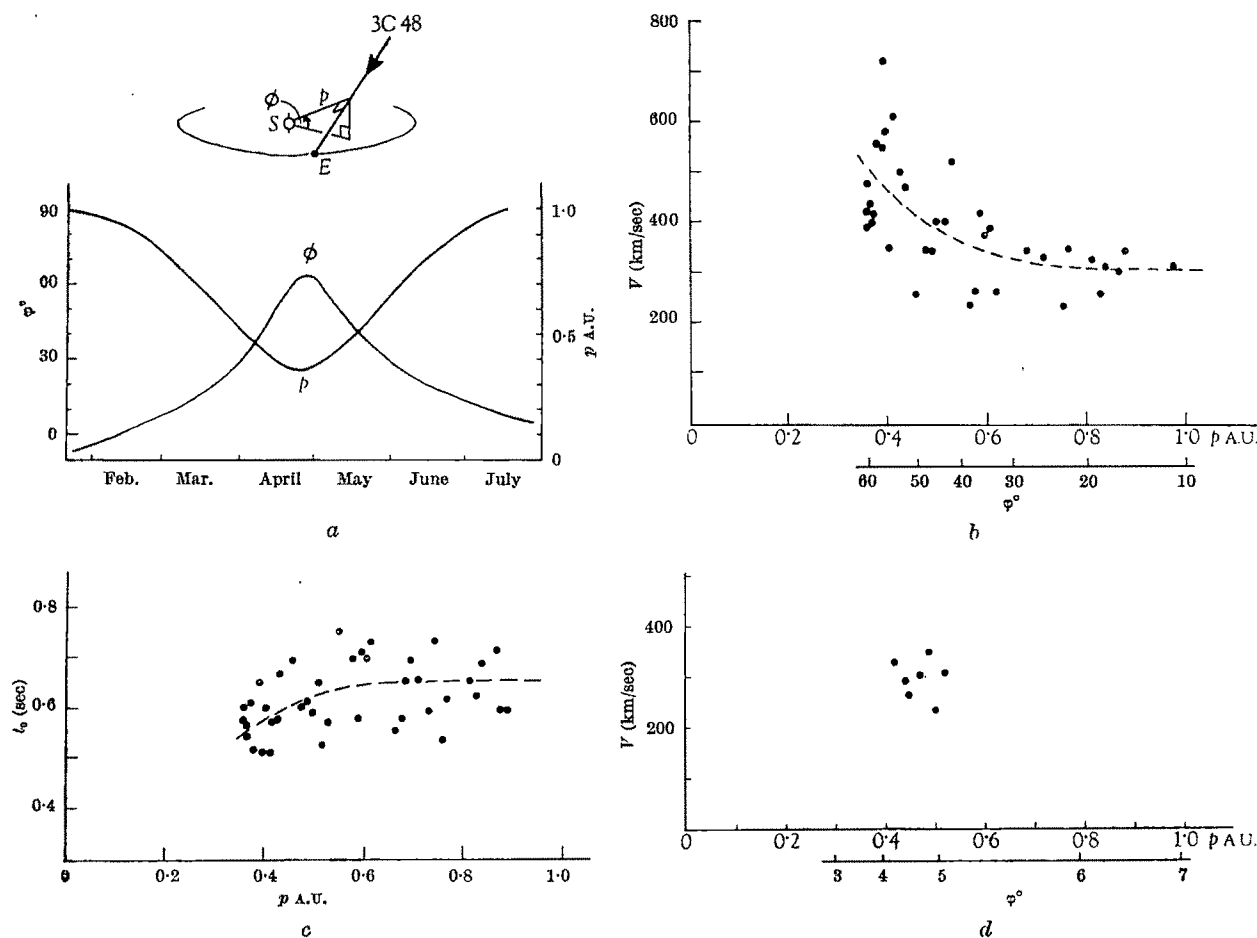


Fig. 3. (a) Heliocentric co-ordinates of the point closest to the Sun on the line of sight to 3C 48. (b) The observed velocity as a function of heliocentric co-ordinates for 3C 48. (c) Width (to  $e^{-1}$ ) of the auto-correlogram of the temporal fluctuations of intensity. (d) Observed velocity derived from observations of the Crab Nebula.

method described by Phillips and Spencer<sup>4</sup> indicates that the spatial correlation falls to  $e^{-1}$  in a distance of about 160 km. Since it has been shown that the interplanetary medium is a weak scatterer for  $p > 0.5$  A.U. (ref. 8), it follows that this remarkably small scale reflects the true size of the plasma density fluctuations themselves. The scale derived from cross-correlation does not differ significantly from estimates of the scale derived from auto-correlation on the assumption that the temporal fluctuations at one site are caused entirely by the drift motion of an unchanging pattern. It follows that the life-time of the pattern must be longer than the correlation time of 0.6 sec. The correlation time observed for various positions of 3C 48 relative to the Sun is shown in Fig. 3(c) where the heliocentric co-ordinates refer to the point on the line of sight closest to the Sun. It is seen that the correlation time decreased as 3C 48 approached the Sun, a result which is anticipated in view of the observed increase of velocity of the diffraction pattern. The decrease of correlation time is, however, not as large as would be expected and this leads to the conclusion that the scale of the diffraction pattern is somewhat greater when the line of sight is closer to the Sun. This effect is probably associated with the changing distance of the Earth from the dominant diffracting region and does not necessarily imply that the plasma irregularities themselves are larger when close to the Sun. For a line of sight such that  $p \approx 0.4$  A.U., the strongest irregularities are 0.9 A.U. distant from the Earth while for  $p \approx 0.9$  A.U. their distance is only 0.4 A.U. Now the diffraction pattern can only contain amplitude variations the lateral scale of which is smaller than the Fresnel zone radius, and it

follows that, if the irregularities in the medium have a range of sizes, then the scale of the pattern will tend to increase as the distance from the diffracting region is increased. This phenomenon could readily account for an increase of scale for small values of  $p$ .

The scale of the diffraction pattern derived from cross-correlation is slightly elongated in a direction parallel to its motion, but the mean axial ratio of the characteristic ellipse is less than 2:1. Such an elongation might be expected since earlier measurements of the angular spectrum of radio waves scattered by the interplanetary medium have given evidence for decreased scattering in a radial direction<sup>6,7</sup>. The possibility that the elongation of the pattern is caused by the shape of 3C 48 itself cannot, however, be eliminated. A detailed consideration of the effects of source diameter has been given elsewhere<sup>8</sup>, and it has been shown that a source extended preferentially in one direction can give rise to an elongated pattern. There is evidence that the angular dimensions of 3C 48 are critical in this respect, since this source scintillates somewhat less than other sources, and further information is needed before the elongation of the pattern can be ascribed to the interplanetary medium. Phillips and Spencer<sup>4</sup> have shown that the simple auto-correlogram analysis can give rise to errors of up to 20 per cent in the derived velocity when applied to a pattern which is elongated by a factor of 2:1. The errors fall to zero when the elongation is along the direction of motion, however, and it follows that the assumption, mentioned earlier, of an isotropic pattern for deriving velocities is justified.

Further attempts have been made to relate the occurrence of scintillation with solar activity. It is possible

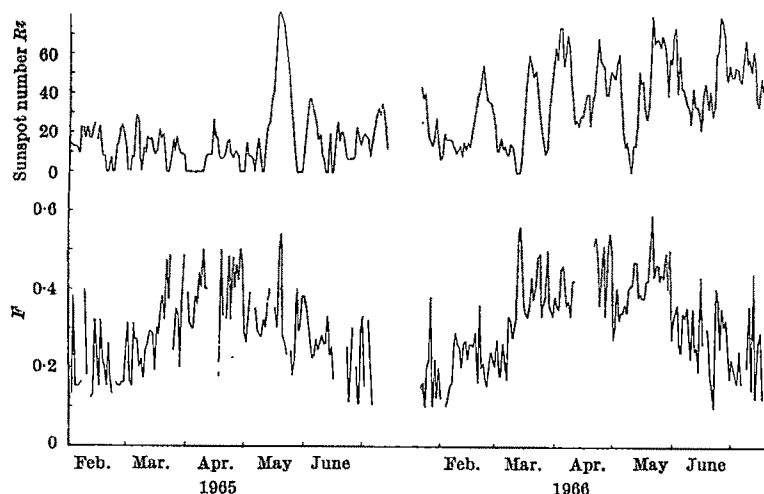


Fig. 4. Day to day variation of scintillation index  $F$  and sunspot number.

that a small correlation with sunspot number exists, but the present data are barely significant. In Fig. 4 the scintillation index observed during 1965 and 1966 is shown together with sunspot number for the same period. The scintillation index on March 13 and 14, 1966, increased to about twice its average value and this may be connected with a limb-flare which appeared on the eastern edge of the disk at heliographic latitude  $20^\circ$  N. on March 14. Radial ejection of material from such a point would have passed close to the centre of the line of sight. Further events of this kind are needed, however, before a definite association may be regarded as established. While the scintillation index varies considerably from day to day, the observed velocities, particularly at low heliocentric

latitude, are remarkably constant. This result contrasts with space-probe measurements of the solar wind which have shown larger variations strongly correlated with  $K_p$ . It appears that variations of this kind must be averaged out by integration along a line of sight which implies that the differences of velocity are confined to relatively narrow streams in the interplanetary medium.

In an earlier paper<sup>6</sup> it was found necessary to assume a slight convergence of the solar wind towards the plane of the ecliptic. This suggestion was put forward to explain the slow radial variation of the measured width of the angular spectrum of radiation scattered by irregularities for  $p < 0.4$  A.U. The present measurements are not sufficiently accurate to detect the difference (about  $10^\circ$ ) from a strictly radial direction predicted by this model.

This work was financed by the Science Research Council, to whom one of us (P. A. D.) is indebted for a maintenance grant. We also wish to thank Viscount Elveden and Mr. Frank Hyde for their help in providing outstation facilities.

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<sup>4</sup> Phillips, G. J., and Spencer, M., *Proc. Phys. Soc.*, B, **68**, 481 (1955).

<sup>5</sup> Little, L. T., and Hewish, A., *Mon. Not. Roy. Astro. Soc.*, **134**, 221 (1966).

<sup>6</sup> Hewish, A., and Wyndham, J. D., *Mon. Not. Roy. Astro. Soc.*, **126**, 469 (1963).

<sup>7</sup> Slee, O. B., *Planet. Space Sci.*, **14**, 255 (1966).

<sup>8</sup> Little, L. T., Hewish, A., and Dennison, P. A., *Planet. Space Sci.*, **14**, 1221 (1966).

## How to count Quasars

by

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Bias in the counting of quasars can arise partly from the scatter of intrinsic brightness and partly because of the shapes of quasar spectra. The corresponding selection effects can be eliminated for any cosmological model assumed. This procedure will be important for the evaluation of future catalogues of radio sources. At present, no decision can be made, from a statistical count of quasars, between steady state and other cosmological models.

THERE is some disagreement in recent papers about the meaning of relations between the observed numbers, red-shifts and brightnesses of quasars. The main source of premature conclusions appears to be the fact that important selection effects have not been taken properly into account.

Veron<sup>1</sup> and others used the uncorrected plots of  $\log N/\log S$ ,  $\log z/\log S$ , or  $\log N/\log z$ . ( $N$  is the number of objects brighter than the flux density  $S$  at some observed frequency,  $z$  is the red-shift.) From the slope of about  $-2$  in the plot of  $\log N/\log S$  for the quasars in the revised 3C catalogue (3Crev), Veron concluded that there are relatively more quasars at larger distances and that one probably would have to explain that as an effect of the evolution of the universe.

Hoyle and Burbidge<sup>2</sup> expressed the opinion that the  $S/z$  relation of quasars is very hard to understand in a cosmological theory. This was contradicted by Roeder

and Mitchell<sup>3</sup>, who computed the intrinsic brightnesses (in special world models) and showed that their large scatter causes the apparent lack of correlation in the  $S/z$  plot. They did not, however, discuss the selection effects and their suggestions about "evolution effects" appear therefore to be premature.

I have found that the slope of about  $-2$  in Veron's plot of  $\log N/\log S$  is a consequence of selection effects and that after the elimination of these effects from samples now available no final conclusions can be drawn concerning the cosmological model and the space-time distribution of quasars. The method I use (which is programmed on a computer) may, however, be useful for the evaluation of future more complete observations. The details will be described in a more extensive report (Kafka, P., *Institutsbericht from the Max-Planck-Institut für Physik und Astrophysik*, in preparation).

Bright sources are seen up to large distances, weak ones only in our neighbourhood; moreover, distant ones are observed at a higher frequency of emission, where (in our case) their spectral intensity curves are lower. To eliminate the selection effects arising from these facts, we have to start with a statistical sample of objects for which all quantities that influence their visibility are known. The sample will be a catalogue of all sources brighter than some limit at some observed frequency. The 3Crev would supply a good statistical sample if all quasars in it were

identified. As this is not yet the case, an additional selection effect may come in that cannot be eliminated, because we do not know why one source is optically identified (and its red-shift measured) and another is not.

To demonstrate the method that should be applied when the red-shifts and radio spectra of all quasars in a complete catalogue (with a given flux limit) are measured, we shall eliminate at least the selection effects that will be important in this case. They can be treated now, although

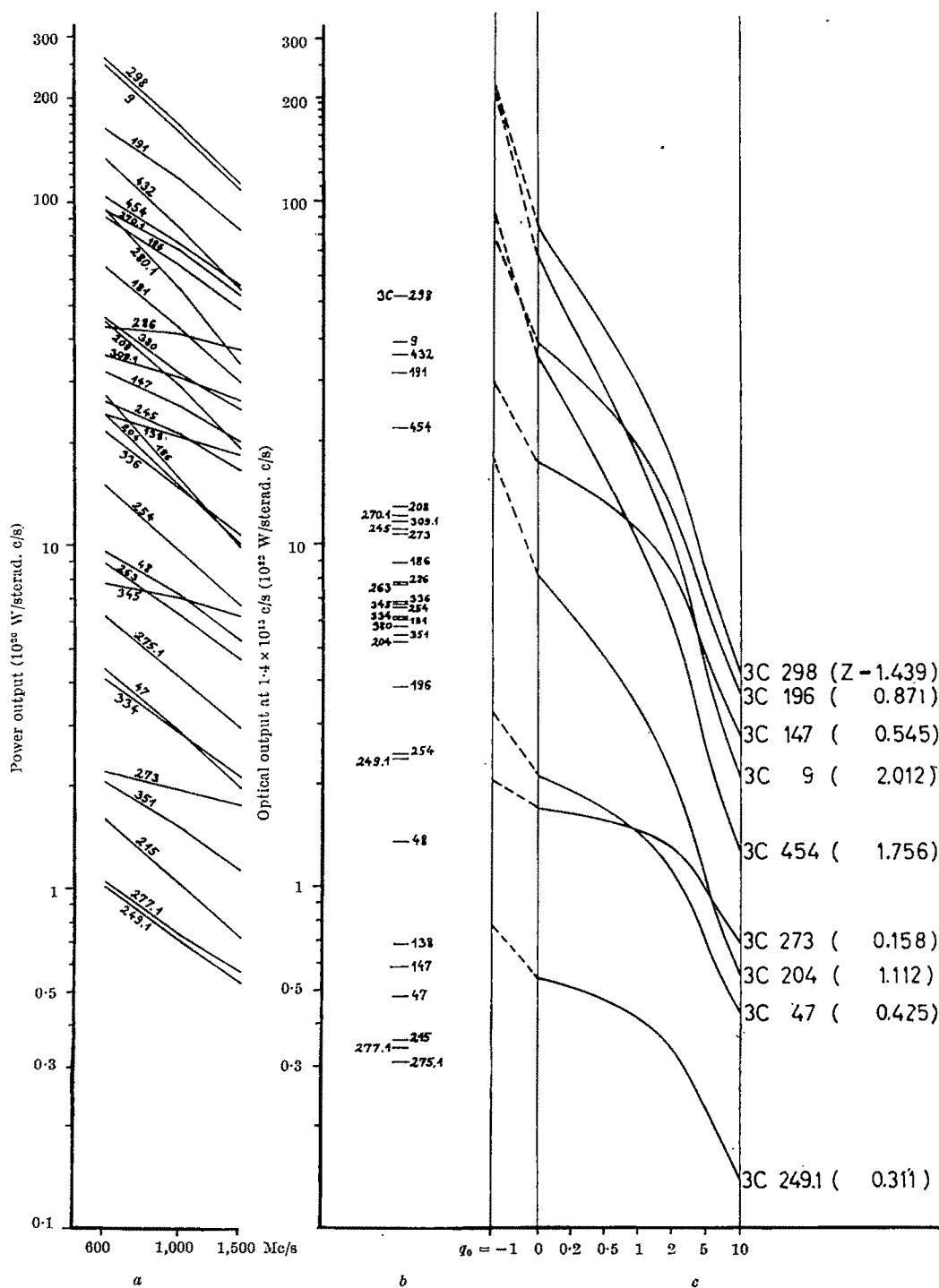


Fig. 1. *a*, Intrinsic spectra for  $q_0 = -1$ ; *b*, intrinsic optical luminosity for  $q_0 = -1$ ; *c*, dependence of the intrinsic brightness (at 1,000 Mc/s) on the model. (Examples for various distances.)

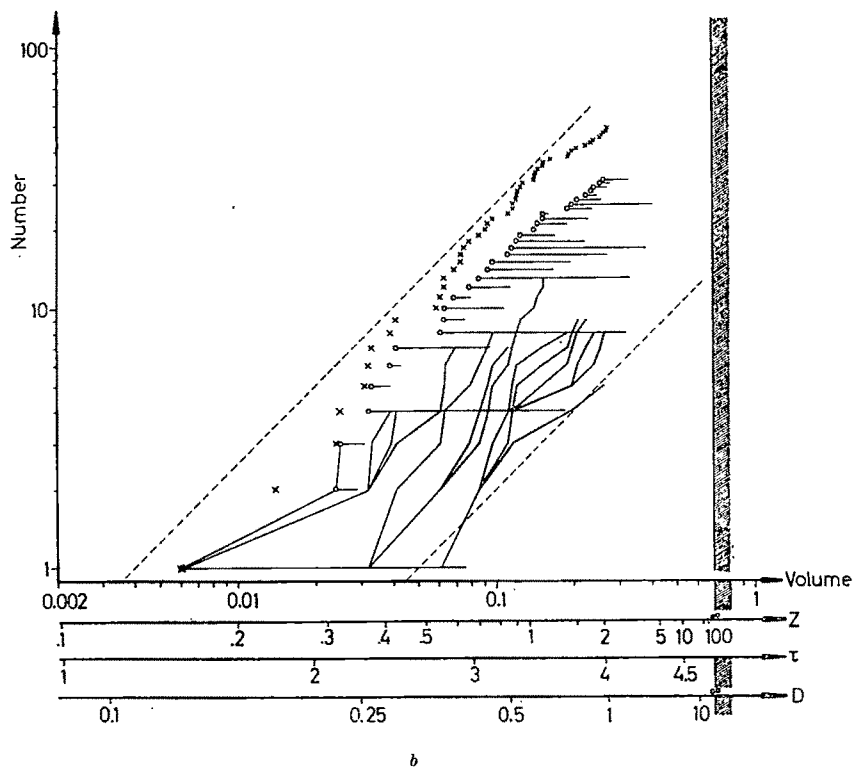
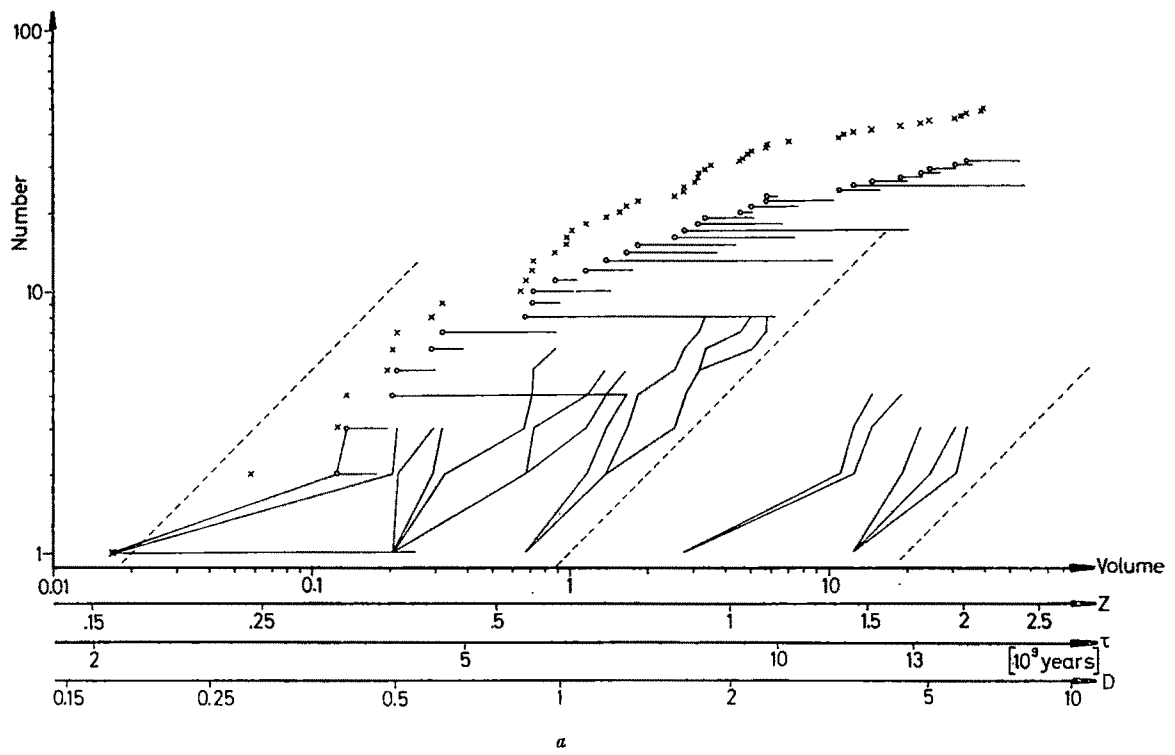


Fig. 2. *a*, Steady-state model ( $q_0 = -1$ ); *b*, steady-state model ( $q_0 = 5$ ).



the present investigation is based on only thirty-one quasars in the 3Crev for which there is sufficient information.

In addition to the 3Crev flux data at 178 Mc/s the flux densities at 750 and 1,400 Mc/s, given in the NRAO catalogue<sup>2</sup>, are used. Thus for an object with red-shift  $z$  the intrinsic spectrum from  $178 \cdot (1+z)$  to  $1,400 \cdot (1+z)$  Mc/s can be computed.

To compute the intrinsic spectral intensities from the observed ones, one has to choose a cosmological model. I have used the Friedmann models without a cosmological constant, for different values of the deceleration-parameter  $q_0$  (which is proportional to the present matter-density), and the steady-state model. (The method can easily be extended to models that introduce unknown forces besides gravitation or the sourceless "force" of a cosmological constant.)

In Fig. 1a a common range of the intrinsic radio-spectra obtained is shown. As their slopes do not depend on the model, they are plotted only for one model. (Here the steady-state model is chosen, because it yields the widest spread of luminosities.) Fig. 1b shows the approximate intrinsic luminosity at  $1.4 \times 10^{15}$  c/s (2140 Å), obtained by interpolation (for mean red-shifts) or by extrapolation (for small and for large red-shifts) from the observed  $V$ ,  $B$  and  $U$  magnitudes. They are plotted for the same model. (From the correlations between intrinsic radio and optical luminosities one might try to find out in which direction a selection effect, arising from the identification problem, might influence results.)

In Fig. 1c the intrinsic luminosities of some quasars at 1,000 Mc/s for different values of  $q_0$  are plotted. The points for each object are connected by a line. The amount of power-output and even the order of brightnesses vary considerably with the model.

We are now ready to eliminate the selection effect which arises from the scatter of intrinsic brightnesses and from the shape of the spectra. From the intrinsic spectra we compute the distance at which each object would have to be in order to be seen (with a given model) at the flux limit of the catalogue, that is, 9 flux-units at 178 Mc/s. Then we compute the spherical space-volume,  $V_{\text{lim}}$  (at the present time), which corresponds to that distance. (The volume,  $V_{\text{lim}}$ , at the present time is proportional to the volume in co-expanding co-ordinates and therefore can be used to compare parts of the expanding homogeneous space.)

Only those objects which could be seen by us if they were anywhere in a chosen volume  $V_0$  (that is, objects for which  $V_{\text{lim}} \geq V_0$ ) are selected. Only those objects must be counted in the volume  $V_0$ . From that subset we plot the logarithm of the number of objects that lie in a volume  $V$  around us as a function of  $\log V$ , for  $V \leq V_0$ . Roughly speaking, choosing a larger  $V_0$  corresponds to counting only intrinsically brighter objects.

Repeating this procedure for increasing values of  $V_0$  a set of lines in the plane  $\log N/\log V$  can be constructed for each model. If these lines are used instead of the uncorrected plots of  $N/V$  the selection effects caused by the scatter of intrinsic brightnesses and the radio-spectra are eliminated.

The plots of  $\log N/\log V$  thus obtained, for different values of  $V_0$ , should be straight lines of slope +1 if the cosmological model chosen is a good approximation to reality and if we do not look too far back in time, where evolution effects may (and for Friedmann models finally must) come in. (Evolution effects in this sense might be a result of quasars originating at different rates or having different luminosities or spectra or life-times at different cosmical epochs. The objects themselves probably do not live very long.)

Figs. 2a and b show the plots of  $\log N/\log V$  constructed for the steady-state model and the Friedmann model with  $q_0 = 5$  (which are two extreme cases). The scale of volume is "cubic-Hubble", where the reciprocal Hubble constant, which may be about 4,000 Mpc (or  $13 \times 10^9$  yr),

is chosen as the length (and time) scale. Parallel to the  $V$  scale we show the red-shift  $z$ , the light-travel-time  $\tau$ , and the luminosity distance  $D$ . At the right-hand side of Fig. 2b the present horizon for this model is shown.

The circles represent the thirty-one quasars. A line through these points would be meaningless. (The crosses show the same plot for forty-nine quasars for which we have optical spectra and red-shifts, but for which we cannot eliminate the selection effect as the sample is not well defined.) The horizontal line beginning at each circle ends at the volume  $V_{\text{lim}}$  computed for the object. At the corresponding distance the object would be just seen with 9 flux-units at 178 Mc/s. Beyond that distance it would not appear in the catalogue.

The oblique zigzag lines connect the points at which the objects have to be counted after the elimination of the selection effects described. Each line corresponds to some value of  $V_0$ . These lines should be straight, with a slope of +1, at least in the region of lower  $z$  (that is, not too long ago), if we had enough objects and picked the right model.

With our small total number the slope is very much disturbed by random fluctuations in the distribution of quasars, and it would not make sense, with our present sample, to rule out one model or the other by statistics. Nevertheless, in the steepness of the lines beyond  $z = 0.5$  for models with high values of  $q_0$  one might see a hint of an evolution effect, if one of these relatively young models should be right.

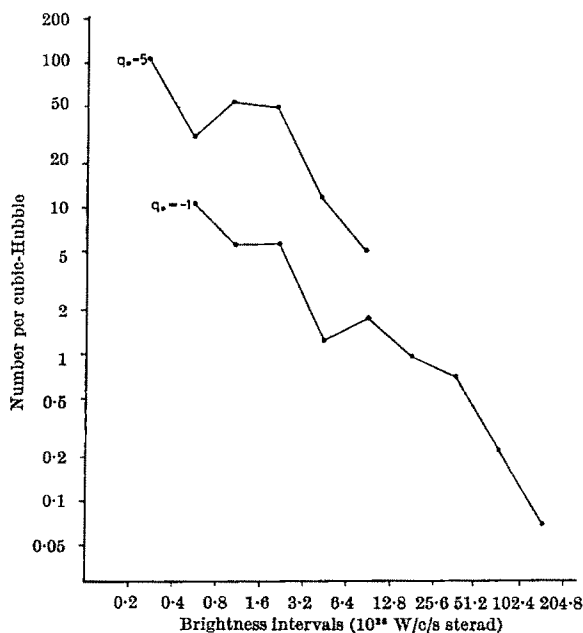


Fig. 3. Density of number of objects with intrinsic brightness (at 1,000 Mc/s) in a chosen interval.

Now we can tentatively compute the luminosity-distribution of the quasars. We select the objects in some chosen interval of intrinsic luminosity at some fixed emitted frequency, count each of these objects once per its volume  $V_{\text{lim}}$  (which is about the same for all objects within the interval) and add the number-densities obtained. We do this for intervals of intrinsic luminosity at 1,000 Mc/s, increasing by factors of 2. The numbers per unit volume obtained are plotted over the intervals.

Fig. 3 shows this plot for our two extreme models. To construct it for the Friedmann model we assume that there

is no evolution effect. This assumption is not very reasonable as the far quasars in young models are already quite near to the horizon, but otherwise we could not simply take the space density of sources. In the steady-state model there is by definition no evolution effect possible.

The distributions obtained for the other models lie between those for the two extremes. Naturally these curves contain all the uncertainty from the random fluctuations that was expressed in the zigzag lines of Fig. 2.

Nevertheless the rise in the number density at low luminosities may be real and important: Then one might find in a catalogue with lower flux limit many intrinsically weak quasars, and a continuous transition to the radio-galaxies. This would be expected if the quasars were the outburst stages of radio-galaxies. The luminosity distribution might then be interpreted in terms of time evolution of objects that are not necessarily very different in their initial power output. In this case the steady-state model—as seen from Fig. 1—would require about twenty-five times more initial power than a model with  $q_0 = 5$ .

Our preliminary result is that with the small number of thirty-one quasars no Friedmann model with reasonable age (when we take into account an uncertainty factor of 2 in the Hubble constant) is ruled out. Also the steady-state model is not significantly ruled out by the quasar counts. The opposite opinion of Sciama and Rees<sup>5</sup> seems to arise from overestimating the statistical significance. In order to be able to rule out with some reliability a model by a  $\chi^2$ -test, examining whether objects of one kind are distributed in space at random (say with a Poisson distribution), we need about 20–30 objects on one

“countline” in our diagrams! The steady-state model would be ruled out if more observations were to show a significant deviation from a slope of +1 in Fig. 2a. For the Friedmann models the situation is more complicated. Here a deviation can mean either that the model is wrong or that an evolution effect is seen. Perhaps this effect will not be important at least for  $z \leq 0.5$  (which corresponds to an age of 0.33 of the cosmical age  $t_0$  for  $q_0 = 0$  and to 0.58  $t_0$  for  $q_0 = 5$ . For  $q_0 = 0$  there is  $t_0 = 1$  Hubble, for  $q_0 = 5$  we have  $t_0 = 0.35$  Hubble.) The right model could perhaps be determined by this method if many weak quasars were found such as would be expected in the transition region to the radio-galaxies. Moreover the distant radio-galaxies could also be treated by this method. In any case very good and rich samples will be needed because the models do not differ very strongly in the region below  $z = 0.5$ .

Probably the method will lead to more positive results when all the quasars in the 3Crev are identified. Then there should be about 100, and we could be sure that no additional selection effect comes in (except possibly some corrections for absorption). Then we may be able to restrict the possible world models and perhaps tell something about what the number-density and the spectra of quasars were in earlier stages of the universe, so that we could learn whether quasars are early or late stages of galaxies or whether they are quite different objects (like, for example, the “birth of matter from singularities”).

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## Oxygen Spectra in Dayglow, Twilight, and during an Eclipse

by

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There is evidence for a morning-evening asymmetry for the  $1.27\mu$   $O_2$  band in twilight, and the evening decay appears slower at high latitude. No appreciable variation in the dayglow intensity has been observed with latitude; the decreased dayglow intensity during an eclipse seems consistent with the theory involving production of  $O_2(^1\Delta_g)$  by photodissociation of ozone.

SINCE the original observation from an aircraft of the  $(0,0) \ ^1\Delta_g - ^3\Sigma_g^-$  band of  $O_2$  in the dayglow and twilight<sup>1</sup>, a number of additional measurements have been made using the same instrument. These include measurements of the  $1.27\mu$  band in the dayglow, morning and evening twilight and during two eclipses. This communication summarizes these observations and compares our results with the balloon measurements reported by Evans *et al.* in the accompanying letter<sup>2</sup>.

Fig. 1 shows the results of several twilight observations at middle and high latitude in 1965. It also shows revised twilight intensities for February 1962; as originally presented by Noxon and Vallance Jones the intensities had not been properly corrected for the  $O_2$  absorption feature at  $1.27\mu$  in the sky background. The pair of solid lines are the balloon twilight measurements of Evans *et al.*; the remaining line is derived from one of the winter twilight observations of the  $(0,1)$  band at  $1.58\mu$  by Gattinger and Vallance Jones<sup>3</sup>. Observation of a discharge through  $O_2$  has allowed us to set a lower limit of 30 on the intensity ratio of  $(0,0)$  to  $(0,1)$ ; the theoretical

intensity ratio is 100/1. All the aircraft observations have been corrected for  $O_2$  absorption above the aircraft using transmission factors calculated by Gattinger and Vallance Jones<sup>3</sup>. Their calculations were based on an  $A$  coefficient for the band of  $1.5 \times 10^{-4} \text{ sec}^{-1}$  which now appears to be about half the correct value<sup>4</sup>; pending a recalculation we have retained their values for the transmission. We can estimate the error as the actual transmission for a given air mass will be about equal to what they calculate for twice that air mass. The 1965 aircraft intensities, measured in the zenith, will thus have to be raised by about a factor of 1.5. The 1962 twilight was observed at  $70^\circ$  zenith angle and the correction is more difficult to estimate; a small extrapolation suggests that here the intensities should be raised by a factor of 2.

Bearing these corrections in mind an examination of Fig. 1 suggests that our evening and morning twilight observations at  $62^\circ$  N. (obtained during a non-stop east-bound flight) are in fair agreement with the balloon observations<sup>2</sup> at  $52^\circ$  N.; not only are the intensities comparable but the dramatic asymmetry between evening

and morning is evident as well. The last two points on the evening of July 27, however, are, if anything, higher than the preceding point and the twilight decay might thus appear to have levelled off at about 1 megarayleigh. But it was just at this time that the aircraft started to pass beneath a bright visible aurora of at least IBC II; this is the only occasion when the airborne spectrometer has been directed at a bright aurora. The anomalous twilight decay could be accounted for by an auroral contribution to the  $1.27\mu$  band of some 500 kilorayleighs; the corresponding intensity of the 0,1 band at  $1.58\mu$  would only be 5–10 kilorayleighs. Although an emission peak of about 100 kilorayleighs has been observed at  $1.58\mu$  in a bright aurora this has been interpreted as being caused primarily by  $N_2^+$  emission<sup>6</sup>. Nevertheless, previous ground based observations at  $1.58\mu$  certainly do not rule out the possibility of  $1.27\mu$  emission in a bright aurora of the magnitude we require and we consider auroral contamination to be the most probable explanation for the abnormally large intensity in this late twilight.

The other evening twilights, February 1962 and July 21, 1965, appear similar to both our July 27 twilight and to the balloon twilight when the error in transmission coefficients is recalled. At the same time it seems clear that the 0,1 band observation implies that in 1961 the 0,0 band was not only brighter but also decayed more rapidly in evening twilight than was the case when it later became possible to observe the band directly.

All the direct measurements of the  $1.27\mu$  band appear to show a rather similar decay rate after sunset, when plotted against solar depression angle, although the decay rate of balloon measurements may be less rapid. An interesting consequence of this is that a great difference exists in the time constant for the decay at different latitudes. When the intensities are plotted against local time the decay is nearly exponential for solar elevation angles between  $0^\circ$  and  $-6^\circ$ ; the evening twilight decay at both  $52^\circ$  N. and  $62^\circ$  N. has a time constant of close to 60 min, almost exactly equal to the radiative lifetime of the  $^1\Delta$  state. At  $35^\circ$  N. the time constant is only 25 min while the 1962 observations at  $20^\circ$  N. suggest a time constant of about 15 min.

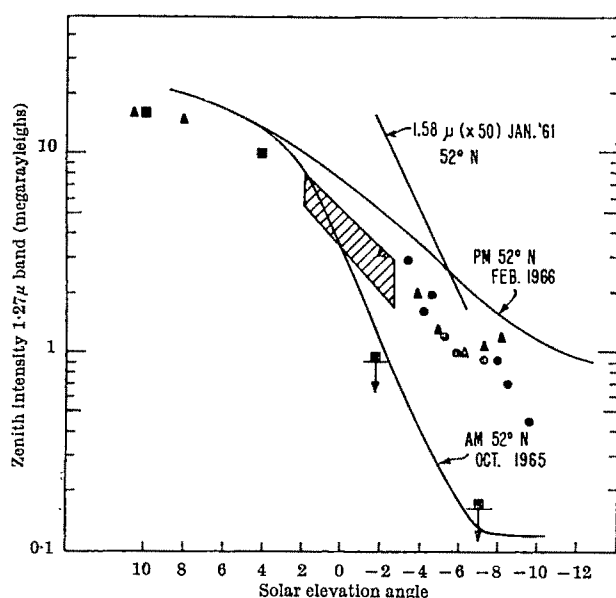


Fig. 1. The points and hatched area show twilight measurements of the  $1.27\mu$  band from aircraft observations; the pair of solid lines are the observations of Evans *et al.*<sup>3</sup>; the 0,1 band data are from Gattinger and Vallance Jones<sup>3</sup>. The aircraft observations have been corrected to zenith intensities using the transmission factors calculated by Gattinger<sup>3</sup>. Our dawn measurements at  $-2^\circ$  and  $-7^\circ$  are upper limits; otherwise the random error is estimated to be 10 per cent.  $\Delta$ , July 27, 1965,  $62^\circ$  N. p.m.;  $\blacksquare$ , July 23, 1965,  $62^\circ$  N. a.m.;  $\bullet$ , July 21, 1965,  $35^\circ$  N. p.m.; hatched area, Feb. 4, 1962,  $20^\circ$  N. p.m.

The simplest interpretation of this is that there is a near equilibrium between excitation and destruction of the  $^1\Delta$  state during the twilight decay, as this will make the twilight intensity dependent only on solar depression angle and not on the latter's rate of change. But this interpretation then makes it more difficult to explain the evening-morning asymmetry; a near equilibrium means a relatively short lifetime for the excited state through collisional quenching and one can no longer take advantage of the long natural radiative lifetime in order to extend the evening decay and retard the morning rise. This was also the problem encountered by Gattinger and Vallance Jones<sup>3</sup> when they attempted to fit their theory to the rapid decay of the 0,1 band observed in 1961; in effect a near equilibrium had to be maintained between excitation of  $^1\Delta$  by photodissociation of ozone and its destruction by collision if the predicted decay was not to be too slow. It does seem possible that, if the theory were applied only to the  $52^\circ$  N. and  $62^\circ$  N. twilights, in 1965–66, a considerable departure from equilibrium might be allowable as the observed decay is no faster than would be expected from radiation alone. Under these circumstances one might expect the theory to produce a noticeable morning-evening asymmetry. But the low latitude decay, being more rapid, would presumably not be consistent with a large departure from equilibrium. In this connexion it is clear that a study of the morning-evening asymmetry at low latitude would be of considerable interest; it is our intention to pursue this in the near future.

In summary, the aircraft and balloon twilight observations both confirm the morning-evening asymmetry observed in the winter<sup>3</sup> and show that it is also very pronounced in the summer. On the other hand, the evening twilight seems almost as strong in summer as in winter; this is a marked contrast to the large seasonal effect observed in 1961 (ref. 3). The aircraft measurements do not reveal any pronounced effect of latitude on the evening twilight, apart from the auroral effect at  $62^\circ$  N. There also seems to be little difference between the February 1962 twilight at  $20^\circ$  N. and the later ones at higher latitude.

A number of measurements of the dayglow have been made from the aircraft: April 1962 at  $45^\circ$  N., July 1963 at  $50^\circ$  N., May 1965 at  $0^\circ$  and  $20^\circ$  N., July 1965 at  $50^\circ$  and  $60^\circ$  N. In all cases the zenith intensity fell between 13 and 20 megarayleighs; with new transmission coefficients we estimate that the range will rise to 20–30 megarayleighs, in reasonable agreement with the 30 megarayleighs reported by Evans *et al.* from their balloon flights in October 1965 and February 1966. There is then no evidence for any large variation with latitude or year in the summer dayglow brightness, nor does there appear to be any seasonal effect, at least in 1965. On the other hand, the intensities<sup>3</sup> for the (0,1) band in early 1961 are so large and decay so rapidly in the evening twilight that the dayglow must have been well above 30 megarayleighs. Fig. 1 makes this clear, particularly if the (0,0) to (0,1) ratio is taken as 100.

There is a very marked difference in the behaviour of the (0,0) band at the eclipses of July 20, 1963, and May 30, 1965. The observations at the earlier eclipse have been reported by Noxon and Markham<sup>6</sup> and have been discussed theoretically<sup>3</sup>. It was necessary to observe at over  $70^\circ$  zenith angle so that the line of sight passed out of totality above 40 km; nevertheless the long duration of partial eclipse and the approach to near totality led to a theoretical prediction of a seven-fold reduction in brightness by the beginning of totality. The observations, however, indicated an intensity of about 12 megarayleighs at second contact with a drop of less than 30 per cent having occurred during the previous 4 min. The implication would thus be that the pre-eclipse dayglow was more than 80 megarayleighs (more than 150 if improved transmission coefficients are employed). Unfortunately we were not able to measure the pre- or post-eclipse dayglow;

even so the dayglow intensities observed at other times make it difficult to accept the required large value. The only other possibilities are that the theory is wrong or that we encountered auroral contamination at 56° N. Although aurora was not observed well above the horizon during totality its presence in the line of sight near the horizon cannot be ruled out.

The observing circumstances were much more favourable in 1965; the eclipse was observed near the equator and in the zenith where totality was complete to well above 100 km; in addition, the duration of totality was 6 min instead of 1 min. The aircraft was not at its maximum altitude of 13 km and so the brighter sky background limited measurement to pre-eclipse and near totality measurements. On this occasion the dayglow was 15 megarayleighs before and after the eclipse, 3.5 megarayleighs at the beginning of totality and less than 1.5 megarayleighs after 4 min of totality, if the old transmission coefficient is used. Although no detailed theoretical calculation has been completed yet for the geometry of this eclipse, one might expect the predicted drop to be similar to that for the previous eclipse; the observed drop of nearly a factor of 5 is thus not likely to be far from the predicted value. The decay during totality has an effective time constant of less than 8 min, noticeably smaller than that for the evening twilight decay observed at low latitude in 1962. This rapid decay is not surprising; not only is the residual illumination cut off entirely but one also expects the bulk of the emission to be lower in the atmosphere with more rapid collisional quenching than during twilight, because of the difference in solar elevation angle. Further discussion requires a detailed theory; it would also be desirable to have a complete set of observations covering the entire course of an eclipse.

The theory for the 1.27 $\mu$  band<sup>3</sup> leaves a number of observations unexplained; these include the morning-evening asymmetry and the seasonal effect so pronounced in 1961. It appears likely that a proper theory will also have to explain a reduction in magnitude of the seasonal effect after 1961 as well as what appears to be the comparative independence of latitude and season on the part of the dayglow. The theory of Gattinger and Vallance Jones<sup>3</sup> is restricted to a pure oxygen atmosphere and also ignores possible dynamic effects resulting from vertical and horizontal transport. At high latitude, for example, zonal winds in the mesosphere sometimes attain velocities comparable with the ground speed of the sunset line and so may either accelerate or retard the apparent change with time in the concentration of long-lived species at twilight. Perhaps even more important is the role of hydrogen compounds in the chemistry of the mesosphere; for example, the recent work of Hunt<sup>7</sup> and Hesstvedt<sup>8</sup>, which indicates that the introduction of water vapour into the model considerably alters the predicted ozone distribution and changes the time constants for variation in this and other mesospheric components. One may hope that a more comprehensive theory which includes such effects may be of benefit in accounting for the behaviour of the 1.27 $\mu$  band.

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### Balloon-borne Observations of Brightness Variations in the (0,0) Band of the (<sup>1</sup> $\Delta_g$ -<sup>3</sup> $\Sigma_g^-$ ) System of Oxygen in the Day and Twilight Airglow

THE (0,1) band of the (<sup>1</sup> $\Delta_g$ -<sup>3</sup> $\Sigma_g^-$ ) system of oxygen was first observed from the ground in the twilight airglow in 1958 (ref. 1). This band, at 1.58 $\mu$ , was subsequently observed often in the evening twilight, but never in the morning twilight.

The (0,0) band at 1.27 $\mu$ , although theoretically about 100 times stronger than the (0,1) band, is difficult to detect from the ground during the day because of re-absorption by oxygen in the lower atmosphere. Since the value of the band extinction coefficient is approximately (1.1 km)<sup>-1</sup>, aircraft observations are also subject to considerable absorption as well as contamination by scattered sunlight. Balloons furnish an ideal observing platform for this emission, because at 30 km absorption is small and the background scattered continuum is reduced by almost an order of magnitude from its value at 13 km.

A successful observation of the (0,0) band of the (<sup>1</sup> $\Delta_g$ -<sup>3</sup> $\Sigma_g^-$ ) system of oxygen in the day airglow, taken with a grating spectrometer in a plane at a height of 13 km, was first reported in 1962 (ref. 2). In 1964, however, Gopshtein and Kushpil<sup>3</sup> reported the results of two balloon flights which had been made in 1956. A "recording spectrophotometer" (filter photometer) was flown on a balloon to a height of 30 km and observations were taken for a few degrees around 20° solar elevation on an enhanced glow in the 1.25 $\mu$  region. Although the authors did not identify the emission it was undoubtedly the (0,0) band at 1.27 $\mu$ .

Recently, in a series of balloon flights with an interference filter photometer, the 1.27 $\mu$  (0,0) band has been observed continuously over a complete day from before dawn until after sunset. The photometer, which utilizes narrow and wide band pass interference filters centered at 1.27 $\mu$ , has a field of view 10° in diameter and was used at a zenith distance of 50°. Absolute brightnesses were obtained by comparison with a standard low-brightness source taken in conjunction with a calculated synthetic spectrum for the (0,0) band. A preliminary analysis of the results has yielded the curves shown in Figs. 1 and 2. The absolute brightness scales, although at this time

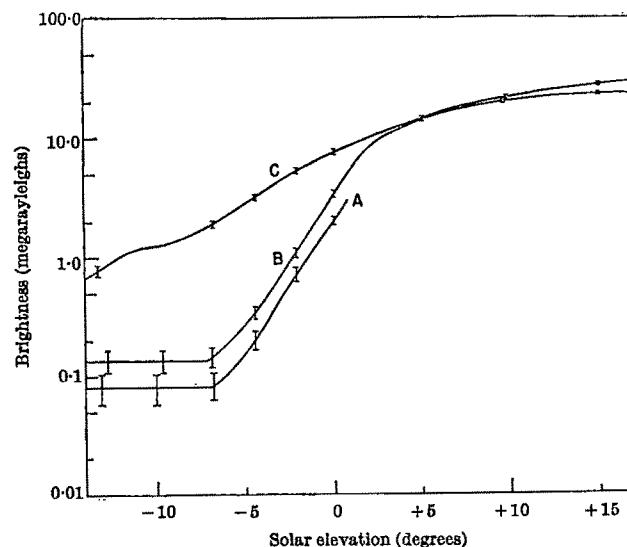


Fig. 1. Morning and evening twilight brightness variations of the 1.27 $\mu$  band of O<sub>2</sub> obtained from balloon-borne measurements at 30 km. A, Morning twilight, April 8, 1965, 58.5° N. (launched from Churchill, Manitoba); B, morning twilight, October 10, 1965, 52° N. (launched from Saskatoon); C, evening twilight, February 24, 1966, 52° N. (launched from Saskatoon). The curves have been drawn through a large number of points; the vertical bars indicate the relative error which may be present in one section of the curve.

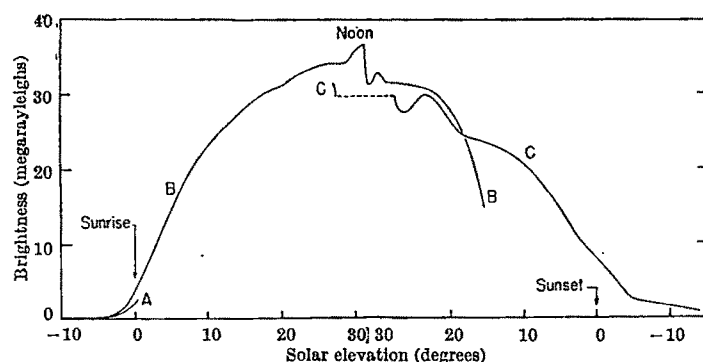


Fig. 2. The diurnal brightness variation of the  $1.27\mu$  band of  $O_2$ . A, April 6, 1965,  $58.5^\circ$  N.; B, October 10, 1965,  $52^\circ$  N.; C, February 24, 1966,  $52^\circ$  N.

approximate, are certainly correct to within a factor of two. These brightnesses have been reduced to zenith approximately by multiplying by the cosine of the zenith angle. An estimate of the relative error possible in each curve is indicated by the vertical error bars.

Curve A in Fig. 1, obtained from measurements at  $58.5^\circ$  N. (launched from Churchill, Manitoba) on April 6, 1965, is believed to be the first balloon observation of the morning twilight rise of the  $1.27\mu$  band. Curve B shows the results of the morning twilight observed on October 10, 1965, at  $52^\circ$  N., plotted on a logarithmic scale as a function of solar elevation angle. The first balloon observation of an evening twilight, measured on February 24, 1966, at  $52^\circ$  N. (launched from Saskatoon), is represented by curve C. The emission increases from a nightglow value of about 100 kilorayleighs to a dayglow value of about 30 megarayleighs at a solar elevation of  $20^\circ$ . The nightglow value compared favourably with a value of approximately 85 kilorayleighs measured from a balloon with a Michelson interferometer by Gush and Buijs<sup>4</sup>. In the morning twilight, the emission begins to increase above the nightglow value at approximately  $-7^\circ$  solar elevation and then rises sharply towards the daytime value. In the evening it falls off more slowly. This is consistent with the relatively long lifetime of the  $O_2$   $^1\Delta_g$  state, estimated to be about 60 min<sup>5</sup> in the absence of collisional deactivation. The evening-morning asymmetry explains why Vallance Jones and Gattinger<sup>6</sup> and Vallance Jones and Harrison<sup>1</sup> observed the evening twilight emission frequently, but could not detect the emission in the morning twilight.

The variation of intensity throughout the day is shown in Fig. 2; here the results from the three balloon flights have been plotted on a linear scale as a function of solar elevation angle. The noon intensity peak in the February flight is not so large as that in the October flight. In October the Sun reached a peak elevation of  $31.5^\circ$ , while in February the Sun rose to only  $27.5^\circ$ ; consequently there is a gap in curve C. A dashed line connects the two portions of the February curve which are to be displaced until they join. For the section of the curves which overlap, the two flights appear to agree well with each other. At noon, on each curve, there appears to be a sharp drop in intensity which results in an asymmetry of about 100 per cent between the forenoon and afternoon values out to approximately  $10^\circ$  solar elevation. The

range over which the drops occur, while small in solar depression angle, is relatively long in actual time as solar elevation angle changes very slowly around noon.

An analysis of the ascent data from the flight of February 24, which was launched at approximately 1130 c.s.t. and reached 100,000 ft. shortly before local noon, indicates that the major portion of the  $1.27\mu$  emission originates from regions above those reached by the balloon photometer. This supports the interpretation of Vallance Jones and Gattinger<sup>6,7</sup>, who attributed the emission of the ( $^1\Delta_g - ^3\Sigma_g^-$ ) bands to the production of excited oxygen molecules by photodissociation of ozone in the Hartley continuum; this mechanism predicts that the emitting regions will be above 30 km.

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## Axial Period of Actin Filaments

Actin is the main constituent of one of the two types of fibres that together operate the sliding filament mechanism of muscular contraction. The form of the actin helix has been examined, first by electron microscopy and, second (page 356), by X-ray diffraction.

### Electron Microscope Studies

CONTRACTION in many muscles is due to an interaction of two kinds of filaments, one containing actin, the other myosin. The positions of actin molecules relative to myosin molecules in the intact system have not yet been defined, even in the case of resting muscle<sup>1</sup>. One of the remaining uncertainties is the form of the actin helix. The most

recently published conclusions<sup>2</sup> are summarized in Fig. 1: they were based on electron micrographs of negatively stained preparations and were also consistent with the X-ray diffraction data of Selby and Bear<sup>3</sup>. These diffraction results did not specify precisely the number of subunits (molecules) per turn of the helix, or the pitch. But Worthington<sup>4</sup> deduced from diffraction patterns that the helix had a pitch of 820 Å and 15 subunits per turn.



The electron micrographs, however, appeared to show that the structure repeated at intervals of about 700 Å, and that if one described the filament as consisting of two strands of subunits wound round each other (Fig. 1), then the number of subunits per turn of the helix in each strand was either thirteen or a non-integral number close to thirteen. These conclusions were based on measurements of the axial period (about 350 Å) and the spacing of the subunits in a large number of filaments, and on a few exceptionally favourable pictures in which it was possible to count the subunits along several turns of the helix.

New measurements of the axial period in electron micrographs of negatively stained preparations have revealed that the results depend on the method used for making the preparation. The previous results<sup>2</sup> have been confirmed, but other methods have been found to give other results.

Filaments from two sources were used in these new studies. Natural filaments were separated from the cross-striated adductor muscle of the scallop *Pecten maximus* (obtained from the Marine Biology Laboratory, Plymouth), and synthetic filaments were made from rabbit skeletal muscle. The method used for obtaining the natural filaments was as follows. A small piece of muscle (approximately a cube of sides 5 mm), cut out of a scallop that could vigorously open and close its shell, was placed in about 25 ml. of 100 mmolar potassium chloride, 6.6 mmolar phosphate buffer, pH 7.0, with 5 mmolar magnesium chloride and 5 mmolar ATP (pH finally adjusted to 7.0). After 30–60 min the muscle was cut into small pieces, transferred to 6 ml. of fresh solution in the flask of a homogenizer, and homogenized for 20 sec at approximately 17,000 r.p.m. A brief centrifugation (a few seconds at about 3,000g) removed large fragments, and the filament suspension was then diluted, using the same medium, and preparations made for the microscope within a period not exceeding 1 h. In all these operations up to the time of making the preparations, the material was kept at 0–4° C. (The reason for choosing this particular isolation medium will be discussed in a later paper.) Synthetic filaments (*F*-actin) were prepared from rabbit skeletal muscle by two different conventional methods (those of Straub and A. G. Szent-Györgyi)<sup>6</sup>. Sometimes the protein was not purified: on other occasions the first *F*-actin was spun down and depolymerized, and then repolymerized using either 0.1 molar potassium chloride or 0.6 mmolar magnesium chloride (ref. 6). None of these variations appeared to affect the results.

All the preparations for the microscope were made on carbon films supported on copper grids. The variations in technique were as follows:

(1) A drop of the filament suspension was placed on the film, left for a few seconds and then washed off with 5–10 drops of 1 per cent uranyl acetate in water (pH 4.4). The last drop of stain was removed with filter paper and the preparation allowed to dry.

(2) As (1) except that several drops of unbuffered 0.1 molar potassium chloride were applied before the stain.

(3) As (1) except that the stain used was 1 per cent phosphotungstic acid adjusted to pH 7.0 or pH 5.8 with potassium hydroxide.

(4) The drop of filament suspension was washed off with 5–10 drops of glutaraldehyde before applying uranyl acetate. 2.5 per cent glutaraldehyde buffered at pH 7.0 with 0.1 molar phosphate was used.

(5) As (4) except that the fixative used was 1 per cent osmium tetroxide buffered at pH 7.0 with 29 mmolar veronal acetate.

Electron micrographs were taken at a magnification of about 40,000 in a Philips 200 instrument (double condenser; accelerating voltage 80 kV; objective apertures 20μ or 30μ). The lenses were switched on at least 1 h before any pictures were taken. The preparations made by the various techniques that have been compared (thirty-two preparations in all) were examined in random order over a period of about 1 year. Magnification was assessed every 2 weeks or so by taking pictures of one particular area in a diffraction grating replica which was always kept in the same specimen holder; the variation found was never more than 2 per cent. Frequent comparisons were made between pictures of this area in the replica taken close to focus and others taken at the particular focal setting that had been used just previously for recording from an actin filament preparation in another specimen holder. These comparisons showed that this source of error in estimating the magnification was less than 2 per cent. Measurements of the axial period in the filaments were made on the original micrographs viewed in a dissecting microscope (magnification  $\times 7$ ). Here the possible error in measurement was not more than 2 per cent. Taking all these sources of error into account, the comparisons between periodicities in the various preparations are therefore judged to be accurate to within 5 per cent.

One other technical point must be mentioned. Each measurement of periodicity was made on between two and six periods, most frequently on three. Longer sequences were not measured because it was interesting to record variations in periodicity along a given filament. On the other hand, it was considered that measurements of single periods would not be sufficiently accurate, because the limits of the periods are not sharply defined, and because under the conditions used for measurement one period spanned only about ten divisions of the scale.

The results are presented as histograms (Figs. 2–8). Those labelled “uranyl” (Figs. 2 and 5) are from preparations made by methods 1 and 2; because the results for these two methods did not appear to differ they have been pooled. For the same reason the results obtained with phosphotungstate at the two pH values used (7.0 and 5.8) have also been pooled.

The wide range of measurements in any one type of preparation is notable. Similar ranges were found in single preparations, and sometimes in the same micrograph or even along a single filament. Synthetic filaments as well as natural ones show these phenomena, which are not therefore due to damage during isolation from the muscle.

Comparing unfixed preparations made with uranyl acetate and with potassium phosphotungstate, the periods measured in the latter are shorter (compare Fig. 2 with Fig. 3 and Fig. 5 with Fig. 6). Because of the large difference in pH between these staining solutions, some preparations were made using potassium phosphotungstate at pH 4.4 (the same pH as the uranyl acetate) and others using a solution containing uranyl acetate at pH 7.0 (ref. 7). The filaments in the latter preparations were so poorly contrasted by the stain that measurements could not be made. No filaments were found in the preparations made with phosphotungstate at pH 4.4. This last result suggests that uranyl acetate, which at the same pH does not dissolve the filaments, must act as a fixative.

Comparing preparations stained with uranyl and made with or without previous glutaraldehyde fixation, the measurements are similar in the two types of preparation (compare Fig. 2 with Fig. 4 and Fig. 5 with Fig. 7). On the other hand, filaments fixed with osmium tetroxide and stained with uranyl have shorter periods than those not treated with osmium tetroxide (compare

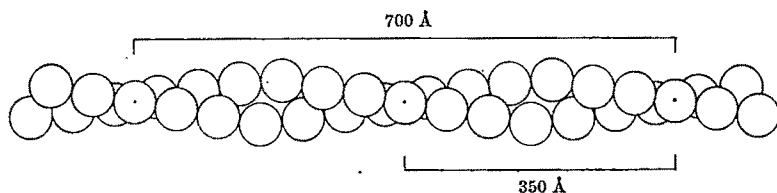


Fig. 1. Model illustrating the conclusions reached by Hanson and Lowy<sup>2</sup> about the form of the actin helix.

Figs. 5 and 8). (The histograms show this only for synthetic filaments; fewer measurements were made in the case of natural filaments, but those showed a similar trend.)

The previous measurements by Hanson and Lowy<sup>2</sup> were mostly made on filaments prepared with potassium phosphotungstate (pH 7.0 or 5.8) or fixed with osmium tetroxide. Their results (mean 349 Å, range 330–363 Å) were similar to those found here for such preparations. The new studies have shown that in other types of negatively stained preparations much higher values can be found for the axial periodicity. The shadowed preparations studied by Depue and Rice<sup>8</sup> gave much lower values, about 310 Å. The conclusion reached by Hanson and Lowy, namely that the actin polymer has thirteen or

nearly thirteen subunits per turn of the helix and an axial periodicity of about 350 Å (Fig. 1), is therefore not generally tenable.

In the studies reported here it was not possible to relate the number of subunits per turn of the helix to the axial periodicity. Counting of the subunits along one or more turns of the helix was usually impossible unless the filament lay across a stain filled hole in the supporting film where, however, distortion of the filament was liable to have occurred<sup>2</sup>. It is therefore not known if the wide range of values found for the periodicity in filaments lying on the supporting film is due to various degrees of shrinkage or if, as seems more likely, it is due to variation in the form of the helix. The spacing of the subunits could not be measured with sufficient accuracy to help distin-

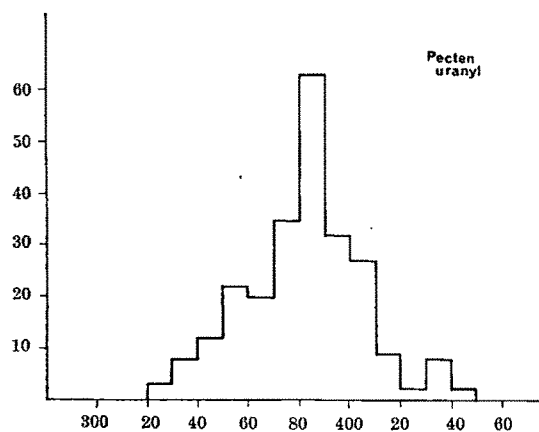


Fig. 2.

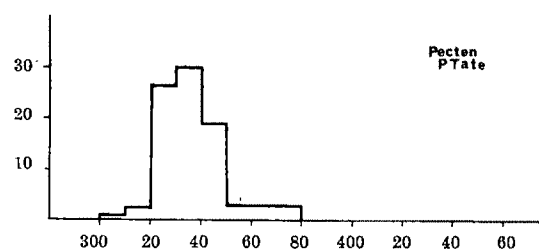


Fig. 3.

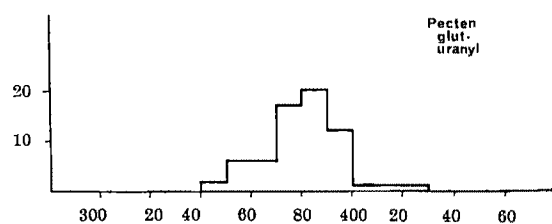


Fig. 4.

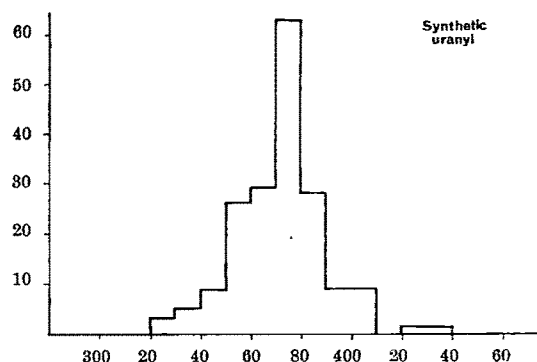


Fig. 5.

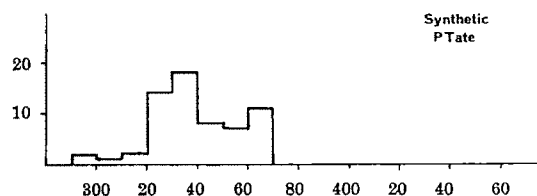


Fig. 6.

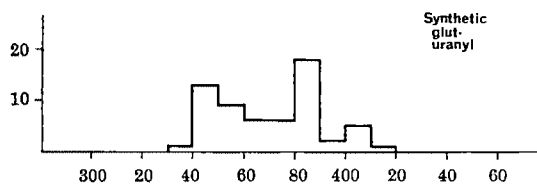


Fig. 7.

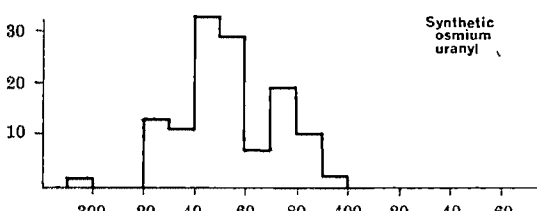


Fig. 8.

Figs. 2–8. Histograms showing axial period measurements (in Å) and numbers of measurements.

Fig. 2. *Pecten* filaments, uranyl acetate. 243 measurements obtained from eight preparations made from five different animals; approximately 2,000 filaments on 107 micrographs were studied to obtain these measurements.

Fig. 3. *Pecten* filaments, potassium phosphotungstate. 87 measurements; eight preparations from three animals; approximately 1,200 filaments on 62 micrographs.

Fig. 4. *Pecten* filaments, glutaraldehyde, uranyl acetate. 66 measurements; three preparations from two animals; approximately 600 filaments on 34 micrographs.

Fig. 5. Synthetic filaments, uranyl acetate. 183 measurements; six preparations made from five different actin samples; approximately 500 filaments on 55 micrographs.

Fig. 6. Synthetic filaments, potassium phosphotungstate. 63 measurements; three preparations from two actin samples; approximately 500 filaments on 54 micrographs.

Fig. 7. Synthetic filaments, glutaraldehyde, uranyl acetate. 62 measurements; three preparations from two actin samples; approximately 500 filaments on 47 micrographs.

Fig. 8. Synthetic filaments, osmium tetroxide, uranyl acetate. 126 measurements; three preparations from two actin samples; approximately 500 filaments on 50 micrographs.

guish between these two possibilities. If the variability in the axial period reflects a genuine variability in structure, present in the filaments before preparing them for examination in the microscope, this would be of considerable interest.

An axial periodicity of about 400 Å (ref. 9) is visible in the *I*-substance in sections of vertebrate skeletal muscle. (The term "*I*-substance" is used to describe the material that extends from the *Z*-line to the *H*-zone and comprises the actin filaments together with other components<sup>10</sup>.) It has been argued<sup>2,9,10</sup> that this periodicity is not due to repetition of the actin helices. One of the bases for this opinion was the finding<sup>2</sup> that the axial period in *F*-actin and in isolated natural actin filaments, negatively stained, measured about 350 Å. But the new studies reported here show that actin filaments do not necessarily have a periodicity that is different from the value found for the repeat in the *I*-substance. There are, however, several other reasons for thinking that the periodicity seen in the *I*-substance may not be due to actin alone. Tropomyosin, one of the proteins that is very probably present together with actin, can form fibrous aggregates which show a repeat of about 400 Å (ref. 11). Moreover, using a similar method to form aggregates of *F*-actin, it has been found that sectioned aggregates of unpurified actin are transversely striated (with a repeat value that depends on the method of fixation but can be about 400 Å), whereas aggregates of actin prepared by methods that eliminate tropomyosin and other impurities do not show this striation in sections—in spite of the fact that similar aggregates of purified actin, examined in negatively stained preparations, show good alignment of the actin helices (Hanson and Weindling, unpublished results).

In current studies actin filaments isolated from muscles in different states (relaxed by removal of calcium, contracting, or in rigor) are being compared with one another and with synthetic filaments to ascertain whether there are any differences in axial periodicity. If differences can be established, then the negative contrast method used might be considered relatively reliable, or at least very useful. Meanwhile, it has to be concluded that electron microscopy has not yet given reliable information about the form of the actin helix.

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### X-ray Diffraction Studies

ACTIN filaments appear to be an essential part of the contractile apparatus in all types of muscle. Electron microscope investigations of negatively stained material isolated from a variety of muscles have suggested that these filaments (about 80 Å in diameter) consist of two

helically wound strands of globular units about 55 Å in diameter, each unit probably representing one monomer of actin<sup>1</sup>. This structural picture of actin filaments is in essential agreement with earlier X-ray diffraction studies on whole muscle by Selby and Bear<sup>2</sup>. Their analysis, however, could not specify the length of the long period (that is, the pitch) of each of the helically wound strands. On the assumption that there is an integral number of monomeric units in each turn of this helix, the long period could be either  $2 \times 350$  Å (thirteen units per turn) or  $2 \times 410$  Å (fifteen units per turn). But there is, in fact, no reason to assume that the number of monomeric units in each turn is integral; the pitch of the helix could also lie anywhere near or between  $2 \times 350$  and  $2 \times 410$  Å.

The distance between the crossover points of the two strands of the helix can be measured directly in the electron microscope. This was done by Hanson and Lowy<sup>1</sup> and also by Peterson<sup>3</sup>, who found values of 350 and 365 Å respectively. A similar measurement (366 Å) was obtained by Huxley<sup>4</sup> for the long axial period in actin filaments which had heavy meromyosin attached to them. These measurements favoured a helix pitch equal to or near to  $2 \times 350$  Å. More recent work, however (see preceding article), has shown that the distance between the crossover points as measured in the electron microscope depends on the method of preparation, is highly variable, and can be more than 400 Å.

It is possible to determine the pitch of the actin filament helix from X-ray diffraction patterns if the reflexion spacings can be measured with sufficient accuracy. Worthington<sup>5</sup> compared the "59" and "27" Å reflexions from actin in dried *Helix* pharynx retractor muscle and concluded that his measurements favoured  $2 \times 410$  Å. On the other hand, W. Brown, K. C. Holmes, H. E. Huxley and A. Klug (personal communication) have compared the "59" and "51" Å reflexions from living relaxed frog sartorius muscle and their results favour  $2 \times 350$  Å. G. F. Elliott and M. Spencer (personal communication), using the whole series of moderate angle actin reflexions from glycerol-extracted rabbit psoas muscle, were unable to decide on a fixed, integral, helix pitch. Their results from some muscles favoured  $2 \times 350$  Å, those from others favoured  $2 \times 410$  Å.

It appears, then, that neither from X-ray diffraction nor from electron microscopy has it so far been possible to obtain a consistent value for the pitch of the actin helix. We now have some low angle X-ray diffraction patterns from living relaxed toad sartorius muscle showing reflexions at angles below those corresponding to the "59" Å reflexions, which, because of their position and shape as compared with the myosin layer lines, appear to be actin layer lines. Although a complete series has not been seen on any one pattern, these reflexions were counted inwards and were found to be consistent with there being six layer lines inside the "59" Å reflexion. This suggests that the "59" Å reflexion may be indexed as the seventh order of a basic periodicity of about 410 Å, giving a helix pitch of about  $2 \times 410$  Å.

In X-ray diffraction patterns from these toad muscles, and also from the living relaxed anterior retractor muscle of *Mytilus edulis* (ABRM) taken with sufficient low angle resolution, a reflexion can be seen at about 400 Å which is of the same general shape as the "59" Å actin reflexion (Figs. 1 and 2) and which is also off the meridian. The relative positions of the maxima along the layer lines are not easy to determine with any accuracy, because the reflexions are very streaked along the layer lines. The "400" Å reflexion appears to lie on the same row line as the "59" Å reflexion (that is, the first row line ( $J_1$ )), although it is just possible that it could lie on the second row line ( $J_2$ ). The  $J_2$  position is required by the model put forward by Selby and Bear<sup>2</sup> (but see their pages 76 and 77). A similar reflexion at 388 Å has been observed in living relaxed frog sartorius muscle by Huxley, Holmes and Brown. This low angle reflexion may also correspond to

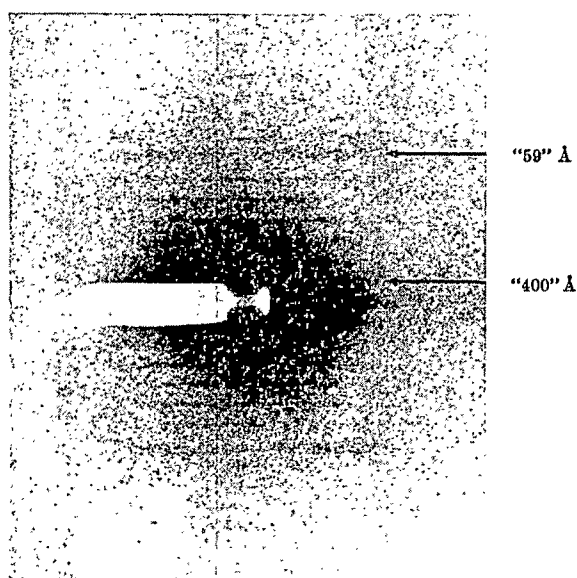


Fig. 1. X-ray diffraction pattern from living relaxed toad sartorius muscle ( $\times c. 8.5$ ). (The "59" Å and "400" Å actin reflexions are indicated by arrows.)

the 400 Å layer line seen by Selby and Bear<sup>2</sup> in molluscan adductor muscle and to the 400 Å layer line seen by Worthington<sup>6</sup> in insect flight muscle. Electron microscope evidence suggests that the actin filaments in all these muscles have the same structure<sup>1,4</sup>, whereas this is not true of the other major component of the contractile apparatus: myosin filaments in frog and toad differ from the thick filaments in insect muscles, and again from the paramyosin filaments in the molluscan muscles. We conclude that the "400" Å reflexion, because of its shape and position, is most probably associated with the actin filaments—and probably lies on the first order layer line.

Using a Franks type of low-angle X-ray camera and a  $40\mu$  focal spot (see ref. 8), measurements were made on X-ray diffraction patterns from ten living relaxed toad sartorius muscles (for examples, see Fig. 1). The ratio of the "400" and "59" Å spacings ranged from 6.71 to 7.00 with a mean of 6.86 (standard deviation of the mean—0.03). This ratio is less than the 7.00 which could be expected from indexing the "59" Å reflexion as the seventh order of a basic period of about 410 Å. In the ABRM, where the "400" Å spacing is more diffuse and

difficult to measure than in the toad muscle, the "400": "59" Å spacing ratio averaged 6.6, over six muscles.

These measurements suggest that the pitch of the actin filament helix in these two types of muscle does not have an integral number of units per turn, but that the structure approximates more closely to one with fifteen units per turn than to one with thirteen. But this may not apply to all types of muscle. In the living striated adductor muscle of the scallop (*Pecten maximus*) there is a normal "59" Å actin reflexion, but the corresponding first layer line is at about 463 Å, giving a ratio of 7.9 (B. M. Millman and G. F. Elliott, unpublished results).

Densitometer traces of our patterns from toad muscle show that the reflexions from the actin filaments are three to four times as broad in the axial direction as those from the myosin filaments (Fig. 2). In general, intrinsic line broadening has one of two causes<sup>9</sup>. Either it arises from the limited extent of the diffracting system; or it is due to some distortion in that system, and the simplest distortion is that in which the parameters vary from point to point within the diffracting specimen. The broadening (ratio) due to a diffracting system of length  $1.6\mu$  (the myosin filament length) is of the order of  $10^{-4}$  at a wavelength of 1.54 Å, and this agrees with the measured broadening (corrected for instrumental effects) of the myosin reflexions on our patterns. In toad muscle, the actin and myosin filaments have (to the approximation valid for this calculation) the same length, so that the diffraction in broadening cannot be due to a difference in filament length.

To explain their observations on the relative intensities of the equatorial reflexions from living frog sartorius muscle, Elliott, Lowy and Worthington<sup>10</sup> suggested that the part of the actin filaments outside the A-bands might be more disordered transversely than the part inside (a radial temperature factor). They noted that it was not necessary to postulate any disorder within the actin filaments, and predicted that the axial actin pattern would be much less affected by the amount of overlap between the actin and myosin filaments. This has now been shown to be the case: patterns from muscles stretched to zero overlap have shown no increase in the intrinsic broadening of the actin reflexions. Also in the ABRM, where the actin filaments are probably very long, the width of the actin reflexions is much the same as in the toad muscle. Therefore the extra broadening of the actin reflexions cannot be due to a limitation either in the length of the whole actin filaments or in the length of the filament overlap, though other factors may be limiting. The extra broadening might be ascribed to a variation in the long period of actin filaments. Broadening due to parameter variation generally increases with diffraction angle<sup>9</sup>. In this case, however, variations in the long period will not greatly affect the axial repeat of the units. This implies that Bessel functions of a given order will be broadened equally, and the broadening of Bessel functions one removed in order may be very similar.

A variation of the long period has in fact been observed in electron micrographs of negatively stained preparations of actin filaments (see preceding article). It is possible that contraction is associated with local changes in this long period, but if so the overall distribution of values for this period does not change on contraction, for we have observed no change in the spacing or broadening of the "400" Å reflexion in our X-ray diffraction patterns from contracting muscle<sup>11,12</sup>.

We thank Professor Sir John Randall for his constant encouragement and Dr. A. Elliott and Professor Jean Hanson for

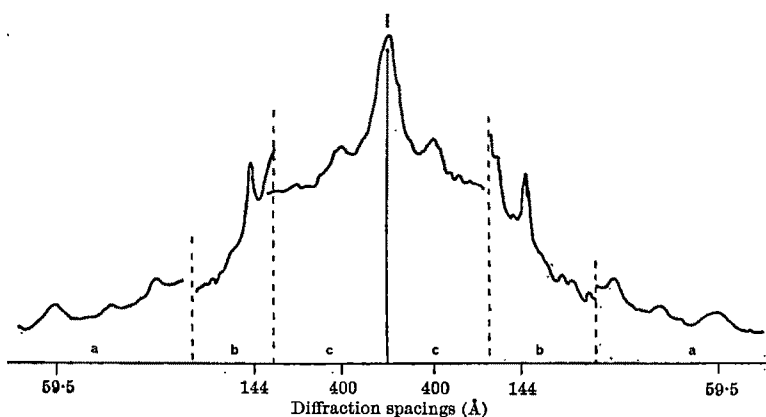


Fig. 2. Microdensitometer tracings from X-ray diffraction pattern from living relaxed toad sartorius muscle. Portions from three similar tracings made in directions parallel to the meridian. *a*, Along row line corresponding to the "59" Å actin reflexion; *b*, along the meridian; and *c*, slightly further from the meridian than *a*. The breadth of the "400" Å reflexion can be compared with that of the "59" Å actin and "144" Å myosin reflexions. ( $\times c. 16$ .)

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## Two Different Excitatory Transmitters acting on a Single Molluscan Neurone

by

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It is known that at least two different mediators (acetylcholine and a non-cholinergic transmitter) are responsible for the excitatory transmission occurring between neurones in the ganglia of the mollusc *Aplysia*. It has now been shown, by the use of selectively blocking chemicals, that excitatory synapses using different mediators sometimes co-exist on the same neurone.

PREVIOUS investigations of molluscan nervous systems<sup>1,2</sup> have shown that in different cells excitatory postsynaptic potentials (EPSPs) can be produced by at least two synaptic transmitters: a cholinergic one, exciting the so-called *D*-neurones, and an unknown non-cholinergic one, responsible for the EPSPs appearing in the conventionally named *H*-neurones. It is possible that other transmitters exist in these same nervous systems<sup>3</sup>. In the present investigation a complication of this pattern of excitatory transmission was observed. In some neurones of the visceral ganglion of *Aplysia* it was found that EPSPs recorded from the same cell could be due to the action of two different chemical transmitters.

Ganglia were isolated from *Aplysia* in a special chamber and perfused with sea water. The neurones were generally impaled with two electrodes, one of which was used for recording and the other for hyperpolarizing the cell to a desired potential level. A full account of technical procedures can be found elsewhere<sup>4</sup>.

The neurones investigated were situated near the caudal pole of the dorsal face of the visceral ganglion. They all had the following properties in common: (a) Orthodromic stimulation of some afferent nerves evoked "inhibition of long duration"<sup>5,6</sup>—the long-lasting hyperpolarization which is the defining characteristic of the CILDA cells (see ref. 3). (b) The proper combination of two adequate inputs to these cells produced presynaptic inhibition—a property analysed by Tauc in the cells labelled *P1* (ref. 5). (c) Ionophoretic injections of both acetylcholine and 5-hydroxytryptamine depolarized these cells<sup>6</sup>. (d) All these cells received complex excitatory inputs.

Excitatory inputs occurred "spontaneously" in these cells, but many EPSPs could also be evoked experimentally by electrical stimulation of the nerves. In order to record and analyse these EPSPs without interference from spike activity, the cells were artificially hyperpolarized ( $-70$  to  $-80$  mV). The nerves were stimulated

at intervals far enough apart to avoid the development of habituation<sup>7</sup> and/or presynaptic inhibition<sup>8</sup>. The polarity of stimulation was regularly alternated in order to avoid polarization of the electrodes. Threshold stimulation of the right pleuro-visceral connective produced in all cells studied a unitary EPSP which was probably monosynaptic. Other excitatory components appeared at higher intensities of stimulation. Stimulation of other nerves, such as the branchial, siphonal, or genital nerves, always produced composite EPSPs. None of the spontaneous or evoked depolarizing potentials was reversed when the potential of the cell was returned to the normal level. Consequently, they all can be considered as truly excitatory.

These synaptic activities were investigated during perfusion of the ganglion with *d*-tubocurarine (dTC). Fig. 1 shows the effects of such a perfusion on the unitary EPSP evoked in the neurone by the stimulation of the right pleuro-visceral connective (Fig. 1A) and on the composite EPSP evoked in the same neurone by stimulation of the branchial nerve (Fig. 1B). It is clear that the dTC progressively blocks the unitary input from the right connective, but does not modify the composite EPSP evoked by stimulation of the branchial nerve even after prolonged perfusions (up to 40 min) of dTC.

The upper records of Fig. 2 also show, in another neurone, the blockage by dTC of the unitary EPSP evoked by stimulation of the right connective. In the lower records it can be seen that dTC affects only partially the "spontaneous" synaptic activity which results from interneuronal firing, even after a long-lasting application.

The selective action of curare can also be observed on the different components of a composite EPSP evoked from a single nerve trunk. Fig. 3 shows such a composite EPSP evoked by strong stimulation of the right connective; the first waves of this EPSP disappear under dTC, while the two late ones persist.



All the effects of dTC could be observed with low ( $10^{-6}$  g/ml.) as well as with high concentrations ( $10^{-3}$  g/ml. —Figs. 1, 2 and 3). This seems to exclude the possibility that the EPSPs resistant to dTC were merely less sensitive to dTC.

It has been shown in sympathetic ganglion cells that some EPSPs resistant to dTC are sensitive to atropine<sup>8</sup>. This suggested the possibility that the two types of excitatory synapses on the CILDA cells be cholinergic, with one involving atropine-sensitive (muscarinic) receptors, and the other involving dTC-sensitive (nicotinic) receptors. It was thus tested to see if atropine would selectively block the dTC-resistant EPSPs. This result, however, was not obtained. Atropine depressed all EPSPs, and in some cases it depressed the dTC-sensitive input more effectively. It should be noted that atropine, unlike dTC, caused an increase in the latency of the EPSPs. Such an effect on the presynaptic fibres could at

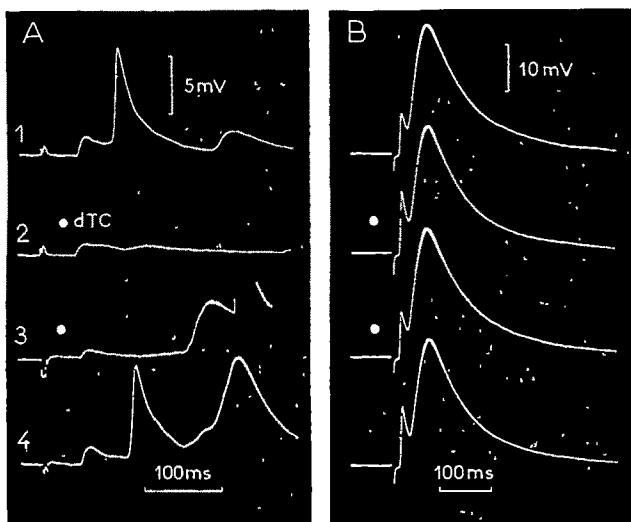


Fig. 1. Differential action of dTC on EPSPs of a CILDA cell. Stimulation of the right connective (A) and of the branchial nerve (B). The first upward deflexion (arrow) in series A to the unitary, monosynaptic EPSP, in series B to an antidromic axonal spike. (1) Control. (2) After perfusion for 1 min with dTC ( $10^{-3}$  g/ml.). (3) After perfusion for 3 min. Note the persistence of "spontaneous" EPSPs in 4. (4) After washing for 30 min with sea water. Differences in artefacts in series A were due to the use of alternated polarities of stimulation (see text).

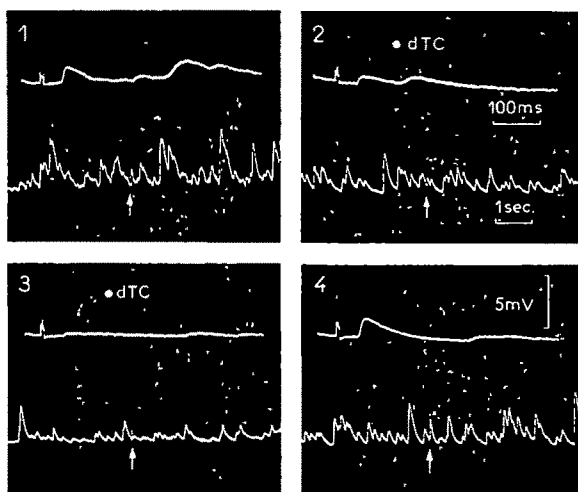


Fig. 2. Action of dTC on evoked and spontaneous EPSPs. In each picture the activity recorded by a single micro-electrode has been registered at two sweep speeds. In the slower recording (lower line) one can observe the "spontaneous" synaptic activity and (arrow) an EPSP evoked by stimulation of the right connective. This monosynaptic EPSP is shown in more detail on the expanded sweep (upper line). (1) Control. (2) After perfusion for 1 min with dTC ( $10^{-3}$  g/ml.). (3) After perfusion for 5 min. (4) Recovery after washing for 20 min.

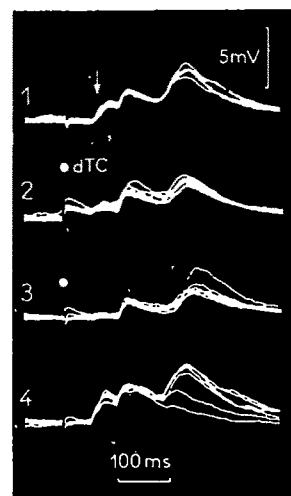


Fig. 3. Differential action of dTC on EPSPs evoked by stimulation of the same nerve trunk. Maximal stimulation of the right connective. The first upward deviation (arrow) corresponds to the unitary, monosynaptic EPSP. (1) Control. (2) After perfusion for 1 min with dTC ( $10^{-3}$  g/ml.). The unitary EPSP is depressed, but the long latency ones persist. (3) After perfusion for 6 min. (4) Recovery after washing for 30 min.

least partially explain its non-specific blocking effects. In addition, it has been observed that atropine has a non-competitive blocking effect on non-cholinergic, 5HT receptors of CILDA cells<sup>9</sup>. Thus, the effects of atropine do not support the hypothesis that the dTC-resistant EPSPs involve muscarinic receptors, but rather imply that these synapses are non-cholinergic and co-exist, on the same cell, with cholinergic synapses. Such a situation has been encountered in the study of Renshaw cells of the cat's spinal cord. These neurones seem to receive a cholinergic input from ventral root stimulation, and a non-cholinergic input from dorsal root stimulation<sup>9</sup>.

The possibility was considered that the EPSPs which were not blocked by curare might be a result of electrical transmission. To evaluate this possibility, we observed the effects of hyperpolarization of the cell on the unitary input from the right connective and on the composite EPSP evoked by stimulation of the branchial nerve. It was found that both the EPSPs sensitive to curare and the EPSPs resistant to curare increased with increasing hyperpolarization, even at polarization levels at which there is a decreased membrane resistance as a result of anomalous rectification<sup>10</sup>. Moreover, the regression lines relating the amplitude changes of both EPSPs to the level of hyperpolarization tended towards the same inversion potential. This suggests that the two types of synapses operate chemically, and may involve the same ionic mechanism.

From the data presented here it can be concluded that in a single CILDA neurone some of the EPSPs are caused by a cholinergic transmitter; others by a non-cholinergic transmitter. The depolarizing effects of 5-hydroxytryptamine on CILDA cells<sup>6</sup> suggest that this amine could be the non-cholinergic transmitter, but definitive evidence of its identification has not yet been obtained.

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# Melanin, a Possible Pigment for the Photostable Electrical Responses of the Eye

by

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Some kinds of biological tissues generate fast electrical responses to intense light and both stable and unstable responses have been detected. The unstable response is known to be associated with visual pigments. The possibility that melanin may be responsible for the photostable response is supported by a study of electrical photoresponses from various tissues.

WHEN a visual receptor in a vertebrate eye is stimulated by an intense flash of light, it generates a fast electrical response—the early receptor potential (early RP) (refs. 1 and 2). Recently it was suggested<sup>3</sup> that a flash of light might generate similar fast electrical responses in all biological tissues containing oriented pigment, and K. T. Brown has since reported such an electrical response in the pigment epithelium-choroid complex (PE-CC) of the eye<sup>4</sup>. Unlike the early RP, this PE-CC response is photostable, being highly resistant to light adaptation. The PE-CC consists of the cell layers immediately behind the retina which are densely pigmented with melanin, which is photostable and therefore the most likely photopigment for this new response, but Brown reported that a similar photostable response could apparently be observed in the eye of the albino rat which is free from melanin. Consequently, on combining this with other evidence, Brown suggested that the photopigment for the PE-CC response was probably related to the visual pigments rather than to melanin, and that this unidentified pigment might be contained in myeloid bodies, which are paracrystalline structures somewhat similar to outer segments of visual receptors<sup>5</sup>, and which are found in the cells of the PE-CC in both pigmented and albino eyes<sup>6</sup>. Because this new response in some ways resembles the early RP from the visual receptors, myeloid bodies seemed likely sources for it.

The purpose of this article is to report that we have found that the action spectrum of this PE-CC response is flat, just as would be expected from a pigment like melanin, and that we have confirmed our previous work in which we found that there was no large photostable response in the eye of the albino rat<sup>7,8</sup>. Thus we conclude, in contradiction to Brown, that the photopigment for the PE-CC response is not related to the visual pigments, and that the myeloid bodies do not generate this response, but instead, that it is generated or augmented by melanin. This new electrical response of the PE-CC therefore appears to be fundamentally different from the early RP, because the early RP depends on visual pigments<sup>2,9</sup>.

Most of the techniques and apparatus used for this investigation have already been described<sup>2,7,8</sup>. The stimulus flashes were produced by xenon flashtubes with input energies of either 60 J (Fig. 1) or 200 J (Figs. 2 and 3). The stimulus flashes were focused on the preparations by lenses of high numerical aperture; their energy and wavelength were controlled by appropriate filters. A second light beam from a tungsten lamp was used as a steady background light to bleach visual pigments. The rate of quanta for the steady background light is about 10,000 times smaller than for the stimulus. Heat filters were placed in the tungsten beam to facilitate rapid bleaching. For whole excised eye recordings, wick electrodes were placed in contact with the cornea and the back of the eye, and the entire retina and PE-CC were uniformly illuminated by both the stimulus flashes and the bleaching light. All electrical artefacts were eliminated by shielding, and the responses were recorded photographically from oscilloscopes.

Almost all the characteristics of the PE-CC response which we have observed confirm or are consistent with

those reported by Brown for the marine toad, *Bufo marinus*<sup>4</sup>. For example, we have observed this new response from the isolated PE-CC layers stripped from the back of the toad eye, and also from the isolated PE-CC layers of frog and cow eyes. We have also observed this response from whole excised eyes of rat, guinea-pig, and goldfish, and in every eye the time-course and amplitude of the PE-CC response are comparable with those reported by Brown for the toad eye. In addition, we have found that the PE-CC response is not only unaffected by a long series of intense flashes such as were used by Brown, but is also unaffected by extended exposures to intense steady light. Thus, the PE-CC response definitely appears to be photostable. Furthermore, we have found that the amplitude of the major peak of the PE-CC response is proportional to the energy of the stimulus flash, even for our maximum flash energy, which was about 0.1 J/cm<sup>2</sup>. There is, however, one crucial point on which our findings differ from those of Brown—a point that is important for Brown's interpretation of his results. As already mentioned, he reported finding a response in the whole eye of the albino rat that was not abolished by a series of fifteen intense stimulus flashes. Because the early RP is photolabile<sup>1,2</sup>, this finding led Brown to assume that this was a PE-CC response rather than an early RP. We have also observed such an apparently photostable response in the albino eye, even after hundreds of stimulus flashes. But from the beginning of our research we have assumed that this is an early RP because it has been established that intense light flashes photoregenerate the visual pigments on which the early RP depends<sup>10,11</sup>. Recently, Arden and Ikeda have shown that the early RP is photoregenerated by such intense flashes<sup>12</sup>. With this in mind, we have always used steady light sources when attempting fully to bleach the visual pigments, and with such light we have always been able to reduce the amplitude of the early RP in the eye of the albino rat to less than 1 per cent of its maximum (dark-adapted) amplitude. In discussing the photolability of the early RP, Brown states that a series of flashes "abolishes" the early RP<sup>1</sup>. We do not find this to be the case, and we have refrained from using the term "abolish", stating only that with continuous light the amplitude of the early RP can be reduced "below the noise level of the preparation"<sup>2</sup> because we find that even after extended exposures to light, especially in the living eye, the early RP slowly recovers in the dark, and at a rate that can be attributed to the regeneration of the visual pigments. This recovery, by the way, provides some measure of assurance that the preparation has not been significantly damaged by exposure to light.

Fig. 1 illustrates the effect of a series of intense flashes on early RP and then, for comparison, the effect of a steady light. The early RP responses shown in Fig. 1 were obtained from whole excised eyes of the albino rat, a different eye being used at each of three different temperatures. The three responses in the top line (a) are from eyes fully adapted to the dark. After recording these responses, the eyes were stimulated at 2 min intervals by the same intense flash and the response to the fifteenth flash is shown in the middle line (b). This procedure for

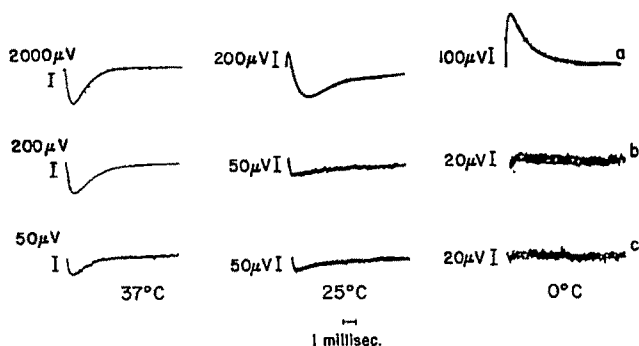


Fig. 1. Effects of light-adaptation on the fast electrical responses of whole excised eyes of the albino rat at three different temperatures. Amplifier bandpass; 1–10,000 c/s. White stimulus flash, 0.7 msec duration. Flash energy such that about 1 quantum was absorbed by each molecule of rhodopsin during a flash. In Figs. 1 and 2 responses of positive polarity at the cornea are displayed as upward deflexions.

light adapting the eye is the same as that used by Brown. It can be seen in Fig. 1 that after such a series of flashes, a sizable response remains, especially at the higher temperatures (note amplification scales). Evidently this is the response that Brown ascribes to the PE-CC chiefly on the basis that it is so resistant to light adaptation. If the eye is exposed to a continuous bright light, however, this response rapidly diminishes in amplitude, and in 5 min the response is as shown in the bottom line of the figure (c). Here the response amplitudes are no more than 1–10 per cent of their dark-adapted values. With continued exposure the amplitudes continue to diminish. Thus, in the albino rat, virtually the entire response recorded from the whole dark adapted eye is photolabile to continuous light. It was important to establish this in our previous work on the early RP because it was necessary to determine whether the early RP was significantly contaminated by photostable responses. Our results show that in the dark-adapted eye of the albino rat, the early RP is essentially uncontaminated.

In the eye of the toad, however, as Brown has clearly illustrated and as we have confirmed, the early RP is severely contaminated by the photostable response of the PE-CC. Because this is an important question for future research on the early RP, we have investigated the extent of contamination of the early RP by the PE-CC response in two other animals—the normal (pigmented) rat for comparison with the albino rat, and the frog. The response recorded from a dark-adapted eye before any visual pigment has been bleached should consist of both the early RP and the PE-CC response. The degree to which the early RP is contaminated by the PE-CC response in

such a dark-adapted eye can be ascertained by comparing this dark-adapted response with one obtained after fully bleaching the visual pigments to eliminate the early RP and thereby to reveal the photostable PE-CC response. Presumably, the same PE-CC response should be present in both cases. To make this comparison we show in Fig. 2 pairs of responses to stimulus flashes of three different colours obtained before and after bleaching the visual pigments. By using different colours the individual contributions of the early RP and the PE-CC response can be further isolated. In the left-hand column of Fig. 2 the stimulus flash contained only light from the far red end of the spectrum. The visual pigments absorbed so little of this light that no early RP could be detected. But the energy of this far red flash was fully effective in stimulating a PE-CC response because, as will be considered later, this response is almost equally sensitive to all wavelengths of light. Thus, in Fig. 2 the responses to the far red flash both before and after bleaching the visual pigments are probably isolated PE-CC responses. These responses are photostable, as can be seen by comparing the first and second traces in each pair. In addition, because the same far red flash was presented to each eye, the relative amplitudes of the photostable responses in the eyes of the frog, rat, and albino rat can easily be compared. It is clear that an appreciable photostable response occurs in both the frog and the rat, but in the albino rat a response is barely detectable, and it is always somewhat reduced by extended exposures to bright light. We have not previously observed this small response in the albino rat eye, and have only observed it now with extremely intense flashes from a 200 J flashtube. It has some characteristics of a PE-CC type response, but its amplitude is an order of magnitude smaller than the amplitude of the PE-CC type response we observe in the normal (pigmented) rat.

In contrast to the PE-CC type responses produced by the far red flash, in the middle column of Fig. 2 a green stimulus flash was used to produce the largest possible early RP with the least possible contamination by the PE-CC response. In the frog, the early RP is relatively small (see amplification scale) and even with this green flash the early RP is significantly contaminated by the PE-CC response. But in the eyes of both the normal and the albino rat, the early RP is much larger, and is essentially uncontaminated, even though in the normal rat there is an appreciable PE-CC response. In the right-hand column of Fig. 2 we used a white flash, with maximum energy, and here, too, the early RP is essentially uncontaminated in both types of rat. In the frog, however, this flash produced a PE-CC response so large that the early RP can only be detected as a slight change in the wave-

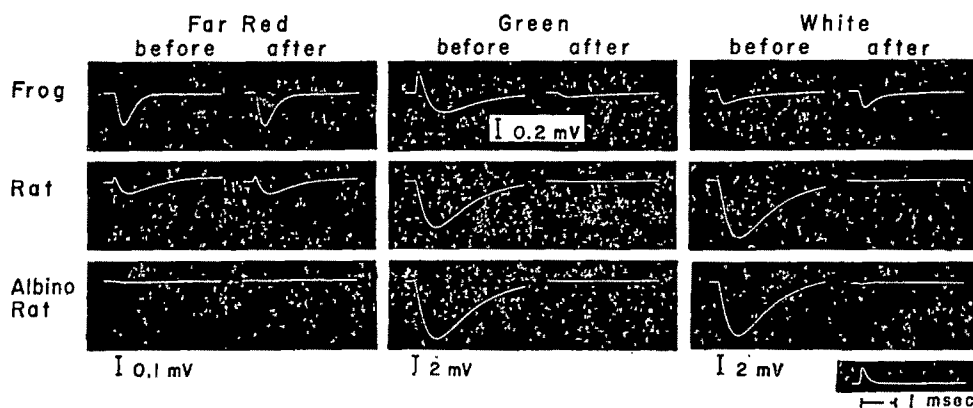


Fig. 2. Fast electrical responses of whole excised eyes before and after bleaching the visual pigments. The first response in each pair of responses was obtained from a fully dark-adapted eye. The second response was obtained with the same stimulus flash but after fully bleaching the visual pigments by exposing the eye for 30–60 min with an intense yellow-green light. This bleaching light was adequately heat-filtered to keep the temperature of the PE-CC from rising significantly. Stimulus flash wavelength bands: far red, 710–1,000 nm; green, 460–560 nm; white, 350–1,000 nm. Approximate flash energy at the retina in J/cm<sup>2</sup>: far red, 0.03; green, 0.008; white, 0.1. Flash duration shown by photodiode tracing. Amplifier bandpass: 10–10,000 c/s. Temperature: 18°C frog; 37°C rat.

form of the PE-CC response on bleaching the visual pigments. In summary, the responses in Fig. 2 show that (1) a PE-CC type response can be isolated in all three animals by using a far red flash, (2) the early RP of the frog eye is significantly contaminated for all three stimulus flashes, and (3) in both the normal and albino rat, the early RP is only contaminated when using far red flashes.

We have observed the action spectrum of the PE-CC response in several animals to help identify the corresponding photopigment. Most of these action spectra were obtained from whole eye preparations to eliminate any possible effects of tissue damage, and also to clarify further the respective contributions of the early RP and the PE-CC responses. Action spectra of whole eye responses both before and after bleaching the visual pigments are shown in Fig. 3 for the same three animals used in Fig. 2. In dark-adapted eyes, the early RP dominates the whole eye response over most of the spectrum and, as has already been reported<sup>7,8</sup>, the resulting action spectrum corresponds to the appropriate visual pigments. At the far red end of the spectrum, however, the whole-eye response changes shape (see Fig. 2) and its action spectrum levels out. This far red response is photostable. Thus, the action spectrum for this response remains unaltered when the eye is exposed to a steady light that fully bleaches the visual pigments. After bleaching, a similar response is observed throughout the spectrum, and the action spectrum for this response is nearly flat. This is the same type of action spectrum as we observe from the isolated PE-CC, and thus the whole-eye response that remains after fully bleaching the visual pigments appears to be an isolated PE-CC response.

The flat action spectra of the PE-CC responses strongly suggest that the photopigments for these responses are not related to the visual pigments, but on the contrary absorb all wavelengths of light equally well. Black pigments such as melanin fit this requirement, and there seems a good possibility that melanin is primarily responsible for generating at least part of the PE-CC response. It is important, however, to note that a small PE-CC type response does occur in the melanin-free albino eye, and that the action spectrum for this albino response is much the same as in the pigmented eye. Thus it also appears likely that melanin may simply augment a response that is already present in the albino eye. In

either case, this evidence begins to rule out myeloid bodies as the source of the photostable PE-CC response. If myeloid bodies generate this response, it should be much larger in the albino eye, where the myeloid bodies are not shielded from the light by melanin. Instead, however, in the albino eye the PE-CC response is at least an order of magnitude smaller, or is absent altogether. Significantly, fast electrical responses quite similar to the PE-CC type response have now been observed from skin, and the action spectra for the fast responses in skin are flat, which again suggests melanin for the photopigment<sup>13</sup>. Moreover, we have observed PE-CC type responses from the back surface of the iris excised from the cow eye, and this surface is densely pigmented with melanin. But in skin, just as in the PE-CC, responses can be recorded from albino skin which are similar to but smaller than those from the corresponding skin containing melanin. Taken together, this evidence suggests that intense light flashes produce a fast electrical response in a variety of biological tissues even in the absence of melanin, but that melanin either augments this response or independently generates a major portion of it.

When melanin is present, all these fast photostable responses from PE-CC, iris, and skin have about the same amplitude for a given energy flash, and they all appear to have flat action spectra extending far into the red end of the spectrum. Brown has pointed out that the PE-CC response is in many ways similar to the early RP, and it is interesting that all these responses bear this resemblance. Because it depends on the visual pigments, however, the early RP is photolabile. Furthermore, for a given flash energy the amplitude of the early RP can be hundreds to thousands of times larger than these fast responses. Moreover, unlike these responses, the amplitude of the early RP saturates at high flash energies<sup>2</sup>. The effects of osmotic pressure and temperature are also different for the PE-CC response and the early RP. (The PE-CC response is greatly affected by glycerol-Ringer solutions<sup>14</sup> but the early RP is not. The amplitude of the early RP is greatly affected by temperature (see, for instance, Fig. 1), but the amplitude of the main peak of the PE-CC response is independent of temperature at least down to temperatures at which the tissue freezes, where changes are seen which are probably due to the same mode of action as the osmotic changes<sup>14</sup>.) These marked differences between the early RP and the fast responses which appear to arise from melanin may simply depend on a different mechanism of generation, or on a difference in photopigments, or on a difference in the distribution or association of the photopigments within the cell. Whether there is a real difference between the two types of responses has yet to be established, but further research with one type of response is certain to offer some insights into the other response.

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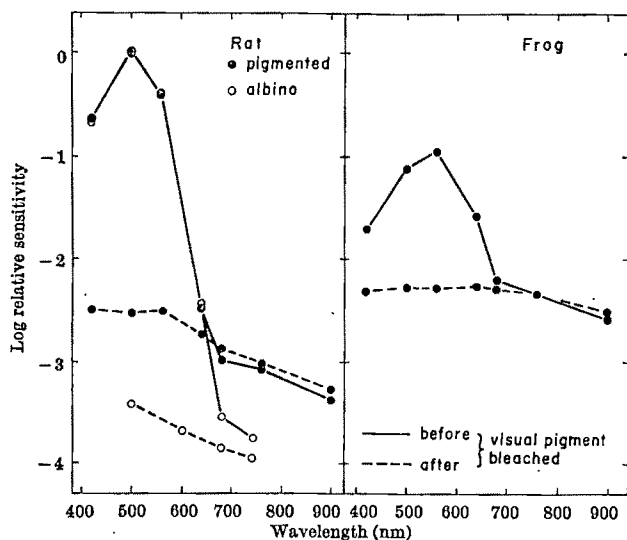


Fig. 3. Spectral sensitivity of fast electrical responses of whole excised eyes before and after bleaching the visual pigments. The sensitivity is the reciprocal of the flash energy required to produce a response of a given amplitude (usually 20  $\mu$ V). The amplitude was measured from the baseline to the point of maximum excursion regardless of the type or mixture of responses present. Well blocked interference filters were used to limit spectral bandwidths to about  $\pm 10$  nm for most points, but points at the end of each action spectrum and all the points for the albino eye after bleaching were obtained with wide-band filters (approximately  $\pm 50$  nm). Temperature: 12°C frog, 37°C rat. Flash duration: 120  $\mu$ sec.

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# Mechanism of Action of Amino-thiol Radioprotectors

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How do amino-thiols protect living cells from radiation? One possibility is that they bind to and stabilize those parts of the DNA helix which are not covered by histones. This reduces both primary and secondary damage, and the DNA replication rate is decreased so that repair processes can act before alterations are replicated.

AMONG the chemicals which are known to protect living organisms against ionizing radiation there is one group, the amino-thiols, which is particularly effective, but despite the numerous papers dealing with the mechanism of action which have appeared, there is still no single theory explaining the prophylactic action of these and other compounds<sup>1-3</sup>.

With few exceptions, the most effective amino-thiols are those whose structures are closely related to that of cysteamine,  $\text{HS-CH}_2\text{CH}_2\text{-NH}_2$  (refs. 4-6). The corresponding disulphides are usually equally active, as are compounds which are readily metabolized to this type of compound<sup>7</sup>. Some generalizations derived from consideration of the structures of these prophylactics are: the amino and thiol groups must not be separated by more than three carbon atoms<sup>8</sup>, a free -SH group is required since thioethers are inactive<sup>8</sup>, alkylation of the amino group reduces, but does not destroy, activity<sup>6,9,10</sup>. Superimposed on these requirements are the subtle effects common in pharmacology; thus, although cysteine is a protector, when the amino and thiol groups are interchanged the product, isocysteine, is not only a non-protector but actually sensitizes living organisms to the action of radiation<sup>11</sup>. At the present time three theories have been thought to offer reasonable explanations, but none is entirely satisfactory alone<sup>1,3</sup>.

The idea that the induction of hypoxia or anoxia was the basis of protection followed from the recognition of the "oxygen effect", that is that the presence of oxygen during irradiation increases damage, but without oxygen natural recovery cannot occur. Another mechanism which has had wide support is that thiols act by destroying the free radicals produced by irradiation<sup>12,13</sup>. The third hypothesis is that of "mixed disulphide formation" advanced by Eldjarn and Pihl<sup>14,15</sup>. These workers believe that the thiol groups of enzymes are the radio-sensitive sites and argue that protective agents form transient mixed disulphides with the enzyme thiol groups. When a mixed disulphide is attacked by a free radical, one of the sulphur atoms is reduced while the other is oxidized so that the damage is reduced by roughly one half.

There are important criticisms of all three theories<sup>1,3</sup>, a common weakness being that neither the structural requirements nor the existence of apparently similar compounds which sensitize organisms to radiation can be explained<sup>16</sup>. Evidently some general unifying principle is needed to rationalize the known facts and to indicate the extent to which any of the above mechanisms may be involved. This article suggests what this principle might be.

There is ample evidence to show that DNA is the site of the primary radiation damage in cells<sup>17,18</sup>. The nature of the damage appears to be single strand breakage followed by deletions and chemical alterations of the bases together with dissociation of histones, if present<sup>19-24</sup>. It has been postulated that the damage is made good by a

repair system<sup>25</sup> and evidence for a most efficient repair system in *M. radiodurans* has recently been obtained<sup>26</sup>. The existence of the repair system is the first requirement for the mechanism proposed in this article.

We can assume that, in order to survive, a dividing cell must successfully replicate a set of nearly normal DNA. We can also agree with Guild<sup>25</sup> that if the repair system has an efficiency of the order of 99 per cent, then small alterations in the speed of repair could alter the amount of residual damage by a factor of three or more. In cases where the repair system is less efficient, however, small changes in the repair rate alone have little effect and the relative rates of three processes, damage, repair and replication, become the governing factors. Crudely we may say that so long as the rate of repair is greater than the rates of replication and damage, the cell should survive. This criterion breaks down, of course, when the rate of replication is so low that essential enzymes which are themselves damaged by radiation are not replaced. The importance of relative rates is borne out by the dependence of the lethal radiation dose on the dose rate<sup>27</sup>. This argument requires that the rates of repair and replication are independent of one another, and there is some support for this in the literature<sup>28</sup>. Thus, control of the three rate processes is required, and we now consider how amino-thiol prophylactics might bring about this control in the cases of replication and damage; there is insufficient knowledge of the repair process for it to be considered at present.

We first note that survival will be favoured by decreasing both the rate of damage and the rate of replication. There is a simple mechanism which would enable both these requirements to be fulfilled—binding to DNA.

If a substance binds to DNA the usual result is that the DNA helix is made more stable. Since DNA replication requires single strand separation<sup>29,30</sup>, the replication rate would be reduced by this increased stability. The mere presence of the binding agent, apart from tending to prevent the original breakage, would also tend to ensure physically that a break in a single strand would not lead to unravelling and consequent secondary damage. There is support for this conclusion in the literature. Thus, histones inhibit DNA dependent RNA and DNA synthesis<sup>29-31</sup>, and deoxynucleoprotein is less sensitive to ionizing radiation than DNA alone when acting as a primer for RNA synthesis<sup>32</sup>.

A feature common to histones is the presence of a large number of amino groups. This, together with the fact that aliphatic diamines are known to bind strongly to DNA<sup>34</sup>, makes it clear that a molecule with two or more amino groups is likely to bind to and stabilize any part of a DNA helix not covered by histone. This conclusion has led to the discovery that the disulphide forms of the amino-thiol protectors also bind strongly to DNA<sup>35</sup>, which explains the need for both the amino and free thiol groups in these protectors. For binding of this type to be relevant to protection, it is necessary for the disulphide form of the prophylactic to be the active one, but on this point there is controversy in the literature<sup>37-40</sup>. The conflicting evidence would seem to reflect the ease of

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reduction of the disulphide (the overall metabolic tendency) and oxidation of the thiol (the *in vitro* tendency).

Binding to DNA is insufficient in itself to afford protection, for the aliphatic diamines, structurally very similar to the protective disulphides, are inactive. Evidently a disulphide link is necessary, and the reason for this may be closely related to the ease of reduction of the disulphide link in living tissue<sup>41</sup>. Certainly cystamine and cadaverine have different effects on RNA polymerase in certain circumstances, for example<sup>42</sup>. Reduction of the -S-S- bond would immediately free the DNA so that replication and repair could take place. This role of the disulphide link is supported by the fact that di(ethylaminoethyl)-sulphide,  $\text{EtNH-CH}_2\text{CH}_2\text{-S-CH}_2\text{CH}_2\text{-NH}_2$ , is inactive, though it should bind as well as, and have half the scavenging capacity of, the active diethylcystamine,  $\text{EtNH-CH}_2\text{CH}_2\text{-S-S-CH}_2\text{CH}_2\text{NH}_2$ <sup>6,11</sup>. With this mechanism in mind, we may proceed to consider the extent to which this hypothesis explains the more obvious aspects of prophylactic activity.

One of the most important structural requirements is that the amino and thiol groups should not be separated by more than three carbon atoms<sup>4-6</sup>. The present hypothesis explains this by referring to the work of Mahler and Mehrotra<sup>34,35</sup> on the effect of a series of normal aliphatic diamines on the melting temperature (stability) of DNA. These workers found that DNA was stabilized only if the number of carbon atoms between the two terminal amino groups was between two and ten. Stabilization of the helix was greatest with diaminopentane, and with diaminodecane had fallen to a very low level. Thus, when ten atoms are present in the chain between two amino groups we may expect very little effect; ten intervening atoms is just the number present in the first non-active aminothiols in its disulphide form, that is  $\text{H}_2\text{N-(CH}_2\text{)}_{10}\text{-S-S-(CH}_2\text{)}_{10}\text{-NH}_2$ .

The work of Mahler and Mehrotra revealed that certain diamines are capable of binding to DNA with a resultant decrease in the stability of the helix. The existence of binding agents which destabilize the helix provides an explanation of the existence of sensitizing agents<sup>16</sup>. Destabilization would lead to an increased rate of replication<sup>43</sup> so that more mistakes arising from primary lesions would be incorporated. Sensitization by compounds with structures very similar to those of protectors has not been adequately explained previously.

Whether a given diamine binds with stabilization or destabilization is dependent on its detailed structure; we can say that the most important factors governing this are the entropy effects associated with the interaction of the surrounding water with hydrophilic and hydrophobic centres in the bound molecule. Since these effects also govern water solubility, it is not surprising that in a closely related series of protectors such as the L-cysteine ester hydrochlorides there is a good correlation between protective capacity and water solubility<sup>2</sup>.

In view of the discovery that histones do, in fact, contain thiol groups<sup>44</sup>, it is tempting to modify the hypothesis slightly in order to include Eldjarn and Pihl's theory of mixed disulphide formation. The same result would obtain if the disulphide were formed between the protector and a thiol group of the nucleohistone; a portion of the DNA normally devoid of histone, and therefore a likely position for the development of damage after a primary lesion, would be stabilized by an easily removable binding agent. The attachment of the histone itself to the DNA would also be strengthened. This possible role of the histones might also provide a rationale for the difference between protection in mammals and in bacteria, for the latter are not known to possess histones.

All three of the earlier theories thus remain admissible as contributing mechanisms, though the present hypothesis suggests that a more fundamental mechanism is also required. The proposed hypothesis is summarized as follows:

Radioprotective aminothiols may act by binding to and stabilizing those parts of the DNA helix not covered by histones. This has two effects. First, apart from helping to prevent the primary lesion, the loose ends resulting from single strand rupture are held in place so that secondary damage arising from shortening or chemical alteration is prevented. Second, the DNA replication rate is decreased so that a repair process can deal with alterations before they are replicated. Binding of this type requires that the disulphide form of the protector is the active one and that the disulphide is necessary for ease of removal, so that repair and DNA and RNA synthesis may proceed. This enables certain structural requirements in the aminothiol protectors, and also the existence of radiosensitizers, to be explained.

The mechanism put forward in the above discussion thus appears to rationalize many features of radioprotection while fitting in with the current view on DNA structure and function. It is hoped that, in spite of the difficulties which will doubtless be found, the general scheme will prove to be valid and result in further understanding in this field.

I should like to thank Prof. Melvin Calvin for useful discussions, the U.S. Atomic Energy Commission for financial support, and the Science Research Council, London, for an abeyant NATO fellowship.

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# Viral Specific RNAs in Infected Cells

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Infection by RNA viruses is followed by the appearance of viral RNA in cells. As well as single and double stranded forms of the viral RNA another viral specific RNA can be found in chicken embryo fibroblast cells infected with the arbovirus Semliki forest virus. This may be concerned in the production of protein specific to this virus.

In cells infected with a variety of RNA viruses, there can be identified viral specific RNA distinct from that incorporated into virus particles (see Levintow<sup>1</sup>). Most often double stranded forms of the viral RNA have been described, but there have also been found in several instances species of viral RNA distinct from both the RNA of the infecting virus and from the double stranded form of viral RNA<sup>2-6</sup>. One of these additional species of RNA is sensitive, or only partially resistant, to ribonuclease; it is of low infectivity, and under appropriate conditions sediments in sucrose gradients between double stranded RNA and the RNA of the infecting virus<sup>2-4</sup>. It has been termed "interjacent RNA" by Martin<sup>6</sup>. There is evidence that the double stranded RNA is an intermediate in the replication of single stranded viral RNA. The role of interjacent RNA in virus growth is not known.

This communication describes the appearance of three distinct species of RNA in chick embryo fibroblasts (CEF) infected with Semliki forest virus (SFV)—an arbovirus containing RNA. A previous communication described a species of RNA in CEF infected with SFV, sedimenting more slowly in sucrose gradients than the RNA contained in the virus particle<sup>7</sup>, and it was thought that this component represented the double stranded form of SFV RNA because it was partially resistant to ribonuclease. Subsequent investigations have shown, however, that this component can be resolved into two distinct RNA species, one of which is highly resistant to ribonuclease. Resolution of the earlier described peak was made possible by comparing its sedimentation in sucrose gradients with that of the reaction product of an RNA polymerase isolated from CEF infected with SFV (ref. 8). This enzyme catalyses the incorporation of ribonucleoside triphosphates into RNA with characteristics of a double stranded molecule, and the reaction product of this enzyme has been shown to be identical with the RNA resistant to ribonuclease isolated from CEF infected with SFV (ref. 8).

The conditions for culturing CEF have already been described<sup>7</sup>. Cells were infected with a cloned stock of SFV at a multiplicity of 20–40 p.f.u./cell in the presence of 2 µg/ml. of actinomycin D by procedures previously described<sup>7</sup>. Under the conditions used, virus production commences at about 4 h after infection and maximum yields of viruses are obtained about 8 h after infection. The maximum rate of synthesis of RNA resistant to actinomycin occurs at 5.5 h after infection, but at 3 h after infection an early peak reaching 40–50 per cent of the maximum value is also seen<sup>9</sup>. RNA was extracted with phenol-sodium dodecyl sulphate from cells which had been exposed to tritiated adenosine or carbon-14 labelled uridine for various periods after infection. Linear sucrose gradients (5–20 per cent sucrose, 0.1 molar potassium chloride, 0.01 molar *tris* hydrochloric acid, pH 7.2, 0.001 molar ethylenediamine tetraacetic acid) were run in the SW 39 rotor of the Spinco model L ultracentrifuge

for 2.5 h. The methods used in the collection of fractions from the gradient, the preparation for counting tritium or carbon-14, and the examination of the infectivity, ribonuclease resistance, and radioactivity of the RNA have been described elsewhere<sup>7,9</sup>. Purification of SFV labelled with phosphorus-32 was by a modification of the method of Cheng<sup>10</sup>. Preparation of the SFV RNA polymerase, assays of the enzyme using tritiated guanosine triphosphate (<sup>3</sup>H-GTP) as the labelled substrate, and extraction of the reaction product have also been described elsewhere<sup>8</sup>.

Fig. 1 shows the distribution in a sucrose gradient of RNA extracted from SFV infected cells incubated with carbon-14 labelled uridine for 7 h after infection. Two main peaks of radioactivity are seen. One sediments at about 45 S and is infectious and sensitive to ribonuclease. The more slowly sedimenting component has a sedimentation constant of about 26 S and, after treatment with ribonuclease, a ribonuclease resistant component sedimenting at about 20 S is revealed. The infectivity in the 26 S–20 S region of the gradient is no more than 3 per cent of that in the 45 S region. The identity of the 45 S RNA with RNA extracted from purified SFV is shown in Fig. 2 where purified viral RNA labelled with phosphorus-32 was mixed with RNA extracted from infected cells labelled with tritiated adenosine, and run together in a sucrose gradient. It can be seen that the phosphorus-32 and tritium labelled RNA sediment in an identical position. RNA extracted from purified virus and 45 S RNA

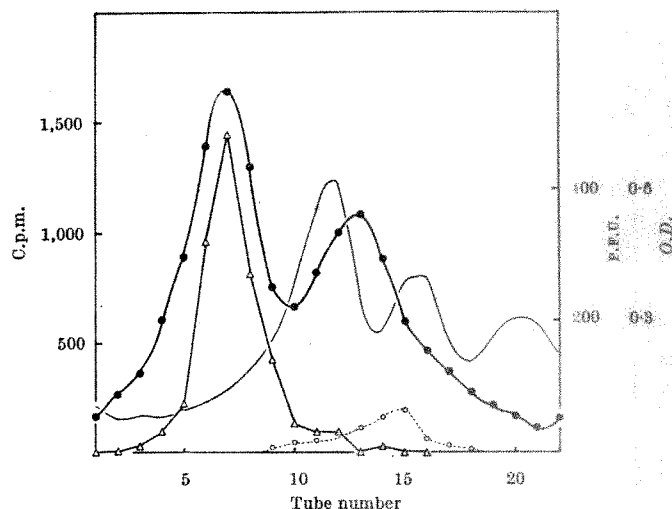


Fig. 1. Sucrose gradient analysis of RNA extracted from CEF infected with SFV. Chick cells treated with actinomycin (2 µg/ml.) were infected with SFV (40 p.f.u./cell) and <sup>14</sup>C-uridine was added. RNA was extracted 7 h after infection, 0.25 mg chick ribosomal RNA was added, and the extract sedimented through a 5 to 20 per cent sucrose gradient. Fractions were assayed for infectivity (Δ—Δ), acid-insoluble radioactivity (●—●), acid-insoluble radioactivity resistant to digestion with ribonuclease (○—○), and optical density at 260 mμ (—).

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had a similar degree of sensitivity to ribonuclease when tested together.

Fig. 3a shows the sedimentation profile of  $^{14}\text{C}$ -uridine labelled RNA extracted from infected cells mixed before centrifugation with a reaction product of the RNA polymerase (labelled with  $^3\text{H}$ -GTP) isolated from CEF infected with SFV. Evidence that this enzyme is a viral specific RNA polymerase and that its product is a double stranded form of SFV RNA is presented elsewhere<sup>8</sup>. The enzyme reaction product sediments in an identical position to the ribonuclease resistant 20 S RNA present in infected cells. In the gradient shown in Fig. 3a, fractions

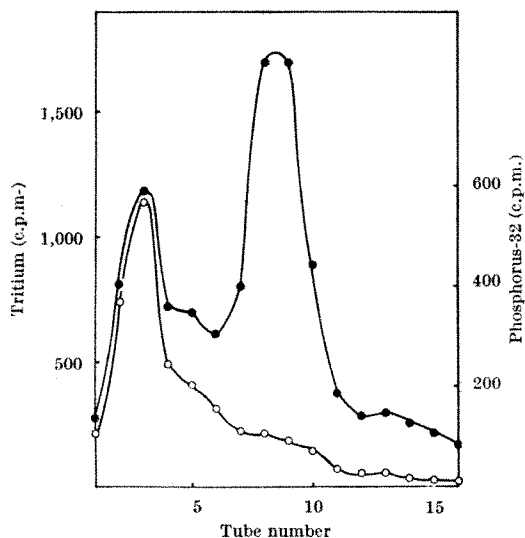


Fig. 2. Sucrose gradient analysis of RNA extracted from infected cells and purified Semliki forest virus. RNA was extracted from cells which had been treated with actinomycin, infected with SFV and then incubated with  $^3\text{H}$ -adenosine from 4 to 6 h. It was mixed with  $^{32}\text{P}$ -labelled RNA extracted from purified SFV and sedimented through a 5 to 20 per cent sucrose gradient. ●—●, acid-insoluble tritium radioactivity; ○—○, acid-insoluble phosphorus-32 radioactivity.

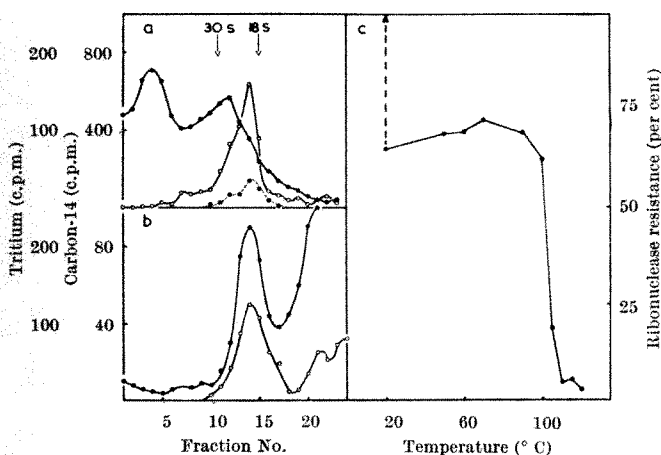


Fig. 3. Characterization of ribonuclease-resistant viral RNA. a, RNA was extracted from cells which had been treated with actinomycin, infected with SFV and incubated with  $^{14}\text{C}$ -uridine from 0 to 6 h. It was mixed with chick ribosomal RNA (0.25 mg) and with RNA extracted from SFV RNA polymerase after incubation in an enzyme assay system with  $^3\text{H}$ -GTP, and sedimented through a 5 to 20 per cent sucrose gradient. Fractions were assayed for acid-precipitable carbon-14 (●—●) and tritium radioactivity (○—○) and for acid-precipitable carbon-14 radioactivity resistant to digestion with 2  $\mu\text{g}/\text{ml}$ . of ribonuclease (●—●). b, As for a, except that the extracts were treated with 2  $\mu\text{g}/\text{ml}$ . of ribonuclease before gradient analysis. c, Fractions from the 20 S region of the gradient shown in Fig. 3a were pooled and diluted with 0.15 molar sodium chloride, 0.015 molar sodium citrate. Portions were heated at various temperatures for 8 min, rapidly cooled to  $-70^\circ\text{C}$ , and then thawed. They were incubated with 5  $\mu\text{g}/\text{ml}$ . of ribonuclease at  $25^\circ\text{C}$  for 30 min and acid-insoluble radioactivity was measured. Results are expressed as a percentage of the radioactivity in the unheated material before digestion with ribonuclease. The 30 per cent loss in radioactivity after treatment of the unheated sample with ribonuclease (dotted line) represents contamination with ribonuclease sensitive RNA.

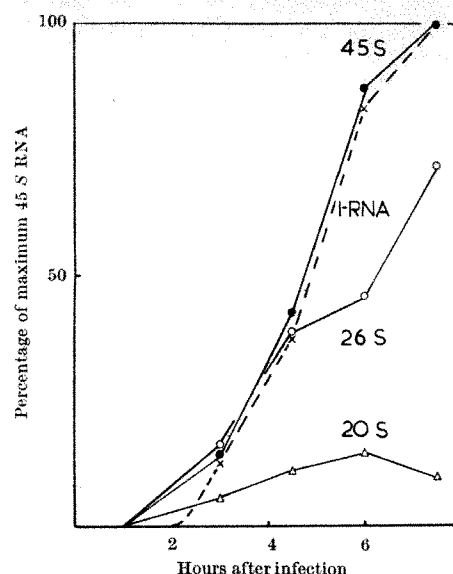


Fig. 4. Synthesis of various RNA species during virus growth. Cells were treated with actinomycin, infected with SFV and incubated with  $^{14}\text{C}$ -uridine from the beginning of infection. RNA was extracted at various times for gradient analysis and determination of specific activity and specific infectivity. The proportions of the various RNA species were calculated from the sucrose gradient and the specific activity of each RNA component determined. The specific activities are expressed as a percentage of the specific activity of the 45 S RNA at 7.5 h after infection. ●—●, 45 S RNA; ○—○, 26 S RNA; △—△, 20 S RNA; x—x, infectious RNA.

were treated with ribonuclease after centrifugation. When the mixed RNA sample was treated with ribonuclease before centrifugation, there was a single peak of carbon-14 and of tritium in an identical position at about 20 S (Fig. 3b). Further evidence for the double stranded nature of 20 S RNA appearing in infected cells comes from studies on its melting temperature. The sensitivity to ribonuclease of RNA isolated from the 20 S region of a sucrose gradient after heating to various temperatures in 0.15 molar sodium chloride is shown in Fig. 3c. A sharp increase in ribonuclease sensitivity after heating to  $103^\circ\text{C}$  can be seen in Fig. 3c. Under the same conditions the reaction product of the SFV RNA polymerase has an identical melting temperature<sup>8</sup>.

The amount of each of the viral specific RNA components present during virus growth was studied by labelling cells with  $^{14}\text{C}$ -uridine at the beginning of infection and extracting the RNA at various times for infectivity determinations, measurement of its specific activity, and sucrose gradient analysis. The relative amount of each of the three RNA components at each time was determined from their distribution in sucrose gradients. From the specific activity of the total RNA a measure could be made of the amount of each of the species of RNA as infection proceeded. Fig. 4 shows the results of such an experiment. Infectious RNA and 45 S RNA increase in parallel, suggesting that most of the infectivity resides in this component (see also Fig. 1). Concentrations of 20 S RNA reach a maximum while 26 S and 45 S RNA are still increasing, and early in infection 26 S RNA shows the highest rate of synthesis. Only in the later stages of infection does 45 S RNA become a major component.

Of the three species of SFV RNA which appear in CEF, 45 S RNA is thus identified as the RNA incorporated into virus particles, and 20 S RNA is a double stranded form of viral RNA. The significance of the 26 S RNA component is unknown. It is unlikely that it represents 45 S RNA associated with ribosomal RNA. The infectivity of 26 S RNA is low or not detectable, and in uninfected cells in which ribosomal RNA had been labelled in advance no evidence of such an association was seen. It seems most likely that synthesis of 26 S RNA is an integral part of virus replication. Early in infection (before 3 h), 26 S RNA is the chief component; thereafter the amount

of 26 S RNA relative to 45 S RNA decreases as infection proceeds (Fig. 4). With brief labelling periods at the time of maximum RNA synthesis radioactivity appears in the 26 S component in advance of 45 S RNA (ref. 7). For example, with a labelling period of 15 min at 5 h after infection, 86 per cent of the radioactivity was distributed in the 20–26 S region of the gradient; this was decreased to 79 per cent with a labelling period of 30 min and to 49 per cent after exposing infected cells to the labelled precursor for 1 h. Even when corrections are made for the ribonuclease resistant RNA component the ratio of radioactivity in the ribonuclease sensitive 26 S RNA to that in the 45 S component still decreases as the time of exposure to the labelled precursor is increased.

RNA with characteristics similar to SFV 26 S RNA has been described in hamster kidney cells infected with foot-and-mouth disease virus<sup>2</sup>, in encephalomyocarditis (EMC) virus infected ascites tumour cells<sup>4</sup>, and in *Escherichia coli* infected with MS 2 phage<sup>3</sup>. Thus it would seem that the formation of this class of RNA is not peculiar to the growth of SFV in CEF. Kelly *et al.*<sup>3</sup> have suggested that 20 S RNA in MS 2 infected *E. coli*, analogous to 26 S SFV RNA, is similar to progeny viral RNA but in the form of a random coil. The sedimentation of SFV RNA (45 S) in sucrose gradients containing 0.1 molar potassium chloride is more rapid than that of EMC viral RNA (37 S), although under identical conditions the double stranded RNA forms of both viruses sediment at approximately the same position (20 S); this would suggest that the RNAs of these viruses have molecular weights of the same order— $2 \times 10^6$  for EMC virus RNA—but that the 45 S SFV RNA may possess a more compact configuration. If these two RNAs differ only in their configurational states, however, then the difference must be a radical one to account for the variance in sedimentation velocities. If the 45 S RNA were circular, possibly through the overlapping of short segments at the ends of the molecule, it would account for the difference. Alternatively, the compactness of the 45 S form may be caused by a low molecular weight component (for example, a polyamine) which is stabilizing the configuration.

Whatever the nature of the structural differences the data on the relative rates of precursor incorporation into the two forms suggests that 26 S RNA is a precursor of 45 S RNA, and that 26 S RNA may be the form in which viral RNA is released from the RNA polymerase. Transition to the 45 S form may occur during incorporation of the RNA into the virus particle; the compact configuration may be imposed on it by the structural proteins of the virus. In addition to serving as a precursor, 26 S RNA could have other functions. For example, it may be in this form that viral RNA acts

as a messenger in the synthesis of viral proteins, or as a template for the viral RNA polymerase.

It should be pointed out that these suggestions need not necessarily apply to other RNA viruses; formation of 26 S RNA may occur as a result of the particular structural characteristics of the viral RNA. There is evidence that Sindbis virus, which is related to SFV, has a high degree of secondary structure compared with poliovirus RNA (ref. 11), and it may be significant that 26 S RNA is such a prominent component of cells infected with SFV compared with other systems.

Since this article was submitted for publication Friedman *et al.* have shown that CEF infected with SFV contain three distinct RNA species similar to those described here. (Friedman, R. M., Levy, H. B., and Carter, W. B., *Proc. U.S. Nat. Acad. Sci.*, **56**, 440; 1966.)

We have now analysed RNA from purified virus and from cells infected with SFV for their base compositions. This was done by incubating actinomycin-treated infected cells with phosphorus-32 for 7 h after infecting and extracting RNA both from cells and extracellular virus (after purification). The RNA preparations were sedimented through sucrose gradients and fractions at the peaks of the 45 S and 26 S regions pooled and hydrolysed with potassium hydroxide. The proportion of radioactivity in each nucleotide was measured after column chromatography according to the method of Katz and Comb<sup>12</sup>. The percentages of adenylic, cytidylic, guanylic and uridylic acids, respectively, in each of the RNAs were as follows: purified virus RNA (mean of duplicates), 27.4, 24.4, 26.1 and 22.2; 45 S RNA taken from infected cells (four determinations),  $27.5 \pm 0.7$ ,  $24.3 \pm 0.7$ ,  $26.2 \pm 0.6$  and  $22.0 \pm 0.5$ ; 26 S RNA from infected cells (four determinations),  $28.0 \pm 0.4$ ,  $24.5 \pm 0.6$ ,  $26.0 \pm 0.4$  and  $21.5 \pm 0.4$ . It therefore seems that the base composition of 26 S RNA does not differ significantly from that of 45 S RNA, which supports our suggestions that these two RNAs may be different configurations of the same molecular species.

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## Mechanism of the Insect Ear

by

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The similarity of the responses and structures of the tympanal organ and an organ in the leg of insects suggests that the former responds to acceleration that induces transient oscillations of the sensory cells.

It was first noted in 1939 that responses could be recorded in the tympanal nerve of *Locusta migratoria* which were synchronous with the amplitude modulation pattern of sounds<sup>1</sup>. It is a general feature of insect tympanal organs that they respond to amplitude modulation and it has been suggested that the species specificity of insect songs lies in the frequency of modulation. Certainly, the songs of insects with tympanal organs are characterized by rapid amplitude modulation, amounting in some cases to pulse modulation.

An intensive investigation of the efficiency of different kinds of amplitude modulation in producing behavioural responses ("phonoresponses") in certain Orthoptera<sup>2</sup> gave results which suggested that "transients" were the essential features of sounds to which the tympanal organs were responsive. There is a basis for confusion here, because transients are normally defined as "phenomena which take place in a system owing to a sudden change in conditions and which persist for a relatively short time after the change has occurred" (British Standards Institution).

They thus involve complex changes in frequency and amplitude. Busnel<sup>2</sup>, however, defines a transient as "a quick rise from intensity zero to maximum intensity or reversed".

Haskell<sup>3</sup> has disputed the important role ascribed to transients by the French school, and in an electrophysiological study of the responses of certain Acrididae to natural and artificial stridulation concluded that the modulation frequency was the only feature of the sound that produced predictable responses in the tympanal organs. Transients (as defined by Busnel) in recordings of natural stridulation produced variable responses.

Autrum<sup>4</sup>, in an electrophysiological investigation of the responses of the tympanal organ of *Tettigonia viridissima*, confirmed that the steepness of modulation, or the rise time, was an important factor in the detection of sound. It was established that constant unmodulated sound of high frequency elicited responses in the tympanal organ of insects. Autrum established that there were two kinds of response: a phasic response, with a relatively short latency, to transients with amplitude changes of sufficient steepness; and a tonic response, with a relatively long latency, to constant tones.

Autrum<sup>5,6</sup> also established that the insect sub-genual organ responded to substratum vibration, and held that vibration of the tibia caused vortices in the blood around the organ, which triggered off activity of the sensory cells. I have investigated the sub-genual organs of the termite and cockroach<sup>7,8</sup>, and found that the electrical responses of the organ to vibration of the leg were similar in many respects to those obtained from tympanal organs in response to airborne sound. With consideration of the structure of the organ and tests on a model, a new theory of the mode of action of the organ was proposed, which can now be elaborated. The same theory would also seem to be applicable to the insect tympanal organ.

A recent examination of the sub-genual organ of the cockroach *in situ*, stained intravitaly with methylene blue, has revealed some features that are not evident in sectioned material. An essential part of the organ is a leaf-like membranous structure attached to the wall of the leg at one end and to the trachea at the other (Fig. 1). This membrane is covered with accessory cells, and is thought to consist of processes derived from them. Sensory scolopale units\* are attached by their tips to the accessory cells. It may be noted that the sub-genual organ of the termite has been described and has a similar structure<sup>9</sup>.

It appears that a sudden displacement of the leg will result in a displacement of the membrane relative to the cuticle. It seems to be a general feature of the insect sub-genual organ that it is anchored to a constricted portion of the trachea (Fig. 1). This means that there is a discontinuity in the structure of the trachea, which will therefore be less resistant to deforming forces acting at this point along the main axis of the trachea. Thus the membrane of the sub-genual organ would appear to be effectively fixed at one end (to the leg wall), and capable of movement at the other; elastic restoring forces are supplied by the trachea. The membrane itself presumably has some elasticity so that it will tend to oscillate in response to a sudden displacement of the tibia. Damping forces will be supplied by the blood in the haemocoel. The same will apply again to each scolopale unit, which is hinged distally, damped by the blood, and subject to elastic restoring forces from the nerve fibre which suspends it.

Electrophysiology has shown that the sub-genual organ of the cockroach generally responds only to the onset of a suitable stimulus or to a sudden disturbance in steady state conditions produced by the stimulus<sup>8</sup>. It was therefore suggested that the receptor responded when it, or some part of it, was set vibrating at a natural (resonant) frequency by a sudden acceleration of the tibia. There appear, theoretically, to be three possible conditions under

which excitation of the sub-genual organ of the cockroach could occur, when: (a) the membrane is in natural oscillation; (b) the scolopale units are in natural oscillation; and (c) both are in natural oscillation at the same time. The last possibility is the only one consistent with the electrophysiological findings, for in the other cases a continuous discharge from the sensilla would be expected at frequencies of stimulation at and around the natural frequency. It can therefore be assumed that a sudden acceleration of the tibia will set the membrane of the sub-genual organ oscillating at its natural frequency, which in turn will cause the scolopale units to oscillate at their natural frequencies. To fit the explanation, it must be assumed that the two natural frequencies are different, which appears evident from the relationships of the structures concerned, so that shear forces will be produced between the two. After the transient natural oscillations have died out the organ will vibrate at the same frequency as the leg, giving rise to a new steady state.

The tympanal organ of the insect is essentially analogous in structure to the sub-genual organ, having a surface that transmits forced vibrations (the tympanum) to which are attached accessory cells, often elongated, to which scolopale units are attached by their tips. If the mode of action of the tympanal organ is similar to that of the sub-genual organ the following characteristics of response are to be expected.

(1) The threshold curves for the tympanal organ should have a shape similar to those typical of the sub-genual organ and suggestive of resonance phenomena. This is well known to be generally true.

(2) If the tympanal organ is an acceleration receptor the optimal threshold displacements for different organs should be inversely proportional to the square of their optimal frequencies. This is seen to be so in the values in the table compiled by Schwartzkopff<sup>10</sup>. A plot of the logarithms of optimal frequencies against optimal threshold intensities gives a regression coefficient of  $-0.62$  ( $P < 0.01$ ).

(3) There should be nervous responses to sudden changes in steady state conditions. These changes could be either in the form of amplitude modulation or "transients" in the sense used by Busnel and his co-workers. The change should impart an acceleration to the sense organ and the minimum acceleration necessary would be defined by the threshold curve. There should be synchronous responses to sound of up to a certain frequency, representing the maximum modulation frequency, providing that the acceleration imparted in each cycle is above the threshold value.

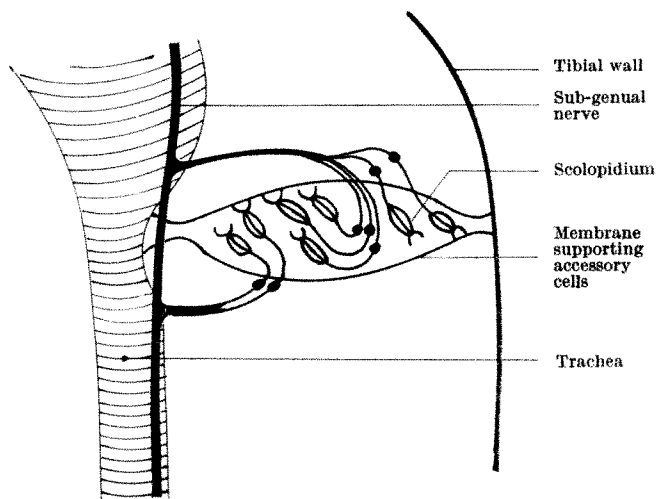


Fig. 1. Diagram of the sub-genual organ of *Periplaneta americana*.

\* The scolopidium minus its accessory cell is referred to here as the scolopale unit.



(4) There should be rapid "adaptation" to a tone of high frequency if the amplitude and frequency are kept constant. There would therefore be a "phasic response" or "on effect". Tympanal organs, however, are clearly very lightly damped systems compared with sub-genual organs and the possibility arises that sound around the optimal frequency would set up strong resonant vibrations of the accessory cells which would be strong enough to keep the scolopale units in continuous vibration, giving rise to a "tonic response". (It is known that substratum vibration of high intensity will sometimes provoke a tonic response in the sub-genual organ<sup>8</sup>.) It follows that the tonic response, although deriving from the same sensilla, would have a longer latency because of the time taken for the build-up of resonance.

(5) At a fixed frequency the rate of production of nerve impulses should be related to the square root of sound intensity, or to the amplitude of displacement of the tympanic membrane, because intensity is equal to  $ka^2f^2$ , where  $k$  is a constant,  $a$  the amplitude and  $f$  the frequency. A limit will be imposed by the non-linear elasticity of the tympanic membrane.

(6) An after discharge should follow a very brief stimulus, and the length of the after discharge reflects the duration of the natural oscillation of the scolopale cells and, therefore, it is proportional to the square root of the sound intensity. It can easily be shown that  $A/A' = t/t'$ , where  $A$  and  $A'$  are initial amplitudes of natural vibration and  $t$  and  $t'$  the respective times required for the vibrations to die down to a given level. An after discharge might also follow the sudden cessation of a constant stimulus because the energy released would result in a free oscillation. This is a special case of (3).

(7) The latency of the response to a harmonic stimulus should vary inversely with (frequency)<sup>2</sup> if the amplitude is kept constant, or inversely with intensity if the frequency is kept constant. This is because the threshold acceleration in a harmonic stimulus will be attained earlier as its frequency or amplitude is increased.

It will be recognized that these are all typical characteristics of the responses of tympanal organs and are illustrated particularly well in orthopteran and lepidopteran tympanal organs, for example, in *Tettigonia viridissima* studied by Autrum and in the tympanal organ of the moth *Prodenia eridania* investigated by Roeder and his co-workers<sup>11</sup>. It is significant in the latter case that touching the sensilla with a fine probe, which would damp any natural vibration, abolished the response to sound.

If the theory proposed here is accepted it is clear that the practice of referring to the tympanal organ as a displacement receptor as opposed to a pressure receptor, while correct, is misleading. It appears that the organ is a pressure gradient receptor<sup>5</sup>, that is, an acceleration receptor. Transients are seen as special cases of amplitude modulation as indeed they are accepted to be, and the importance of a short rise time in provoking responses in the tympanal organ is then clear, because the shorter the

rise time the greater the acceleration imparted to the tympanum. This is not to say that other features of modulation of a sound would not affect the perception of a transient there. Frequency modulations involving the order of change of acceleration we are considering are probably rare in nature and would be difficult to achieve without simultaneous changes in amplitude. Detection of changes in frequency would, however, be possible in some form if sensilla of graded sizes, and therefore differing natural frequencies, were present in a receptor. It is then possible that there is a "typical intensity" of a sound of a certain frequency, which results in the stimulation of a certain proportion of all sensilla, because the optimal and natural frequencies may be equated, and the higher the optimal frequency the lower the threshold at this frequency (see note 2). Sound of greater or lesser intensity would activate more or less sensilla. Evidence for a typical intensity is found in the work of Busnel and Loher<sup>12</sup>, who found an increase in phonoresponses in *Chorthippus brunneus* to sounds up to a certain intensity, after which there was a falling off in the number of responses. At 85 dB with a time rise of 2 msec they found a maximum of 45 per cent positive responses, but only 9.5 per cent at 100 dB. The same sort of condition could arise with a shift in the frequency of a sound stimulus with constant amplitude, and this would explain very well certain unexpected findings. In the tettigoniid *Gampsocleis buergeri* Katsuki and Suga<sup>13,14</sup> found an increase to a maximum in impulse frequency in central neurones and then a decline as the frequency of sound was increased at constant intensity. They found evidence for the existence of two groups of neurones. Those of one group were activated only by sound of higher frequencies, and were more sensitive to stimuli than those of the other group which were activated by sounds of lower frequencies. Horridge<sup>15</sup>, studying *Locusta migratoria*, *Schistocerca gregaria* and *Acheta domesticus*, also found evidence for two different groups of receptors, sensitive to higher and lower frequencies, and showed that the discrimination took place at the level of the sense organ.

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## Origin of Eyes and Brains

by

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The evolution of the mechanism of visual perception must entail the separate elaboration of eyes and brain. But which came first? Or is this a "hen and egg" problem?

ALL knowledge comes through the senses, and this suggests an intimate link between the evolutionary development of brains and eyes. Somehow brains and eyes are developed by the random steps which form the evolutionary ladder according to the dictates of survival value at each rung.

The restraints imposed on the development of perception by natural selection give some clue as to the way in which eyes and brains came into being. The fossil record is of no use in tracing the origins of these organs because the crucial steps have not been preserved. An attempt



may, however, be made to put existing "primitive" forms into sequence according to various criteria. I shall not attempt a detailed account but rather endeavour to outline how the principal forms of eye could have come about and consider the "hen and egg" problem: which came first—the eye or the brain?

"Simple" eyes have a single optical system serving many photoreceptors. "Compound" eyes, found in arthropods, have up to many thousand individual optical systems, each with its own photoreceptor. Both kinds of eye must have appropriate neural systems to handle the information they provide. There is then a "hen and egg" problem, for it is very difficult to imagine how an eye could develop unless there were some suitable neural system already present to handle its information. Why, however, should a visual "computer" arise before there was an eye to feed it with information? If there is an answer to this, there is then the question of why there should be the two great classes of eyes.

Animals live in a world of objects, some edible, others dangerous, some protecting, such as crevices in rock, and others irrelevant to their survival. Objects have many characteristics beyond shape and colour, which alone are represented by optical images. It is these other characteristics which are biologically important. One cannot be attacked and eaten by an image—hence the vicarious pleasures of the cinema—and neither can one feed on images. It follows that information given by eyes is only of indirect use to living creatures, and to make any use of visual information considerable computing is required. Other senses, especially touch and taste, do, however, give information of immediate value and thus it can be assumed with some confidence that these senses were the first to develop. Touch and the chemical senses directly monitor biologically vital features of the environment, and their information requires but a minimal "computer". It is therefore reasonable to suppose that vision is a lately acquired sense. How did it arise? How did a neural computer develop which was capable of reading significance into the non-biologically important optical images? The problem is especially acute because the significance of the images is not in the present state of affairs, but rather in what they presage of the immediate future. Touch and the chemical senses signal the state of the environment in immediate contact with their owner, but vision signals distant events, and may give warning of the future. The visual computer cannot be a simple reflex affair, which acts as soon as possible to a "stimulus". It must transform optical information into hypotheses of the nature of distant objects, which may be friend or foe, food or disaster. The eyes give time to compute; they allow brains to devise strategies more subtle than reflexes in response to stimuli.

*Origin of the simple eye.* The first eyes<sup>1,2</sup> were merely regions of the outer skin which were sensitive to light, perhaps originally responding more to heat than to light. These first light-sensitive regions could have fed the original touch neural systems. Simple tropisms and immediate reflex action to photic stimuli could have been mediated by the touch neural system. It is not too difficult to imagine a gradual improvement in the sensitivity to light and that certain cells became specialized photoreceptors in regions where they would be most useful. The ends of the animal were favoured with the first true eyes—gradually deepening pits lined with specialized cells sensitive to light. The pits served to increase the contrast of shadows, reducing ambient light like the shafts dug in the ground by ancient astronomers to view stars in day-time. Plato describes how Thales fell down such a pit; obviously he should have covered his pit to prevent such accidents. It seems that the eye pits of primitive creatures did develop such protective covers—in the form of transparent windows—to prevent blockage by alien bodies, such as particles in suspension and grains of sand. It is usually held that these windows gradually thickened

towards their centres, for this would increase the intensity of light at the bottom of the eye pits. From such a process a lens would have evolved, and thus give true formation of images. With improvement in the resolution of the lens the acuity would increase to match it, transforming skin which was sensitive to touch into a true retina. The optical reversal of the lens must have imposed the biggest jolt since life began, for the ancient touch inputs were suddenly reversed, evidently requiring the dramatic reorganization of the nervous system which we see today in the crossings of the chiasma. Retinal information is different from the information gained from touch, for not only is the pattern reversed but also it refers to distant objects. The crossings of the chiasma probably served to relate the reversed retinal patterns to touch information from the body<sup>1,3</sup>. Primate perceptual systems can compensate for systematic translations of their retinal images<sup>4,5</sup>, but there is no evidence of this ability in creatures as advanced as even the amphibians<sup>6</sup>, and so explicit reconnection seems to have been necessary at the early stages of perception. The retinal image is not only reversed: it shrinks as represented objects become more distant. It seems that this is compensated by scaling mechanisms, which give size constancy<sup>7</sup>, to maintain an effective connexion between the neural representations of touch and vision despite changing image size.

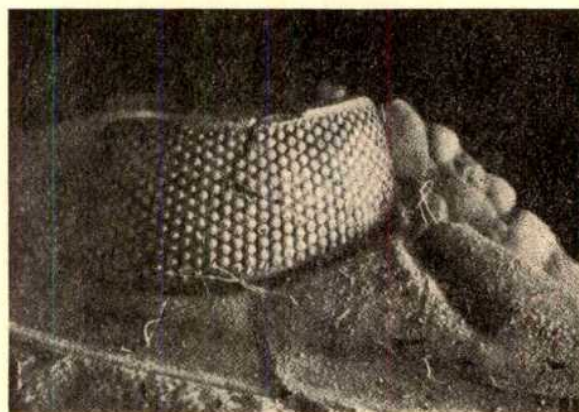


Fig. 1. An early compound eye of a trilobite from the Cambrian.

*Origin of compound eyes.* Each unit, or ommatidium, of a compound eye consists of three basic elements: the corneal lens, crystalline cone and the photoreceptor, or rhabdomere. Compound eyes are found in early fossils, in trilobites from the lower Cambrian rocks of about 600 million years ago (Fig. 1). They appear to be essentially the same as modern insect eyes, but because there are no earlier examples in the fossil record it is only possible to guess at the earlier stages, using living creatures as examples of what has occurred, however uncertain the order may be. There is a special difficulty with the compound eye: what could give rise to many separate and identical elements, each complete with lens and photoreceptor? It is difficult enough to imagine how the simple eye developed—but how could several hundred, all at once? A single element would seem quite pointless—or is it?

I have supposed that the simple eye took over existing touch neural mechanisms, and will later point out that there are basic reasons for supposing that touch information was essential for the first vision. There are two kinds of touch, which involve entirely different neural mechanisms. These are skin pattern touch and limb probe touch. Pattern touch involves the reception of patterns by contact with areas of skin, while probe touch is very different and



requires exploratory movements of a limb. Pattern touch gives information only of structures lying on the two dimensional surface of the skin, while probe touch gives information in three dimensions, within the reach of the limb. Pattern touch is mediated by many parallel neural channels simultaneously sending pattern information to the central nervous system; active touch is essentially single channel, signalling structure in three dimensions but spread out in time as the probe explores external space. The neural systems for the two kinds of touch must always have been very different; one requires many parallel channels, the other a single channel transmitting information of space traced in time.

If the first retinal images were accepted by the original nervous system responsible for pattern touch, could the compound eye be a multiplication of a single moving element, signalling down a single channel to explore space in a manner similar to probe touch?

An example exists of a living creature in which a single unit eye seems to work as a photic probe. Exner<sup>8</sup> described a small copepod, *Copilia*, living in the bay of Naples. He described that it has a pair of strange eyes, like telescopes with two lenses; the second "eye piece" lens is deep in the body, and in "continual lively motion". We have examined living specimens, and it appears that these eyes could be single channel scanning probes<sup>9,10</sup>.

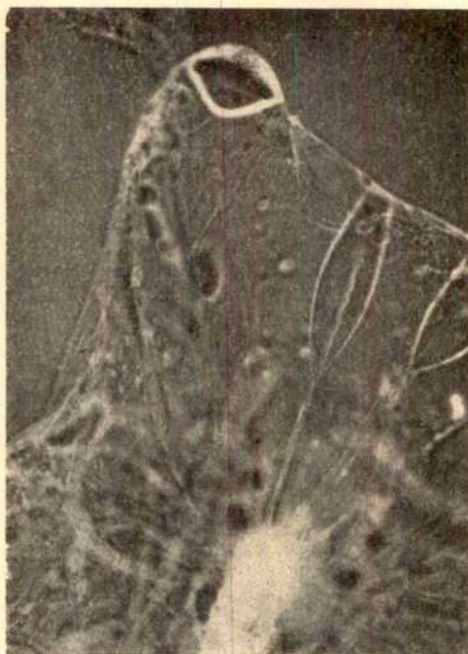


Fig. 2. Photograph of a single channel scanning eye of *Copilia quadrata*.

The female of *Copilia quadrata* is 4-5 mm long, 1 mm wide, and entirely transparent; there is no colouring except for an orange pigment which surrounds the single photoreceptors of each eye. Under the microscope the internal structure of *Copilia* is clearly visible, including that of the eyes and the optic nerve. The two eyes occur on opposite sides of the animal, which has an unusual shape for a copepod because it is very wide at its anterior. There are two large lenses placed far apart in front. Each eye has a large anterior lens and a second posterior smaller lens which is shaped like a pear. Almost half the total volume of the animal is taken up by the two eyes. Attached to the posterior lens, which is situated deep in

the body of the animal, is a long orange pigmented structure which extends back from it and curves inward, but does not touch its counterpart from the other eye. The posterior lens is situated at the image plane of the large anterior lens, and moves horizontally across the image plane of the first lens with a "saw-tooth" scan. The "scanning" rate varies from about one scan/2 sec to about five scans/sec. The movements are synchronized; the posterior lens and photoreceptor of each approach and recede together. In the resting position the optical units are separated from each other, near the tough transparent outer cuticle of the creature, from which position they rapidly approach each other and then slowly separate across the image planes of their respective anterior lenses.

The optic nerve arises from about half way down the pigmented photoreceptor, and enters the brain which is located at the centre of the creature. *Copilia* has very simple mouth parts and, because it is a filter-feeder, does not have to seek its "prey". It is almost certain that sexual reproduction occurs; obviously this requires recognition of the male. It is not known, however, what use *Copilia* makes of its eyes; however, it does seem clear that each eye is an optical unit, which works by temporal scanning somewhat like a simple television camera. Detailed examination reveals that the elements of the eye are extremely similar to corresponding elements of an ommatidium of a modern compound eye. The spacings between the anterior and posterior lenses, however, are quite different—distant in *Copilia* but almost touching in normal compound eyes. The lenses themselves and the single "rod" photoreceptors, however, are almost identical.

*Copilia* could be a surviving form with a prototype single channel scanning eye. The compound eye could have developed by multiplication of these units. It seems easier to suppose this than that a hundred or a thousand units sprang up together. Why should the prototype single element scanning eye not develop further, but rather multiply to form the compound eye? The answer to this seems fairly clear—the basic engineering limitation of scanning systems is the amount of information which they can transmit by the single channel. The channel capacity of any nerve fibre found in nature is extremely low compared with electronic channels; at best, the maximum frequency response approaches only  $10^3$  pulses/second; while, for comparison, to transmit television pictures about  $4 \times 10^6$  cycles/second are needed. Sophisticated perceptual systems save channel capacity by various tricks<sup>11</sup>, but a vast discrepancy still remains. If, however, a scanning eye were duplicated, the elements could send down information simultaneously. This would be rather like exploring a structure with two fingers at once. There could be three fingers, four fingers . . . a thousand fingers or ommatidia. There is no need to sweep the eye of a thousand optical units across the structure, for the entire pattern can be signalled by simultaneous transmission down the many channels. Then scanning can be abandoned. The compound eye started by using a single channel, with the kind of temporal information processing of probe touch, but it can be supposed to have developed many static parallel units, to become, like the simple eye based on the pattern touch neural system, a mosaic eye. Compound eyes which have too few elements to have entirely abandoned scanning (or active exploration) by each photic probe are still found. In *Daphnia* (Fig. 3) we find a compound eye which consists of about twenty elements, and this eye is in continuous oscillatory movement. This seems to be an eye which is part-way up the sequence from the single channel scanning eye of *Copilia* to the fully developed static compound eye. In *Daphnia*, there are few units and it seems that the deficiency is made up by oscillatory scanning very like the single unit eye of *Copilia*. All compound eyes with but few elements can be assumed to scan.



I have outlined, in general terms, a possible sequence of events leading to simple and to compound eyes. Each must have taken over and developed for its own use the primordial neural mechanisms mediating touch. The simple eye took over the pattern touch system; the prototype scanning unit, which later formed compound eyes by multiplication, took over probe touch neural mechanisms. By supposing that already existing touch mechanisms were taken over for vision, the "hen and egg" problem of which came first, eye or brain, is answered. There is some justification for supposing that the first eyes took over earlier touch neural systems, which then developed to process distant information. Jumping a thousand million years or so, there may be other and deeper reasons for believing this.

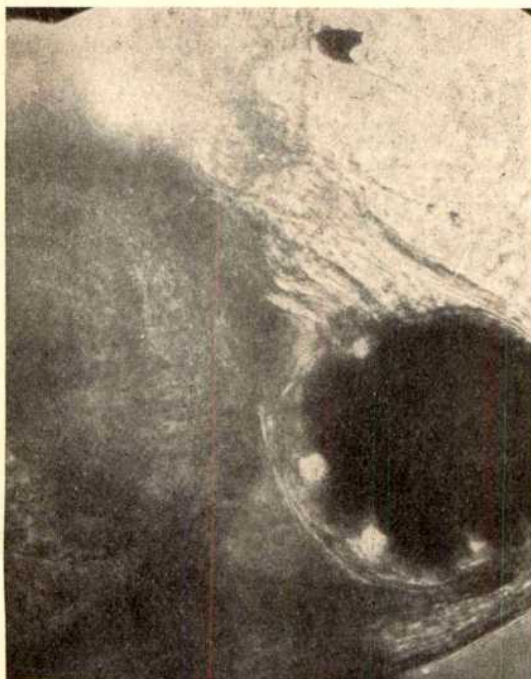


Fig. 3. Photograph of the eye of *Daphnia*; possibly a scanning eye.

*Origin of visual perception.* What are the essential differences between perception in primitive creatures and in higher animals, including man? It is generally believed that all primitive perception is a matter of reflex neural mechanisms, activated by more or less specific "stimuli". Perceptual learning is certainly minimal in primitive creatures; and these special visual patterns can be supposed to have taken on significance through ancestral disasters just as in the development and inheritance of more obvious structural characteristics through natural selection. Some insects do show visual learning (for example, bees learn key features of the terrain around their nests for navigation) but this ability is much greater in mammals, especially primates. Clearly perception, as it developed phylogenetically, became less and less tied to specific visual patterns, so that finally a large variety of patterns elicit the same behaviour. We may say that perception becomes geared to the response to objects, no matter how they are presented to the senses. Finally, retinal images become indicators, symbols, identifying objects. What happens is that we perceive far more than is actually sensed in each moment of perception. We "see" that a table is hard, and a chair safe to sit on. We "see"

from a smile that a person is pleased. This goes far beyond the given sensory data and yet it is usually correct. This ability of the human perceptual system to go beyond immediate data is brought out most dramatically by considering cartoons<sup>10</sup>. A few lines convey an entire story with the personality of each person and his mood. It is useful to think of perceptions as hypotheses based on, but certainly not limited by, current sensory data.

When visual information leads to behaviour appropriate to non-visual features (such as the hardness of a table, though the image of the table is not hard), then the retinal image is acting as a sign. Retinal images are symbols, like words in a language; however, like any other symbols there must be a process of initial association to acquire significance, or the symbols are in a logical vacuum and cannot represent any reality. Furthermore, retinal images are but flat projections of a three dimensional world and yet they give perception of three dimensions. There must be direct, non-visual, information of the third dimension. Other information comes from touch. It appears that any conceivable device for perceiving which relies on two dimensional images must use, at some stage, direct touch information if it is to interpret its images in terms of the three dimensions of surrounding space.

Dependence on early touch experience for visual perception is supported by cases of recovery from congenital and early blindness<sup>12,13</sup>, and in animals reared in the dark<sup>14,15</sup>. What is true for development of perception in the individual should also be essentially true for the development of vision in evolution, for touch must have preceded vision if touch information is required to make retinal images effective symbols of the non-optical world of objects.

If simultaneous information from the eyes and from touch is required to develop correlations between them, regions of the body which could not be seen could not give correlations. Held and Hein, in an address to the Congress of Psychology in Moscow, have shown that a monkey which is denied the sight of one of its fore limbs does not develop normal eye-limb co-ordination, though the other limb is normal. It is the fore limbs, and especially their movements, which are available to vision but not pattern touch—which is hidden from the eye by (non-transparent) objects in contact with the skin. It is active rather than passive limb movement which gives visual learning<sup>16</sup>.

In the human being we see preserved almost all the stages in the developments of vision from the simplest reflex (closing of the eyes on sudden change of illumination), to pattern recognition, and identification of objects from unusual points of view, with prediction of the immediate future based on the past. Such feats cannot be simulated with even the most advanced computers.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Gravitational Red-shifts in Quasi-stellar Objects

It has already been pointed out that the very large energies,  $\sim 10^{60}$  erg or more, associated with some radio sources imply the existence of supermassive objects and strong gravitational fields<sup>1,2</sup>. The subsequent discovery of large red-shifts associated with quasi-stellar objects<sup>3-6</sup> might well have been taken as confirming the existence of such strong fields. Greenstein and Schmidt<sup>7</sup>, however, gave an argument to show that the observed large red-shifts are not of gravitational origin, and this argument has generally been thought to demonstrate the cosmological nature of the quasars. The purpose of this communication is to argue that, while the conclusion of Greenstein and Schmidt<sup>7</sup> is correct for their assumed model, a different model can be postulated, one which permits a gravitational explanation for the red-shifts of the quasars.

The unusual picture of gravitational reddening is that quanta emitted from the surface of an object of mass  $M$  and radius  $R$  experience a wavelength shift given by  $\Delta\lambda/\lambda_0 = z = (1 - 2GM/Rc^2)^{-1/2} - 1$ . Apart from the difficulties discussed by Greenstein and Schmidt<sup>7</sup>, there is the further difficulty in this picture that even the maximum permissible value of  $z$  is still not adequate to explain the observations. For a perfect fluid, the well known Schwarzschild interior solution gives  $z_{\max} = 2$ . The perfect fluid case is unreal, however, in that the transmission velocity within the fluid exceeds the speed of light. An analysis by Bondi<sup>8</sup> leads to the conclusion that, if appropriate conditions on stability and on the equation of state are imposed,  $z_{\max}$  cannot exceed about 0.62. Yet values of  $z$  somewhat in excess of 2 have been observed<sup>9-11</sup>.

We propose that the model be changed to one in which the observed emission lines come from the centre of an object, not from its surface. Instead of the system comprising a single coherent cloud of gas, we imagine a large number of comparatively compact subunits with radii  $\ll R$ , the probability for an arbitrary direction through the centre intersecting any of the subunits being small. As an example of the kind of structure we have in mind, we could equate the subunits to clusters of neutron stars or other highly collapsed objects. We could take  $M = 10^{12} - 10^{13} M_{\odot}$ ,  $R = 10^{18} - 10^{19}$  cm, in which case the probability of an arbitrary direction from the centre intersecting a star is  $\ll 1$ . If the cloud at the centre has radius  $\sim 10^{16} - 10^{17}$  cm, say, light emitted by it passes essentially freely through the star distribution. In this new model the main mass serves to generate a strong gravitational field. Continuum emission is taken to be of non-thermal origin (although faint stars could make a contribution in the near infra-red) while emission lines come from the gas cloud at the centre.

The numerical values for  $R$ ,  $M$ , and for the radius of the central cloud, can be changed from those already mentioned. The foregoing values seem to accord with the observed properties of quasars, but the model is evidently not critically dependent on these particular values.

The gas cloud at the centre lies at the bottom of a gravitational potential well. Writing the metric in the form

$$ds^2 = c^2 e^{\nu} dt^2 - e^{\lambda} dr^2 - r^2 d\Omega^2 \quad (1)$$

$e^{\nu}$  is effectively constant throughout the cloud. Emission lines from the cloud are red-shifted by  $1 + z_c = e^{-\nu/2}$  where the subscript  $c$  denotes central values. The immediate advantages of the present model are that (a) gas naturally tends to fall to the centre of the system, and (b) the whole central gas cloud is subject to the same red-shift. Because of (b), emission lines from the cloud are not smeared out, as they would be in the model considered by Greenstein and Schmidt<sup>7</sup>.

There is a third important advantage. The red-shift  $z_c$  from the centre can be much larger than the shift  $z_s$  associated with emission from the surface. For example, we have

$$\frac{1 + z_c}{1 + z_s} = \frac{2}{2 - z_s} \quad (2)$$

in the Schwarzschild interior solution. In the limiting case in which the central pressure  $\rightarrow \infty$ ,  $z_s \rightarrow 2$ , so that  $z_c \rightarrow \infty$ . This property is general for all static structures. For a given  $M$ , and for a simple equation of state,  $p = p(\rho)$ , there is an infinity of equilibrium solutions corresponding to different choices for  $R$ . As  $R$  decreases,  $p_c$  increases until  $p_c \rightarrow \infty$  for a certain  $R$ , when  $z_c$  also  $\rightarrow \infty$ . A distribution of subunits, star clusters, for example, will in general have a more complicated "equation of state" than this, but the same property must hold. As a further explicit example, in the "standard model" studied by Tooper<sup>12</sup>

$$\frac{1 + z_c}{1 + z_s} = (1 + f\alpha)^{4/f} \quad (3)$$

Here  $f$  is a number satisfying the inequalities  $2.5 \leq f \leq 4$  and  $\alpha$  is proportional to the central value of the ratio of the pressure to the baryon number density. The limiting case for such a model occurs when  $\alpha \rightarrow \infty$ , in which case  $z_c$  again  $\rightarrow \infty$ .

Radiation pressure does not play a critically important part in the study of static stellar atmospheres because gravity and radiation pressure which act on an ion depend similarly on the radial co-ordinate, so that radiation pressure cannot come into adjustment with gravity through a change of  $r$ . The situation is different in the present case, however. For ions outside the central gas cloud, radiation pressure decreases with  $r$ , whereas gravity increases with  $r$ , provided  $r$  does not become too large. (For a stationary particle in the metric (1) the radial acceleration  $d^2r/ds^2 = -\frac{1}{2}e^{-\lambda}dv/dr$ , which increases in magnitude with  $r$  because  $dv/dr = 0$  at the centre. Eventually, the acceleration falls off as  $r$  continues to increase.) This means that stable equilibrium positions are possible in principle for clouds of ions that surround the central cloud. It is possible that the absorption lines found in several quasars arise from such floating ion clouds. This suggestion would require  $z$  for absorption lines to be less than  $z$  for emission lines—at any rate for a static ion cloud. It also suggests that  $z$  may be different for different ions. Motions of the ion clouds or departures from spherical symmetry will lead to other observational possibilities.

The value  $M = 10^{12} - 10^{13} M_{\odot}$  mentioned previously would accord with the possibility that quasars may give rise to galaxies, or even to whole clusters of galaxies. For the usually accepted mean intergalactic density of about  $10^{-28}$  g cm<sup>3</sup> the total mass within 100 Mpc is about  $10^{18} M_{\odot}$ . Most of this mass is unaccounted for—the galaxies which are spread uniformly throughout space give about  $3 \times 10^{-31}$  g cm<sup>3</sup>. If the "missing mass" were condensed entirely in quasars of mass as large as  $10^{13} M_{\odot}$  there would still be about  $10^5$  quasars within 100 Mpc.

A further consequence of the proposed model is that a simple magnitude-red-shift relation for quasars such as that derived on cosmological grounds, for example, would not necessarily be expected.



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### Possible Large-scale Clustering of Quasars

Strittmatter, Faulkner and Walmesley<sup>1</sup> have recently pointed out that quasars with large red-shifts do not appear to be distributed isotropically. The quasars with red-shift  $z > 1.5$  are mainly confined to two regions, one near the North Galactic Pole and the other in the South Galactic Hemisphere. These groups each have an angular diameter of about  $30^\circ$ . Quasars with intermediate  $z$  also appear to be distributed anisotropically, but with a larger angular diameter. Strittmatter, Faulkner and Walmesley have suggested that, if future observations confirm these trends, then either (a) the universe is anisotropic for  $z \geq 1$ , or (b) quasars are not at cosmological distances.

We would like to propose a third possibility (c) that the universe is inhomogeneous on a scale corresponding to  $z \sim 1$ . This seems more plausible than anisotropy because the radio sources in the 4C catalogue are distributed isotropically<sup>2</sup>, and the excess microwave background<sup>3-5</sup> which probably comes from regions with  $z \gg 1$  (ref. 6), appears to be highly isotropic. (A value of 3 per cent is quoted by K. S. Thorne in a communication to be published in the *Astrophysical Journal*.) Moreover, the existence of large scale inhomogeneities is compatible with an overall isotropic, homogeneous point source model of the universe.

We assume that the universe now contains regions of dimensions  $R \sim 1,500$  Mpc in which the density exceeds that outside by the factor  $x$ . If the higher density facilitates the formation of quasars, prolongs their life-time, or increases their intrinsic radio luminosity, then the number of quasars per unit volume with a given limiting radio flux density will be higher inside clusters, by a factor, say,  $x^n$  ( $n > 1$ ). If  $n$  is large, a correspondingly smaller value of  $x$  yields a given clustering of quasars. An upper limit on  $n$  is, however, set by the observed log  $N$ -log  $S$  relation for quasars<sup>7-10</sup>. Following Davidson and Davies<sup>7</sup> and Longair<sup>8</sup> we find that  $n \leq 3$ . A detailed discussion would not be justified but for illustration we take  $x \sim 3$  and  $n \sim 2$ . Then if clusters occupy about one-third of space, they would contain more than 80 per cent of all quasars.

We must also avoid excessive anisotropy in the effective temperature  $T'$  of the microwave background. We would

expect  $\frac{\delta T'}{T'} \sim \frac{G \delta M}{c^2 R}$  (ref. 11), where  $\delta M$  is the excess mass

in a cluster. We find that, if  $\frac{\delta T'}{T'} \leq 3$  per cent, the present mean density  $\rho \leq 10^{-30}$  g/c.c. (for  $x \sim 3$ ). This is consistent with the observed lower limit of  $3 \times 10^{-31}$  g/c.c.<sup>12,13</sup> and implies a small deceleration parameter  $q_0 (\leq 0.025$  if the

cosmical constant is zero). Such a low density universe would be consistent with the observed lack of absorption on the short wavelength side of Lyman  $\alpha$  in the spectra of quasars with  $z \sim 2$  (refs. 14-18), since any intergalactic gas present would be ionized by ultra-violet radiation from quasars<sup>15,19</sup>. Furthermore, a low density universe can be more easily reconciled with the observed X-ray background at 3-8 Å (refs. 20-24), and with the low helium abundance in certain old stars<sup>24-29</sup>.

Since quasars of low  $z$  occupy a large region of the sky, it is likely that we are inside a cluster. These quasars are, however, more numerous, relative to quasars of high  $z$ , in the Northern Galactic Hemisphere, so we are presumably situated appreciably off-centre. A schematic diagram of a possible arrangement of clusters is shown in Fig. 1. We emphasize that this diagram is purely illustrative, and may require modification when further red-shifts are determined. Two features are, however, worth noting. First, it provides a natural explanation of the fact that quasars with  $z \sim 3$  have not yet been observed, although on grounds of radio flux density and apparent optical magnitude such red-shifts would be expected. (This point has been stressed, in conversation, by Prof. E. M. Burbidge.) Second, we can derive a relation between the angular diameter  $\theta$  of a roughly spherical cluster and its minimum and maximum red-shifts  $z_1$  and  $z_2$ . In a low density universe with  $x \sim 3$ , the self-gravitation of clusters would be unimportant. A straightforward calculation then gives

$$\tan^2 \theta / 2 = \frac{4(z_2 - z_1)^2 (1 + z_1 + z_2 + z_1 z_2)}{(z_1 + z_2 + z_1 z_2)^2 (2 + z_1 + z_2)^2 - (2 + z_1 + z_2 + z_1 z_2)^2 (z_2 - z_1)^2}$$

The existing data suggest that  $z_1 \sim 1$ ,  $2 < z_2 < 3$ , for which  $24^\circ < \theta < 33^\circ$  (a result which depends insensitively on the cosmological model). Thus, the computed angular diameter is in reasonable agreement with the observed angular diameters. Our own cluster, as drawn, would be comparable with the other two observed clusters.

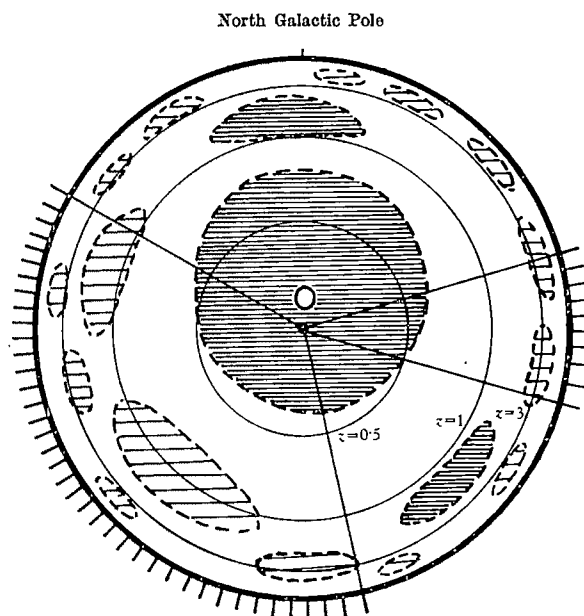


Fig. 1. Schematic diagram of a possible arrangement of (spherical) quasar clusters. The diagram shows a section of the world picture as seen by an observer O on our Galaxy. Circles of constant red-shift  $z$  are drawn for  $z = 0.5, 1$  and  $3$ . The section is chosen to pass through the North Galactic Pole and galactic longitude  $l \sim 110^\circ$ . It then passes through the centre of the two quasar clusters the existence of which is suggested by the distribution of quasars with large red-shift (ref. 1). These two clusters, and the cluster within which we may be situated, are heavily shaded. The position of the other clusters (lightly shaded) is purely conjectural, but the diagram shows a possible distribution, assuming that they have roughly equal masses and occupy about one-third of space. The directions within which quasars have not been observed, either because of galactic absorption or because of the low declination, are indicated by the shading outside the outer circle.

We now consider whether the large scale fluctuations we postulate could arise in a point source model universe. Little progress has been made in understanding the origin of the larger amplitude smaller scale fluctuations represented by clusters of galaxies. The mechanism which does produce galaxy clusters might, however, also produce our larger scale (but smaller amplitude) fluctuations, because in the early stages of the universe the background radiation is dynamically dominant and is likely<sup>30,31</sup> to smooth out density fluctuations contained within an observer's field of view<sup>32</sup> at that time (although larger fluctuations might persist). When the radiation ceases to dominate, instabilities on a scale smaller than the radius of the observable universe presumably grow, some of them developing into galaxy clusters. The largest possible such scale depends on  $T$  and  $\rho$ . For  $T \sim 3^\circ \text{K}$  and  $\rho \sim 5 \times 10^{-31} \text{g/c.c.}$  this scale would now be about 1,000 Mpc, in reasonable agreement with the apparent scale of the observed clustering. A theoretical determination of  $\alpha$  would require a detailed theory of the instabilities.

Finally, we consider some testable consequences of our model. It seems possible that one or two other clusters in the range  $1 \leq z \leq 3$  might be observable if a determined search were made at low galactic latitudes. In particular the sources in the region  $l \sim 210^\circ$ ,  $b \sim 20^\circ$  (not shown in the diagram) may be at the edge of a cluster. The fact that one of the observed clusters lies almost in the direction of the North Galactic Pole would, in our model, be a coincidence. If  $\rho \sim 10^{-30} \text{g/c.c.}$ , however, the microwave background radiation may exhibit anisotropy of up to a few per cent, especially in the general direction of the North Galactic Pole. We suggest that such anisotropy be looked for—its absence would, on our model, place a stringent upper limit on  $\rho$ . Another question concerns the isotropy of the radio source counts. Since there is a large dispersion in the intrinsic power of radio galaxies and quasars, radio source catalogues include, at the same flux density, intrinsically faint sources well within our own cluster, and very powerful sources in remote clusters. The large scale clustering suggested here might thus be consistent with the observed isotropy of the source counts<sup>3</sup>. Our proposed clustering would be most apparent if one were able to select objects from a small number of clusters—that is, in terms of their red-shift rather than their radio flux density—and this is precisely what Strittmatter, Faulkner and Walmesley<sup>1</sup> have done. A conclusive test of their proposal clearly requires more red-shift determinations. These should also show whether the quasars are indeed clustered in the manner suggested here.

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## Anisotropic Distribution of Quasars with Large Red-shift

Strittmatter, Faulkner and Walmesley<sup>1</sup> have suggested that there is an anisotropy in the distribution of quasars with large red-shift over the sky, pointing out that most of those discovered to date fall in two regions near the galactic poles. In this communication we present evidence that this anisotropy may be merely an effect of observational selection.

We confine our attention to sources identified from the revised 3C catalogue. Such identified sources might be expected to form a fairly homogeneous sample, and the identifications are about 80 per cent complete<sup>2</sup>.

In Fig. 1 we plot the visual magnitude of quasars identified from the 3C catalogue against their flux density at 1,400 Mc/s. Of the ten quasars in 3C known to have red-shifts greater than 1.38, seven are optically fainter than 18 magnitudes and have flux densities<sup>3</sup> at 1,400 Mc/s fainter than 3 f.u. ( $10^{-26} \text{Wm}^{-2} (\text{c/s})^{-1}$ ): this suggests that red-shift is correlated either with distance, as the cosmological theories and the simple single explosion local model require, or inversely with optical and radio luminosities. Hoyle and Burbidge<sup>4</sup> have pointed out that there is no clear red-shift magnitude relation for quasars either optically or at 178 Mc/s. We do not dispute this, but claim that most quasars with large red-shift have faint apparent optical magnitudes and radio flux densities at

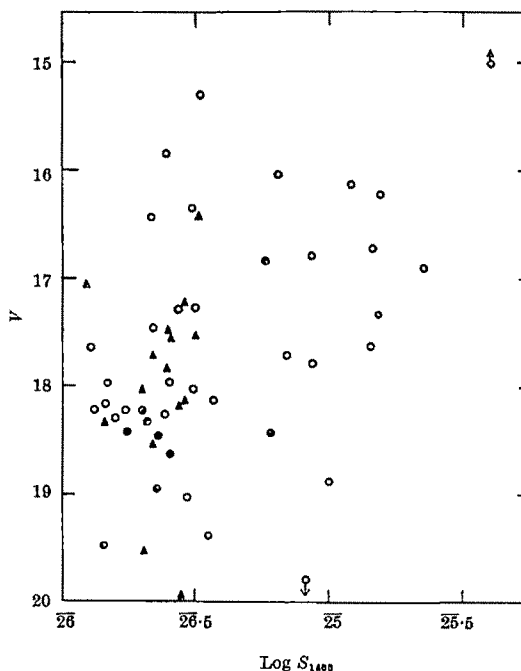


Fig. 1. Plot of  $V$  against  $\log S_{1400}$  for 3C quasars. ●,  $z \geq 1.38$ ; ○,  $z < 1.38$ ; ▲, no  $z$  available.

1,400 Mc/s. (At 178 Mc/s even this weaker relationship does not obtain, because most of the sources which have the highest flux densities at 1,400 Mc/s have radio spectra which either curve down at lower frequencies, or have smaller values of the spectral index  $\alpha$  (where  $S(\nu) \propto \nu^{-\alpha}$ ). This correlation between high flux density at 1,400 Mc/s

and flatter radio spectra may also account to some extent for the steeper number-count slope found by Veron<sup>2</sup> for quasars ( $-2.2$  as opposed to  $-1.85$  for all sources.)

In Fig. 1 we see more 3C quasars without large redshifts have flux densities less than 3 r.u. at 1,400 Mc/s than are optically fainter than 18 magnitudes. Thus it is

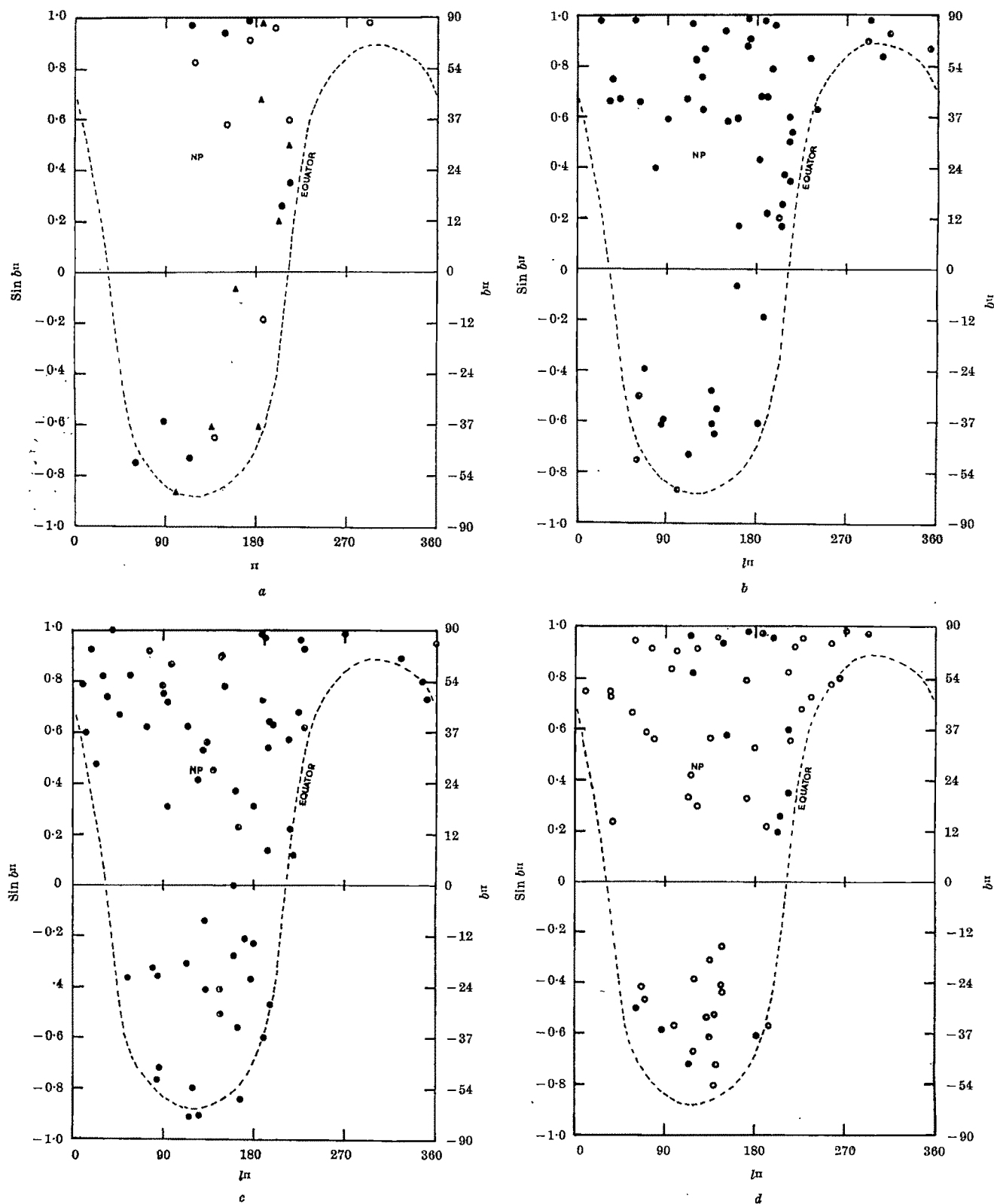


Fig. 2. Plot of  $\sin b_{II}$  against  $l_{II}$  for: a, 3C quasars with  $V \geq 18$  (symbols as in Fig. 1); b, 3C quasars all  $V$ ; c, 3C galaxies with  $V \geq 18$ ; d, 3C quasars with  $V \geq 18$  (●) and 3C unidentified sources (○). (d includes only radio sources with  $\log S_{1400} < 25.5$ .)

clear that apparent optical faintness is a more characteristic property of 3C quasars with large red-shifts than apparent radio faintness at 1,400 Mc/s.

In Fig. 2a we plot  $\sin b_{II}$  against  $l_{II}$ , where  $b_{II}$  and  $l_{II}$  are the galactic co-ordinates, for all 3C quasars which are visually fainter than 18 magnitudes. This is an equal area projection, but the galactic poles correspond to whole top and bottom margins of the diagram. The celestial equator is marked as a dotted line and the north celestial pole is marked "NP". No 3C sources lie far below this dotted line. As a result of galactic obscuration, few identifications can be made near the galactic plane ( $b_{II}=0$ ).

The distribution of points in Fig. 2a is markedly anisotropic: there is none in the region  $b_{II} > 0$ ;  $300 < l_{II}$  and  $l_{II} < 100$ . The eight quasars with large red-shift ( $\geq 1.38$ ) do not appear, however, to be distributed more anisotropically than the other sixteen quasars, eight of which have measured red-shifts less than 1.1. To see that the quasars identified in 3C are, as a whole, not especially anisotropically distributed Figs. 2b and 2c should be compared. Fig. 2b contains all quasars identified in 3C so far, while Fig. 2c contains all those radio galaxies listed by Wyndham<sup>4</sup> as being fainter than 18 magnitudes. We think that the anisotropy shown by those quasars in Fig. 2a with smaller red-shift may be an effect of selection. If this is the case, the anisotropy noticed by Strittmatter, Faulkner and Walmesley may have the same cause.

Our contention is thus that the primary anisotropy is in the distribution of quasars with  $V \geq 18$  rather than of those with large red-shifts (Strittmatter, Faulkner and Walmesley remark "observers in their quest for larger red-shifts may tend to investigate only fainter objects"). It is natural that any observational bias will be more severe for the fainter objects. Although Fig. 2c shows that there is no similar anisotropy for radio-galaxies with  $V \geq 18$ , galaxies may be identified with radio-sources from the *Palomar Sky Survey* alone, whereas quasars require three colour photometry to be performed. The region in which there are no optically faint quasars corresponds to the northern summer sky (right ascension 13 h 40 min to 22 h 40 min). Possible causes of observational selection may be: (i) shorter nights in the summer; (ii) the absence of the main quasar observers from California during the conference season; or (iii) difficulty in obtaining accurate radio positions through the galactic foreground radiation in the vicinity of the north polar spur.

In Fig. 2d we have superimposed the distribution of 3C quasars which are optically and radio faint on that of those 3C sources which are radio faint and not in regions of high absorption which are still unidentified ( $E$ ,  $E?$ ,  $?$ , in Veron's<sup>2</sup> notation). This distribution displays far less anisotropy than Fig. 2a, so that if some of the unidentified sources in the summer sky are quasars with large red-shift, then isotropy could be maintained. We await any further information on these unidentified sources with considerable interest.

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## PLANETARY SCIENCE

### Enhanced Interplanetary Scintillations associated with Solar Flares

DURING the past year, a survey of radio sources at 195, 430, and 611 Mc/s has been undertaken at the Arecibo Ionospheric Observatory to determine the sources which scintillate as a result of irregularities in the interplanetary medium. The degree of the intensity scintillations observed on the Earth depends on the integrated fluctuations in the electron density along the line of sight in the interplanetary medium and on the scale size of these fluctuations<sup>1</sup>. Thus, by monitoring those sources which scintillate, some of the gross features of the interplanetary medium can be deduced. In particular, it should be possible to plot the passage of shock waves and the ejection of turbulent plasma associated with large solar flares, as the disturbance moves out from the Sun into the interplanetary region.

At the end of March 1966, a number of class II and class III flares appeared in two plage regions, *KD 07* and *KE 23*, situated near the west and east limbs, respectively, of the solar disk at latitude 20° N. Although we were unable to plot the passage of the associated disturbances, we were able to detect their presence on two occasions, through an anomalous increase in the value of the scintillation index,  $m$ , defined by

$$m^2 = \frac{\langle \Delta I^2 \rangle \text{ on source} - \langle \Delta I^2 \rangle \text{ off source}}{\langle I \rangle^2}$$

$\langle \Delta I^2 \rangle$  and  $\langle I \rangle$  are the variance of the intensity fluctuations and the mean intensity of the source. On March 27 the scintillation index was four times larger than expected for quiescent solar conditions for the sources 3C 454 and 3C 454.3, and on March 31 eight times larger for *OTA 21*.

The scintillation indices and the elongations for 3C 454 and 3C 454.3 are given in Table 1 for five days between March 6 and April 15. The data for *OTA 21* are presented in Fig. 1 as a plot of the scintillation index against elongation for the 4 week period beginning on March 26. The solid lines represent the best fit to the experimental points and display the observed dependence of the scintillation index on the source elongation for conditions of quiescent solar wind. For the range considered in this report, the dependence of the scintillation index on elongation is similar for most sources. Thus the expected quiescent behaviour for 3C 454 and 454.3 on March 27 can be deduced from the graph of *OTA 21* using the scintillation indices listed in Table 1 for the other 4 days of observation. This results in an average increase due to the presence of the disturbance of a factor 4.

Table 1

Source	Frequency (Mc/s)	6th	March 13th	27th	3rd April	15th
3C 454						
Scintillation index	195	0.11	—	0.78	—	0.20
	430	0.10	< 0.10	0.25	—	< 0.10
Elongation		25°	24°	28°		40°
3C 454.3						
Scintillation index	195	—	—	0.62	< 0.10	0.18
	430	—	—	0.35	< 0.10	< 0.10
Elongation				26°	31°	39°

The observed increase in the scintillation index reflects an increase in the root mean square fluctuations in the electron density  $\Delta n$ , produced by the disturbance. For *OTA 21* at 430 Mc/s, the scintillation index is less than 1, and the observer-disturbance distance can be taken to be about the same as the Fresnel range ( $\sim 1$  AU). Then  $\Delta n$ , and the half width of the scintillation frequency spectrum,  $b$ , as given by Salpeter<sup>1</sup> and Cohen<sup>2</sup> are

$$m_i \doteq \sqrt{(2) (2\pi)^{1/4} \lambda r_e (a_i L_i)^{1/2} \Delta n_i} \quad (1)$$

$$b_i \doteq 2.4 \frac{2u_i}{\pi a_i} \quad (2)$$

In these expressions  $a_i$  is the scale size of the fluctuations in the electron density,  $L_i$  is the line of sight screen thickness,  $u_i$  the mass velocity of the medium perpendicular to the line of sight, and  $r_e$  the classical radius of the electron  $= 2.83 \times 10^{-13}$  cm. The subscript  $i$  has the value 1 for the parameters associated with the disturbance on March 31 and the value 2 for those expected on the same day for quiescent solar conditions. Taking the ratio of the scintillation indices for these two conditions produces

$$\frac{\Delta n_1}{\Delta n_2} = \frac{m_1}{m_2} \cdot \frac{(a_2 L_2)^{1/2}}{(a_1 L_1)^{1/2}} = 8 \frac{(a_2 L_2)^{1/2}}{(a_1 L_1)^{1/2}}$$

where the numerical value 8 for the ratio  $(m_1/m_2)$  has been obtained for CTA 21 from Fig. 1.

As the scale size and the thickness of the disturbance are not known, it is necessary to appeal to the work of other authors and to make a number of assumptions regarding these parameters in order to obtain an estimate for the increase in the fluctuations in the electron density.

We have experimentally observed the increase in the width of the scintillation frequency spectrum ( $b_1/b_2$ ) to be a factor of 2. Wolfe *et al.*<sup>3</sup> and Neugebauer and Snyder<sup>4</sup> have observed an increase of 2 in the streaming velocity of the interplanetary medium after substantial solar activity, so  $u_i$  can be taken to be approximately equal to  $2u_2$ , indicating that  $(a_2/a_1)^{1/2}$  can be taken to be of the order of 1. The density fluctuations during quiescent solar conditions are observed to obey a  $1/r^2$  law<sup>2</sup>. For this dependence Cohen *et al.*<sup>2</sup> have shown that the main contribution to the scintillation phenomena is produced by an effective screen thickness in A.U. of  $L = (2/\sqrt{3}) \sin$  (elongation). If it is assumed that the density fluctuations associated with the disturbance also have a  $1/r^2$  dependence, the ratio  $(L_2/L_1)^{1/2}$  can be set greater than or equal to 1, depending on whether the disturbance along the line of sight is smaller or larger than the effective screen given here for the elongation occurring on March 31. This leads to a minimum value of eight for the increase in the fluctuations of electron density associated with the disturbance.

Because the intensity scintillations are dependent only on the fluctuations in the electron density, the actual increase in the overall mean density associated with the disturbance cannot be determined.

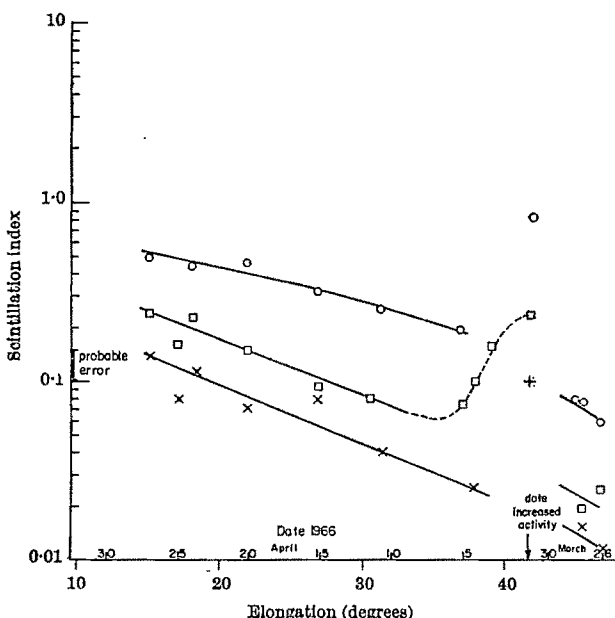


Fig. 1. The scintillation index for the source CTA 21 at frequencies of 195 (○), 430 (□) and 611 (×) Mc/s, plotted as a function of time with the source elongation included on the abscissa. The graph shows a marked increase in the value of the scintillation index on March 31 after type II flares on the solar disk.

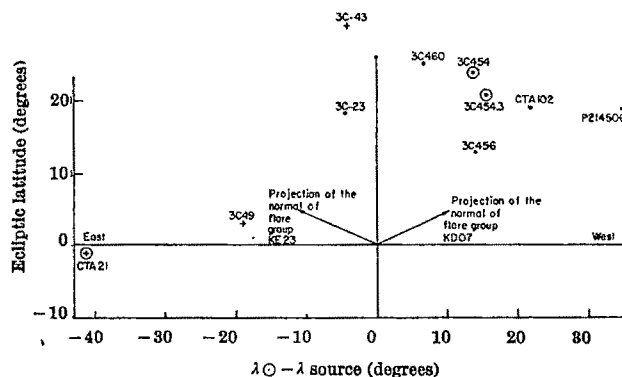


Fig. 2. The relative position of the sources observed on March 27 (●) and 31 (+) with respect to the Sun and the ecliptic in the plane of the sky. The latitude and the relative longitude with respect to the Sun are in ecliptic co-ordinates. The circles represent those sources which exhibited a large increase in their scintillation index.

In Fig. 2, the relative positions of the sources are plotted with respect to the Sun in geocentric ecliptic co-ordinates. 3C 454 and 3C 454.3 with elongations of 28° and 26°, respectively, are positioned to the north-west of the Sun while CTA 21 is to the east near the ecliptic at an elongation of 42°. On the same diagram seven other sources observed during this period have been plotted. They are known to scintillate but did not display a large increase.

It is interesting to note from Fig. 2 that (1) the activity on March 27 was observed in a region 40° N. of the projection of the flare normal on the plane perpendicular to the line of sight, and (2) that on March 31 either the main part of the disturbance had passed the position of 3C 49 or else the angular direction of the disturbance with respect to the ecliptic was less than 15° N. For the latter case, as with the activity of March 27, the data suggest that the disturbance can propagate at an oblique angle to the flare normal. A definite velocity for the disturbance cannot be deduced because the activity of March 27 was preceded by some ten well separated class II or III flares, while the activity of March 31 was preceded by five well separated class II flares after March 27, when the KE 23 group was first observed on the east limb. Nevertheless, assuming the disturbance travels faster than the solar wind, the data for March 31 are consistent with the velocities of 500–600 km/s found by Wolfe *et al.*

The large increases in the interplanetary scintillations observed after substantial solar activity suggest that the careful monitoring of a grid of sources known to scintillate will be a powerful method for the determination of the influence of such activity on the interplanetary medium and the structure and propagation characteristics of the resultant shock waves.

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## Interpretation of the Continuous Spectra of Comets

COMETARY dust, as well as being one of the basic features of comets, is of importance in several major problems of the solar system: it is most nearly representative of solid particles in the primordial solar nebula<sup>1</sup>; it is a contributor to zodiacal light<sup>2</sup>; and, although Harwit<sup>3</sup> has questioned its effectiveness, it plays some part in processes in interplanetary space<sup>4</sup>. Information concerning the nature of the dust can be obtained at present only by an analysis of scattered sunlight in the heads and tails of comets.

Mie scattering calculations for single particles and for cumulative scattering functions<sup>5</sup> were carried out on a computer at Goddard Space Flight Center<sup>6</sup>. These results were applied to measurements of the continuum in two ways.

Quantitative calculations were carried out for size distributions of the form

$$f(a) = Ca^a, a_1 \leq a \leq a_2 \quad (1)$$

where  $a_1$  and  $a_2$  are minimum and maximum radii and  $a$  took on integral values between  $-4$  and  $2$ . Five size ranges were used for each distribution:

- case (a)  $5 \times 10^{-7} \leq a \leq 1.5 \times 10^{-4}$  cm
- (b)  $5 \times 10^{-7} \leq a \leq 5 \times 10^{-6}$
- (c)  $1.5 \times 10^{-6} \leq a \leq 1.5 \times 10^{-4}$
- (d)  $5 \times 10^{-6} \leq a \leq 1.5 \times 10^{-4}$
- (e)  $5 \times 10^{-5} \leq a \leq 1.5 \times 10^{-4}$  cm

Both intensity and degree of polarization were computed and compared with observations of the comets 1957 III and 1957 V (ref. 7). They follow the same procedure as used previously<sup>8</sup>.

Cumulative scattering functions<sup>5</sup> are defined as

$$F_j(X) = \int_0^X i_j(x) dx \quad (2)$$

where  $X = 2\pi a_{\max}/\lambda$  and runs from 0.1 to 25 in steps of 0.1 and the subscript  $j$  represents either of the two polarization components. These functions were used to obtain qualitative characteristics of scattering by dielectric and iron spheres. In this analysis there was no restriction on the shape of the particle distribution nor of the size range for  $X$  less than 25. Comparisons were made between these data and observations of comets 1957 III and 1957 V.

In each investigation it was found that the observed degree of polarization, about 20 per cent, was too low to be caused by a distribution of iron spheres as proposed by Liller<sup>9</sup>. The small variation of polarization with wavelength also supported this. We conclude that iron grains can make only a small contribution to the scattering in comets. The observations can be explained, however, by the presence of a cloud of dielectric particles.

Some preliminary calculations indicate that scattering from carbon grains will resemble scattering from iron. For scattering angles close to 90°, calculations for spheres agree rather closely with measurements on non-spheroidal particles<sup>5</sup>.

Infra-red measurements were made on the comet Ikeya-Seki<sup>10</sup> by Beklin and Westphal in four wavelength intervals: 1.5–1.8 $\mu$ , 2.0–2.4 $\mu$ , 3.0–3.8 $\mu$  and 8.4–13.5 $\mu$ . Infra-red emissivities were derived on the assumption that molecular emission could be neglected. The calculations were corrected for scattered sunlight. Spectra in the 2.2 $\mu$  region are quoted as showing less than 10 per cent line emission. A survey of predicted infra-red emissions in comets<sup>11</sup> shows that the 3.4 $\mu$  region should be rich in molecular emission, much more so than the 2.2 $\mu$  region. The 10 $\mu$  region was not examined nor were atomic lines in the infra-red. Without spectroscopic study, it is not safe to assume that the measurements of the 3.4 $\mu$  and 10 $\mu$  filters are not affected by atomic or molecular emis-

sions. It is well known that, to make intensity measurements of the continuum in the visible, great care is required in the choice of wavelength regions passed by the filters.

In seeking to match their deduced emissivities Beklin and Westphal<sup>10</sup> used the optical properties of bulk material and suggested that iron gave a satisfactory fit. Cometary grains cannot be larger than a few microns radius without requiring excessive mass<sup>12</sup>. A similar limiting radius is required for the grains to be carried along by the escaping molecules<sup>13</sup>. For sizes less than the limiting radius the emissivity depends on the ratio radius/wavelength as well as index of refraction. Thus, even for the emissivities derived by Beklin and Westphal, it is not valid to say that iron is satisfactory without determining the emissivities of small grains.

Weinberg<sup>14</sup> has obtained extensive measurements of the intensity and polarization of the continuum of the comet Ikeya-Seki at 8 wavelengths throughout the visible spectrum. The analysis of this material will be of great value for the interpretation of the continuum of comets. The difficulty of making a unique determination of the nature of a cloud of scattering particles from optical observations is well known. Measurements of intensity and polarization over a wide wavelength interval and over as large a range of scattering angles as possible are necessary for interpreting the continuum of comets.

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## Secular Movement of the Zone of Auroral Blackout

THE magnetic dip pole in 1922 and 1942 was close to the position 71° N. 97° W. whereas in 1955 and 1965 it was near 76° N. 101° W.<sup>1</sup>. A test has been made to find whether this movement was accompanied by changes in the rate of occurrence of radio blackout near Inverness (57.5° N., 4° W.) using the  $f_{min}$  tables from the vertical incidence data taken in this area between 1942 and 1963. The ionograms are reasonably comparable for the period. Data for months on which the number of blackout entries ( $B$ ) were abnormally large because of low critical frequencies at night were rejected. Independent studies were made for epochs near solar minimum, 1942–43 and 1962–63, and near solar maximum, 1946–47 and 1957–58, and significant differences

in the numbers of days with blackout were found for both sets of data.

At Inverness blackout mostly occurs on days with at least one value of  $Kp \geq 6$  and there were appreciably fewer such days in 1962-63 than in 1942-43. Correcting to equal effective levels of magnetic activity, the ratio of the number of days per month with blackout for the periods before 1950 to those after 1950 was 1.7 in both cases. This strongly suggests a real change in the position of the blackout zone.

The network of ionospheric stations in Northern Europe is not sufficiently dense to allow more than a rough estimate of the long term change in geomagnetic latitude of the lines which represent a constant number of days of blackout. The best estimate from these data is between  $3^\circ$  and  $6^\circ$ , but there is a large possible error caused by systematic differences in the relative sensitivity of the stations and errors in analysis. It is therefore not known whether the rate of change of blackout with latitude is the same as that of zenith aurora or is slower, as suggested by the absorption data.

Using the number of days with zenith aurora between  $58^\circ$  and  $63^\circ$  magnetic latitude over the U.K. (Paton, J., private communication) and assuming that blackout and zenith aurora have the same rates of change with latitude, the most probable shift in the lines of constant blackout activity between the epochs 1942-47 and 1957-63 is about  $2^\circ$ . There therefore appears to have been a real change in the position of the blackout zone.

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## CHEMISTRY

### Separation and Analysis of some *cis,trans* Isomeric Olefines on Activated Alumina

PREVIOUS investigations have shown that gas-solid chromatography (GSC) on activated alumina is useful in separating paraffins and olefines from  $C_1$ - $C_8$  (refs. 1-3). The physicochemical characteristics of the solute and its affinity for the adsorbent determine the efficiency and limitations of alumina GSC. The choice of carrier gas for adsorption chromatography has a marked effect on retention times and column efficiencies<sup>4</sup>. Solute characteristics that influence the degree of separation attained include differences in boiling points, molecular branching, and the presence of double and triple bonds.

Jacobs<sup>5</sup> has separated *cis,trans* butenes from automotive exhaust gases by temperature programming from  $-55^\circ\text{C}$  to  $140^\circ\text{C}$ , using a 150 ft. capillary gas-liquid chromatographic column in conjunction with a flame ionization detector. Chromatographic curves show that isomeric 2-butenes were well separated and retention times are tabulated for  $C_5$  and  $C_6$  isomers. No separations of the isomeric 3-heptenes were obtained, however, and data for higher molecular *cis,trans* isomers were not presented. Isomeric 2-butenes have a comparatively large difference in boiling points and have been separated on propylene carbonate-modified alumina<sup>6</sup>. Such analytical columns, however, are of limited value for higher homologues because of low thermal stability of the liquid phase. Although distinguishing between low molecular weight *cis* and *trans* hydrocarbon isomers by infra-red spectra is difficult<sup>7</sup>, the technique has been used<sup>8</sup>. The method requires skilled application, because Sadler's infra-red index<sup>9</sup> shows the spectra for both *cis* and *trans* 2-butene to be virtually identical. The spectra for 2-pentene pub-

Table 1. PHYSICAL CONSTANTS OF SOME *cis,trans* HYDROCARBONS<sup>10</sup>

Isomer	Boiling point (°C at 760 mm)	Heat of vaporization (Kcal/mole) at bp	Difference
<i>cis</i> -2-Butene	3.72	5.57	
<i>trans</i> -2-Butene	0.88	5.43	0.14
<i>cis</i> -2-Pentene	36.94	6.24	
<i>trans</i> -2-Pentene	36.35	6.22	0.02
<i>cis</i> -2-Hexene	68.84	6.91	
<i>trans</i> -2-Hexene	67.87	6.89	0.02
<i>cis</i> -3-Heptene	95.75	7.53	
<i>trans</i> -3-Heptene	95.67	7.52	0.01
<i>cis</i> -2-Octene	125.64	8.05	
<i>trans</i> -2-Octene	125.0	8.03	0.02

lished in this index do not indicate which isomer was used for establishing the absorption curves. The need for a rapid method to separate and quantitate each isomeric component is apparent.

Table 1 shows a series of *cis,trans* isomeric olefines separated by GSC on activated alumina. In all isomeric pairs the *trans* component exhibited the lower boiling point. Differences in boiling points between individual isomers, however, do not account for the observed degree of separation between the components. For example, the 2-pentenenes, of which the boiling points are nearly identical, are about as well separated as the 2-butenes that boil about  $3^\circ\text{C}$  apart.

Heats of vaporization at the boiling points are lower for the *trans* isomer<sup>10</sup>. Heats of adsorption reportedly have the same magnitude as the latent heats of vaporization<sup>11</sup>, and although the differences shown in Table 1 are small they are consistently higher for the *cis* isomer. Enhanced adsorption of *cis* relative to the *trans* isomer was pointed out by Scott<sup>12</sup> to be a general phenomenon for solid surfaces. He indicates that separation on gas-solid columns can be achieved with only a fifth or a tenth of the plates needed in gas-liquid columns. Our separations of *cis,trans* isomers on short columns of activated alumina indicate a high degree of adsorption. Because GSC separations are based on adsorption rather than partition between gas and liquid phases, the electrokinetic interactions between solute and adsorbent contribute the physical forces necessary to resolve components.

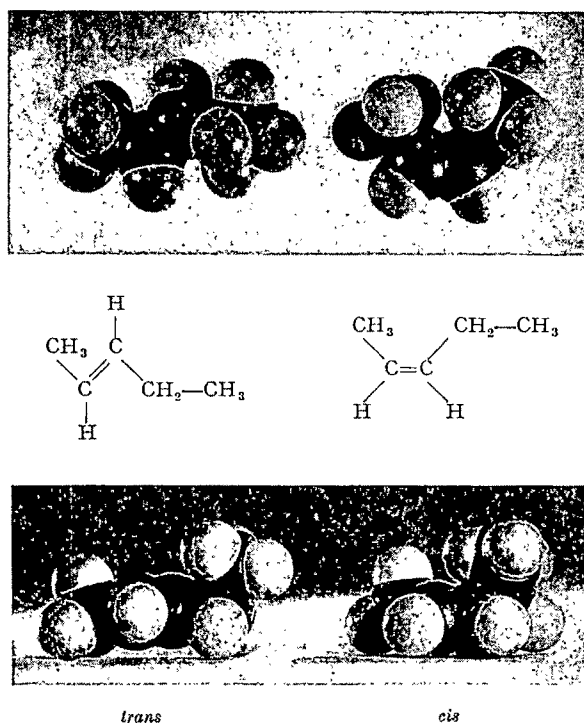


Fig. 1. Photographs of molecular models of *cis* and *trans*-2-pentene. Upper, a top view; lower, a side view.

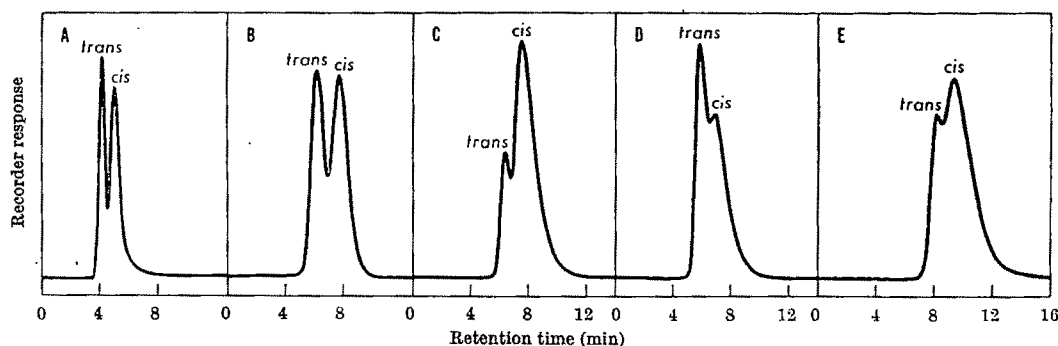


Fig. 2. Separation of some *cis,trans* isomeric hydrocarbons on activated alumina: A, 2-butene; B, 2-pentene; C, 2-hexene; D, 3-heptene; and E, 2-octene. The instrument used was an F and M 1609 flame ionization detector with column 3 ft.  $\times$  1/16 in., 60/80 mesh activated alumina; chart speed 30 in./h, carrier gas, nitrogen 40 c.c./min; and column temperatures isothermal: A at 75°C; B at 100°C; C at 115°C; D at 150°C; and E at 175°C.

From a photograph of a molecular model of *cis* and *trans* 2-pentene (bottom of Fig. 1), it is evident that electron clouds surrounding a *cis* double bond can lie closer to the alumina surface than can those of the *trans*. The *cis* isomer, more strongly bound than the *trans*, desorbs less readily. This retentive mechanism accounts for their observed elution order<sup>12</sup>. Giddings<sup>13</sup> states that a small bore column should give optimum results for GSC separations.

This investigation shows it to be useful for even the difficult task of separating *cis,trans* isomers.

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Table 2. ANALYSIS OF 2-BUTENE ISOMERS

Known composition (per cent)		Found by gas solid chromatography (per cent)	
<i>trans</i>	<i>cis</i>	<i>trans</i>	<i>cis</i>
9.7	90.3	10.5	89.5
35.1	64.9	37.0	63.0
53.7	46.3	53.7	46.3
70.0	30.0	67.4	32.6
100.0	—	100.0	—
—	100.0	—	100.0

Fig. 2 shows GSC separations of a homologous series of  $C_4$ - $C_8$ -*cis,trans* isomeric olefines. Although  $\frac{1}{8}$ -in. diameter columns gave adequate separation of the isomeric 2-butenes and 2-pentenenes, they were unsatisfactory for resolution of the isomeric 2-hexenes, 3-heptenes and 2-octenes. A 3 ft. standard column of outer diameter 1/16 in. gave good separation of all components under the conditions indicated. Even though the higher  $C_{6,7,8}$  members were not so completely resolved as the lower  $C_4$  and  $C_5$  isomers, results indicate that the separations are useful for qualitative analysis of hydrocarbon mixtures containing such geometrical isomers. Resolution achieved for the butenes and pentenes permits integration to quantitate their proportions, and the results for most samples agreed closely with the suppliers' analyses. One sample, however, erroneously labelled mixed 2-butene isomers, was analysed and found to be 100 per cent *trans*. Variation in amounts of geometrical isomers in a commercial 2-butene is reported<sup>14</sup> within the limits of 50-67 molecular per cent *cis* content based on infra-red and freezing point. Our analysis of this sample indicated a *cis* content of 72 per cent. Analyses of mixtures of butenes containing 100 per cent *cis* to 100 per cent *trans* 2-butenes have shown reasonable agreement with the known isomer contents (Table 2). The 2-butene mixtures analysed and reported in Table 2 were prepared by mixing known volumes of pure *cis* and *trans* 2-butenes in a manometric apparatus equipped with mercury levelling bulbs. The accuracy of this method is limited to how accurately one can read the burette used in the apparatus. Complete resolution of the two isomers was not obtained (Fig. 1) and errors in integration are inevitable.

Under proper chromatographic conditions of column length and diameter, sample size, column temperature, and choice of carrier gas, alumina GSC lends itself well to volatile hydrocarbon analysis. It can separate saturates and unsaturates, as well as iso- and cyclic hydrocarbons<sup>1</sup>.

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### Role of $O^-$ in the Gas Phase Radiolysis of Alkane-Nitrous Oxide Mixtures

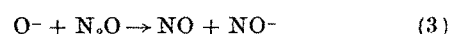
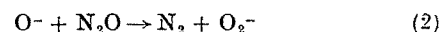
WHEN an alkane gas is irradiated in the presence of nitrous oxide, nitrogen is formed. This has been shown to result from the dissociative capture of electrons by nitrous oxide<sup>1</sup>



At constant dose rate the yield of nitrogen,  $G(N_2)$ , obeys the expression

$$\frac{1}{G(N_2)} = \frac{1}{G(N_2)_\infty} \left( 1 + \frac{A}{[N_2O]} \right)$$

expected for competition between electron capture and ion recombination.  $G(N_2)_\infty$  is a constant, for a given hydrocarbon, and represents the yield of nitrogen when all electrons are captured. The fact that  $G(N_2)_\infty$  is greater than the yield of electrons,  $G_e$ , calculated from the  $W$  value<sup>3</sup>, has been ascribed to the subsequent reaction of  $O^-$  with nitrous oxide<sup>1,2</sup>. Evidence has been found, in the mass spectrometer, for reactions (2) and (3) (ref. 4). The relative rates of these reactions are not, however, reported.



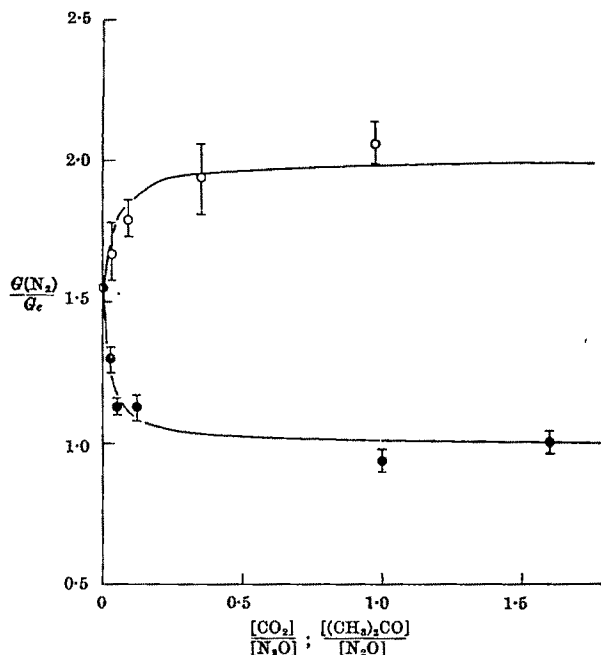
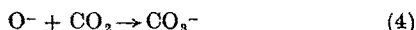


Fig. 1. The effects of carbon dioxide (●) and acetone (○) on  $G(N_2)$  from a propane (600 mm. mercury)-nitrous oxide (25 mm. mercury) mixture.

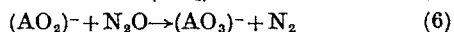
On the basis of the ethylene dosimeter\* ( $G(C_2H_4 \rightarrow H_2) = 1.31^3$ ),  $G(N_2)_\infty$  for the  $C_2$ ,  $C_3$  and  $C_4$  alkanes is found to be  $1.55 G_e$  (to be published). This can be explained by assuming a rate constant ratio,  $\frac{k_2}{k_3} = 1.22$ .

It has been found, in the mass spectrometer, that the predominant negative ion formed in oxygen containing carbon dioxide is  $CO_3^-$  (ref. 5). This has been attributed to the fast reaction



Carbon dioxide is not known to react with the radical or ionic species produced in alkane radiolysis. It was therefore thought that the addition of carbon dioxide to alkane-nitrous oxide mixtures might provide unambiguous evidence for reaction (2) by competing with nitrous oxide for  $O^-$  thereby decreasing  $G(N_2)_\infty$ . Carbon dioxide was, in fact, found to decrease  $G(N_2)_\infty$  even for  $[CO_2]/[N_2O]$  as low as 0.02 (Fig. 1). Higher concentrations of carbon dioxide further decreased  $G(N_2)_\infty$ , in the limit, to  $G_e$ . From other experiments the ratio  $k_4/k_2 + k_3$  has been determined to be 44 (to be published). The curve in Fig. 1 was computed using this ratio in the kinetic expression, for the dependence of  $G(N_2)_\infty$  on  $[CO_2]/[N_2O]$ , derived by a steady state treatment of reactions (2), (3) and (4). The agreement between this curve and experiment, together with the known reactivity of carbon dioxide towards  $O^-$  and the decrease of  $G(N_2)_\infty$  to  $G_e$ , is taken as conclusive evidence for the occurrence of reactions (2) and (3).

It has been found that the addition of small amounts of acetone to alkane-nitrous oxide systems increases  $G(N_2)_\infty$ , in the limit, to  $2G_e$  (Fig. 1). This can be explained by a competitive reaction of  $O^-$  with acetone (AO) to produce a species which later reacts with nitrous oxide quantitatively to form nitrogen.



The upper curve in Fig. 1 was computed from the expected variation of  $G(N_2)_\infty$ , in such a competition, using a value of  $k_5/k_2 + k_3 = 23.5$  obtained from other data (to be published).

\* Based on  $G(C_2H_4 \rightarrow H_2) = 1.31$ ,  $G(N_2O \rightarrow N_2)$  was found to be 10.0 in agreement with the latest reported value<sup>6</sup>

It is possible that the anomalously high yields of nitrogen (that is,  $> G_e$ ) observed with solutions of nitrous oxide in high pH water and liquid alkanes<sup>7-9</sup> also result from the subsequent reaction of  $O^-$  with nitrous oxide. The use of carbon dioxide as an  $O^-$  scavenger in the latter system should, therefore, prove of interest. Further investigations of the reactions of  $O^-$  with other additives are in progress.

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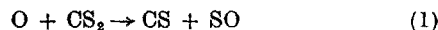
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### Measurement of the Rate Parameters for Reaction of $O(2^3P)$ with Carbon Disulphide and Olefins, by Flash Spectroscopy

THIS communication reports the measurement of the rate parameters for some reactions of  $O(2^3P)$  by a new application of flash spectroscopy, which could be adapted to a wide range of fast reactions. The intermediate, the reactions of which are under investigation, is directly produced by the photolytic flash; the method requires the selection of a substance which reacts with the intermediate to produce a molecule which has intense light absorption at a wavelength suitable for kinetic spectroscopy. The reaction rate is determined by observing the rate of formation of this product, and the relative rates of competing reactions with added compounds can be measured by observing the diminishing yield of the "marker". The rate of a reaction, in which neither reactants nor products exhibit convenient absorption spectra, can then be determined by the spectroscopic technique. In this investigation carbon monosulphide was produced by reaction of atomic oxygen with carbon disulphide and served as an ideal spectroscopic marker, because it possesses an intense band system at about 2600 Å. and because its lifetime is very long compared with the time in which measurements need to be made.

Mixtures of nitrogen dioxide and carbon disulphide, in an excess of argon, were flashed to produce  $O(2^3P)$ . By filtering the light through 'Pyrex', the production of excited oxygen atoms and the direct photolysis of carbon disulphide were avoided. The addition of argon prevented any significant temperature change and ensured rapid quenching of electronically excited nitrogen dioxide produced by absorption at long wavelengths<sup>1</sup>. The subsequent fate of  $O(2^3P)$  was monitored by spectroscopic observation of carbon monosulphide formed in the reaction

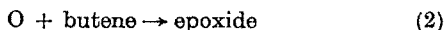


The rate constant of (1) was determined with  $[NO_2]/[CS_2]$  ratios of about 1:10; with a flash energy of 2,500 J; only a very small fraction of the atomic oxygen reacted with residual nitrogen dioxide. The absolute concentrations were adjusted to produce carbon monosulphide in a pseudo first-order reaction with a half-life of about 100 μsec. The finite duration of the photolytic flash was considered in the analysis of the results which gave

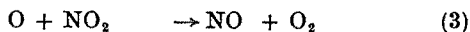
$$k_1 = 2.5 (\pm 0.3) \times 10^9 \text{ l. mole}^{-1} \text{ sec}^{-1} \text{ at } 305^\circ \text{ K}$$

$$\text{and } \log k_1 = 9.8 (\pm 0.2) - \frac{600 (\pm 300) \text{ cal/mole}}{2.3 RT}$$

From a study of the reduction of the yield of carbon monosulphide on addition of iso-butene or but-1-ene to mixtures of nitrogen dioxide and carbon disulphide the rate constants for addition of oxygen atoms to the olefines were obtained.



The high pressure of argon (100 mm) prevented fragmentation of the "hot" epoxide which Cvetanović has observed at low pressures in his detailed study of these reactions<sup>2</sup>. Concentrations of carbon monosulphide were measured at 900  $\mu\text{sec}$  and from plots of  $[\text{CS}]^{-1}$  v  $[\text{butene}]$   $[\text{CS}_2]^{-1}$ , the ratios  $k_2/k_1$  were determined. The rates of the reactions



and



have also been measured with this technique and are required for the accurate evaluation of  $k_2$  (ref. 3). The rate constant derived for reaction (3) is in excellent agreement with that recently reported by Klein and Herron<sup>4</sup>. For iso-butene,  $k_2/k_1 = 3.1$  at 298° K and 2.5 at 410° K, and for but-1-ene,  $k_2/k_1 = 0.78$  at 298° K and 0.83 at 410° K.

These results are compared with previous measurements in Table 1. Cvetanović has measured the rate constants relative to  $k_3$  and the conversion to absolute values has been made with  $k_3 = 3.28 (\pm 0.33) \times 10^6$  l. mole<sup>-1</sup> sec<sup>-1</sup> (ref. 4). His activation energies are relative to that for O + tetramethyl ethylene which is set equal to zero.

Table 1. RATE CONSTANTS (L. MOLE<sup>-1</sup> SEC<sup>-1</sup>  $\times 10^6$ ) AND ACTIVATION ENERGIES (KCAL/MOLE)

	This work		Cvetanović <sup>2</sup>		Elias <sup>5</sup>		Avramenko <sup>6</sup>	
	$k_{298}$	$E_a$	$k_{298}$	$E_a$	$k_{298}$	$E_a$	$k_{298}$	$E_a$
Iso-butene	7.6 ( $\pm 1.2$ )	0.1 ( $\pm 0.4$ )	7.5	0.47	10	0.4	0.03	2.5
But-1-ene	1.9 ( $\pm 0.3$ )	0.8 ( $\pm 0.4$ )	1.7	1.40	3.1	0.85		

The following comparisons are noted: (a) the first three sets of rate constants are in moderately good agreement but differ very significantly from those of Avramenko and co-workers; and (b) the absolute rate constants found in this work at 298° K are in excellent agreement with those of Cvetanović.

The measurements of Elias depend on consumption of atomic oxygen in a flow-system and removal of atoms in secondary reactions could result in slightly high values for  $k_2$ .

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## THE SOLID STATE

### Arrangement of Rotating Molecules in the High-temperature Form of Normal Paraffins

NORMAL paraffins undergo a phase transition near the melting point into a crystal form which has been described as hexagonal. This form was first found by Müller<sup>1</sup>, who obtained hexagonal dimensions from X-ray powder data and therefore concluded that the molecules rotate in the lattice. Later, Bernal<sup>2</sup> reported a hexagonal cell for dodecanol in which the molecules lay along triad axes. Crystal forms with the chains arranged according to the same type of chains have then been found in other long-chain compounds, for example, simple esters and glycerides, and they have been termed  $\alpha$ -forms. The name  $\alpha$  will also be used for the high temperature crystal form of  $n$ -paraffins here, since it is inadequate to term it hexagonal as will be shown below. The nature of the molecular

motion in this crystal form of normal paraffins has been studied extensively, and the results indicate that the molecules are undergoing some kind of concordant rotation<sup>3-5</sup>.

The present author has examined  $\alpha$ -forms of mono- and tri-glycerides which occur in fat crystallization<sup>6</sup>. It was then realized that a detailed knowledge of the molecular packing in the simplest  $\alpha$ -form, that of paraffins, is of fundamental importance for the understanding of the behaviour of general  $\alpha$ -forms. An X-ray single-crystal investigation of the  $\alpha$ -form of a normal paraffin will be reported here.

$n$ -Nonadecane ( $\text{C}_{19}\text{H}_{40}$ ) was chosen because it exhibits an  $\alpha$ -form over a relatively wide temperature range near room temperature (21.9°–32.0° C). All the experiments were performed in a room where the temperature was kept constant at 25° C. Crystallization from solvents was tried in order to obtain the best conditions for crystal growth, although it was not certain that an  $\alpha$ -form could be obtained in this way. The behaviour on crystallization was very remarkable. A transparent and flexible film of paraffin was formed over the solvent (chloroform). Because the film prevented evaporation it was necessary to cut out openings in order to grow films thick enough to handle. The whole film proved to be a single crystal of the  $\alpha$ -form. Small pieces of the soft material were cut out for X-ray work, and oscillation and Weissenberg photographs were taken using copper  $K\alpha$  radiation.

A unit cell which corresponded to orthohexagonal axes was chosen in conformation with the earlier hexagonal description. The dimensions are:

$$a = 8.30 \pm 0.08 \text{ \AA}, b = 4.79 \pm 0.05 \text{ \AA}, c = 52.8 \pm 0.5 \text{ \AA}$$

The recorded X-ray data were of poor quality, but the reflexions were resolved, so that the orthorhombic symmetry and the dimensions in the reciprocal lattice could be established. The unit cell contains four molecules arranged in two molecular layers with vertical hydrocarbon chains. These layers are parallel to the film which is formed when the solvent evaporates, and the chain packing within each layer can be described by a hexagonal sub-cell. The lattice is shown in Fig. 1, where it is evident that the symmetry is not hexagonal. This is simply an effect of the relative displacement of adjacent molecular layers. Fig. 1 also illustrates the general features of the packing. The structure is viewed along the chain direction, and the molecular axes in the two molecular layers of the unit cell are then represented by circles and dots respectively. Many crystals were examined and disorder effects were frequently observed. The disorder corresponds to the occurrence of irregularities in relative displacement of adjacent layers, and may be due to sliding of molecular layers along (001) planes when the crystals are cut. The distribution of the reflexion intensities is in general agree-

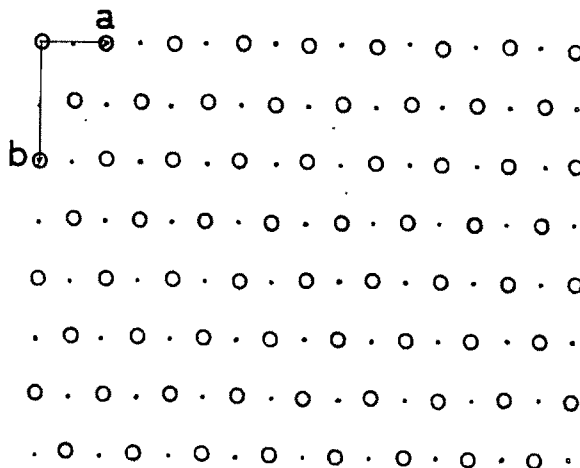


Fig. 1. Lattice of the  $\alpha$ -form of  $n$ -nonadecane. The lattice points denoted by circles are situated  $c/2$  above (or below) those denoted by dots.



ment with the theoretical predictions for rotating chains by Vainshtein<sup>7</sup>. Electron diffraction photographs of the 2-form have also been recorded which confirm the earlier proposal of rotating chains (a diffuse halo was observed which fits very well with the calculated Fourier transform of a rotating chain).

Further analysis of the X-ray single crystal data of  $\alpha$ -forms in other long-chain compounds and additional studies by electron diffraction are in progress.

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## PHYSICS

### Two-dimensional Nucleation of Ice

THE customary theory for the nucleation of ice on a crystalline substrate is based on the probability that a spherical cap of ice will grow, by statistical fluctuations, to a size such that the volume free energy and the surface free energy counterbalance<sup>1,2</sup>. Although this model is mathematically tractable, its validity has been questioned<sup>3,4</sup> on the grounds that the macroscopic values of the physical properties of ice may not apply to aggregates of only a few molecules. No alternative theory has yet been proposed to describe the molecular process by which ice deposits on a substrate. It is the purpose of this communication to show that the first stage in the nucleation of ice on organic nucleators is the growth of monolayer patches of ice on the nucleator surface.

The existence of an ice monolayer at the interface between a nucleator and supercooled water is not easy to detect directly, but because the thermodynamic properties of the monolayer differ from those of bulk ice it is possible, by the application of high pressure, to reach conditions under which bulk ice is unstable, yet the monolayer of ice can be formed and its properties can be studied.

The properties of the monolayer of ice can be investigated by studying the effect of pressure on the nucleation of Ice I by organic nucleators. The apparatus, which has been described previously<sup>5</sup>, enables the freezing point of a small droplet of an aqueous suspension of the nucleator to be measured under pressure. Temperatures are accurate to  $\pm 0.25^\circ\text{C}$ , and pressures are accurate to  $\pm 20$  bars. Of a large number of organic nucleators which have been studied, phloroglucinol dihydrate is a typical representative, and in this communication its behaviour is quoted as an example.

Fig. 1 depicts the conditions under which ice nucleates on phloroglucinol dihydrate under pressure, the supercooling required at any particular pressure being the vertical distance between the lines *CYC* and *DDZ*. Nucleation is enhanced by pressure and the supercooling necessary to nucleate ice falls from  $-6.5^\circ$  at atmospheric pressure to virtually zero at 1,500 bars. These results apply to droplets frozen for the first time. If, however, the droplets are melted, then frozen again, the second nucleation takes place at a temperature which depends on the maximum temperature to which the droplet is subjected during the melted stage. Specifically, if after melting the temperature is kept below  $-1^\circ\text{C}$  (for up to 20 min), the second freezing requires no supercooling,

while if the temperature exceeds  $+1^\circ\text{C}$  the original supercooling is required. This so-called memory effect is well known in other systems<sup>6</sup>, but on no previous occasion has a memory for ice persisted above the melting point of bulk ice<sup>7-9</sup> except for a passing observation by Bridgman<sup>10</sup> with regard to one of the dense polymorphs of ice. Because memory is lost above  $0^\circ\pm 1^\circ\text{C}$  regardless of pressure (*AA*, Fig. 1) it is apparent that at 2,500 bars the memory is retained  $29^\circ$  above the bulk melting point of ice, whereas at atmospheric pressure no memory can be detected.

The phenomenon of memory can arise in two distinct ways—either in the form of an unmelted ice layer or in the form of a structure imprinted on the phloroglucinol dihydrate surface by previous contact with ice. In the latter case, loss of memory would be a process akin to diffusion and would take place over a relatively wide temperature range in the vicinity of the Tammann temperature<sup>11</sup> of the nucleator. On the contrary, the results show that memory is lost over a narrow range between  $-1^\circ\text{C}$  and  $+1^\circ\text{C}$ , not only for phloroglucinol dihydrate but for the compound  $\alpha$ -phenazine the Tammann temperature of which differs markedly from that of phloroglucinol. Further, it has been found that when deuterium oxide is substituted for water, the memory is lost between  $+2^\circ\text{C}$  and  $+4^\circ\text{C}$ —a clear indication that the ability to retain a memory is a property of the water phase. That only a single monolayer of ice remains unmelted in the region *AACC* can be deduced in the following way. Because the melting point of the ice layer is not detectably altered by pressure (*AA*, Fig. 1) the volume change on melting the layer must be very small (compare the Clausius-Clapyron equation,  $dT/dP = \Delta V/\Delta S$ ). This condition can only be met by a single monolayer of ice because the packing density of water molecules in a single [0001] layer of ice is very close to that of liquid water, whereas a second layer of ice would drastically reduce the average density of the two layers to a value midway between that of ice and that of water.

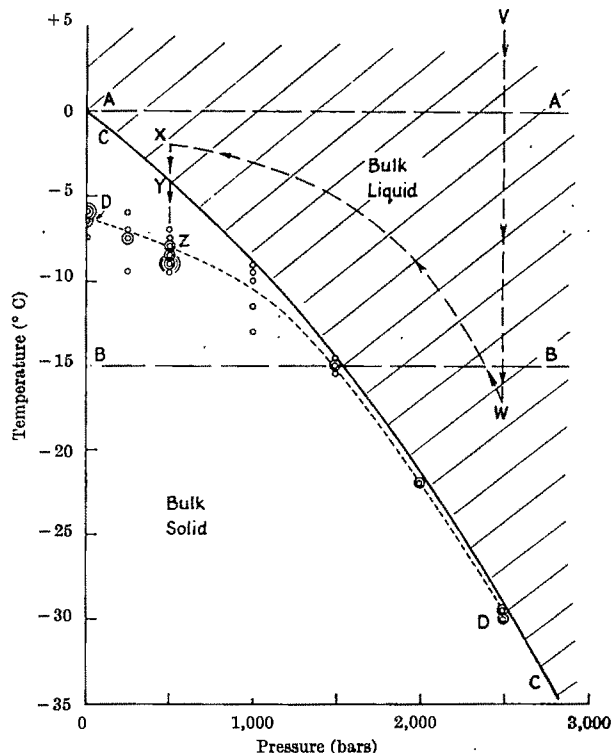


Fig. 1. The  $P$ - $T$  phase diagram of Ice I. Curve *CYC* (—) is the melting point of ice as a function of pressure; curve *DDZ* (---) is the best line through the data points (○) which represent the temperature of the "first freeze" of ice by phloroglucinol dihydrate under pressure. Line *AA* (—) is the melting point of an ice monolayer; line *BB* (—) is the temperature at which an ice monolayer becomes fully developed.

The foregoing experiments show that once ice has been formed on phloroglucinol dihydrate, a monolayer persists at temperatures up to 0° C. To show how the ice monolayer participates in the initial nucleation of a water droplet, a further hypothesis must be made, namely, that although the ice monolayer melts at 0° C it does not freeze at 0° C but develops gradually as the temperature is lowered below 0° C. This hypothesis has been proved by carrying out experiments of the type described by the sequence *VWXYZ* (Fig. 1). *VW* represents a drop of water containing phloroglucinol dihydrate being cooled at 2,500 bars from room temperature to a temperature  $T_w$  which is above the melting point of bulk ice. *WX* represents decompression to 500 bars, keeping the temperature always between *AA* and *CC*. *XYZ* represents cooling at 500 bars. During this last step, *XYZ*, bulk nucleation takes place. It is found that the supercooling, relative to the point *Y*, at which bulk nucleation takes place, depends on the temperature,  $T_w$ , at the point *W*. As  $T_w$  is lowered the necessary supercooling during step *XYZ* diminishes, reaching a value of zero when  $T_w$  is -15° C or below. Clearly, the monolayer has progressively developed during stage *VW* without the prior formation of bulk ice, and having once developed it persists during step *WX* and is therefore available to promote bulk nucleation during step *XYZ*. At -15° C the monolayer may be defined as "fully developed", that is, the monolayer is capable of nucleating bulk ice at zero supercooling. This definition does not imply that the organic surface is completely covered by an ice monolayer. By carrying out the step *VW* at various pressures between 1,500 bars and 3,500 bars it can be shown that the monolayer becomes fully developed at -15° C regardless of pressure. It is a reasonable extrapolation to deduce that at atmospheric pressure also the temperature at which the monolayer becomes fully developed is -15° C (*BB*, Fig. 1).

In general, it may therefore be stated that at all pressures the nucleation temperature of bulk ice is the temperature at which the monolayer becomes sufficiently well developed to act as a nucleator for bulk ice at the ambient bulk supercooling. Thus the influence of pressure on the temperature of nucleation of bulk ice (*DD*, Fig. 1) is explained as follows. At atmospheric pressure, bulk ice nucleates at -6.5° C because the monolayer, though poorly developed, is able to nucleate the bulk at a bulk supercooling of 6.5°. At 500 bars, bulk ice nucleates at -8° C, because, although the bulk supercooling of 4° is less than that at one bar, the monolayer is better developed at the lower temperature. At pressures exceeding 1,500 bars, zero supercooling is required because the monolayer is already fully developed by the time the temperature falls to the bulk melting point.

An important corollary to the present work is the demonstration that, in reality, the first stage in the nucleation of ice by an organic nucleator is the development on the nucleator surface of an ice monolayer—presumably in patches centred on the sites of lowest interfacial energy. Such a process which is equivalent to the nucleation of an intermediate phase is not considered by theories which treat the growth of the ice embryo as a dynamic equilibrium, dependent on statistical fluctuations.

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## Difference between Prompt and Delayed Fluorescence Spectra

It is commonly assumed that the emission spectrum of the delayed fluorescence of an organic material is identical with that of the prompt fluorescence, because the two spectra arise from a radiative transition between the same two electronic levels, although the origin of the excitation differs. Recently a difference has been reported between the prompt and delayed fluorescence spectra of fluorescein in boric acid<sup>1,2</sup> and of tryptaflavin in polymethyl methacrylate<sup>3</sup>. Three possible causes of the phenomenon have been suggested<sup>2</sup>: (1) the possible existence of two metastable (triplet) levels,  $T_1$  and  $T_2$ , in some molecules; (2) intermolecular migration of the triplet excitation energy; and (3) a possible difference in the transition probability distribution for prompt and delayed fluorescence.

The effect has now been observed in proflavin in polymethyl methacrylate solid solution. Proflavin (BDH Laboratory Chemicals) was purified, recrystallized, dissolved in ethanol and mixed in suitable proportions with methyl methacrylate. The solutions were acidified by the addition of a small amount of hydrochloric acid, so that the final solutions consisted of methyl methacrylate + 5 per cent ethanol + 0.1 per cent hydrochloric acid + proflavin. The solutions were placed in glass tubes, of 3 mm internal diameter, and the residual space was evacuated (to eliminate oxygen which quenches the proflavin phosphorescence), filled with nitrogen, and flame-sealed. The solutions were then polymerized thermally in the dark.

A xenon lamp was used for luminescence excitation at a wavelength of 365 nm, and the emission spectra were measured with an Aminco-Keirs spectrophosphorimeter. The luminescence was observed normally to the direction of incidence of the exciting light. No correction was applied for resorption and secondary luminescence, because the spectra of the prompt and delayed fluorescence were observed under the same conditions, and should therefore be affected in the same way by any such effects.

Fig. 1 shows (1) the prompt and (2) the delayed fluorescence spectra at room temperature of specimen *A* with a proflavin concentration of  $3 \times 10^{-6}$  g cm<sup>-3</sup>, and Fig. 2 shows similar spectra of specimen *B* with a proflavin concentration of  $2 \times 10^{-4}$  g cm<sup>-3</sup>. The prompt and delayed spectra were observed in identical geometry. A few hours before measurement of the prompt spectra the upper ends of the specimen tubes were broken to admit atmospheric oxygen and quench the proflavin phosphorescence.

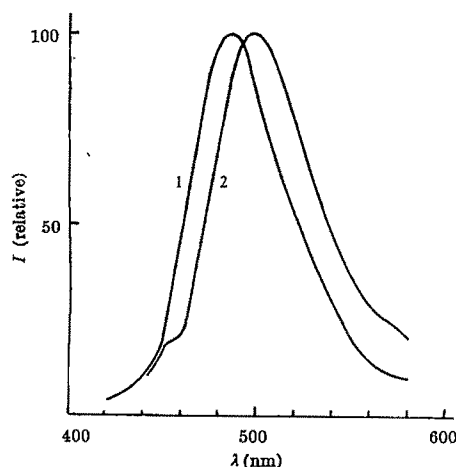


Fig. 1. Prompt (curve 1) and delayed (curve 2) fluorescence spectra of  $3 \times 10^{-6}$  g cm<sup>-3</sup> proflavin in polymethyl methacrylate. Intensity in relative units.

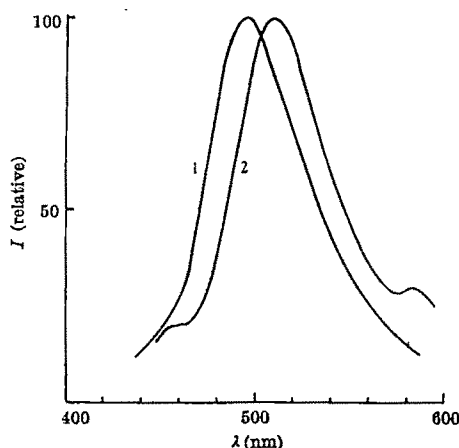


Fig. 2. Prompt (curve 1) and delayed (curve 2) fluorescence spectra of  $2 \times 10^{-4}$  g cm $^{-3}$  proflavin in polymethyl methacrylate.

The maxima of the prompt and delayed fluorescence spectra of *A* are at  $\lambda_p^A = 486$  nm and  $\lambda_d^A = 498$  nm, respectively, and those of *B* at  $\lambda_p^B = 497$  nm and  $\lambda_d^B = 510$  nm, respectively. In each case there is a similar shift in the position of the fluorescence maximum of  $\Delta\lambda^A = 12$  nm and  $\Delta\lambda^B = 13$  nm, respectively. These results agree with those reported previously<sup>2</sup>, namely, that  $\lambda_d > \lambda_p$  and that  $\Delta\lambda (= \lambda_d - \lambda_p)$  is independent of the dye concentration. They appear to confirm that intermolecular triplet migration does not contribute to the effect, because otherwise a dependence of  $\Delta\lambda$  on dye concentration would be expected.

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### New Method of Thickness Measurement with the Interference Microscope

THE optical path difference (OPD) between an object and the surrounding medium, as measured with a transmission interference microscope using axial illumination, is given by

$$\text{OPD} = T(\mu_o - \mu_m) \quad (1)$$

where  $T$  is the thickness of the object, and  $\mu_o$  and  $\mu_m$  are the refractive indices of the object and medium, respectively.

The usual method of measuring the thickness and refractive index of an object with the interference microscope is to make readings of the OPD with the object successively mounted in two media of different refractive index. The pair of equations thus obtained can be solved for  $T$  and  $\mu_o$  (ref. 1).

Nomarski has pointed out (personal communication) that the information necessary for the calculation of  $T$  and  $\mu_o$  can be obtained from measurements in a single medium, if the OPD is first measured in the usual way, and then with the object tilted relative to the optic axis of the microscope, using a universal stage. This note proposes an analogous but more convenient method,

namely, the measurement of the thickness and refractive index of a microscopical object mounted in a given medium, by means of two OPDs, one measured with axial parallel light, and the other with an oblique beam of light obtained by using only marginal rays from a wide-angle condenser. As the technique depends on the use of different parts of the aperture of the condenser, it may be called a two-aperture method of interference microscopy.

In Fig. 1, based on one given by Ingelstam<sup>2</sup>,  $T$  is the thickness of a plane-sided object of refractive index  $\mu_o$ , mounted in a medium of refractive index  $\mu_m$  and illuminated by a parallel beam of light inclined at an angle  $DEA$  to the optic axis  $AE$  of the microscope. The numerical aperture of the beam is given by  $NA = \mu_m \sin DEA = \mu_o \sin BEA$ , where  $CBE$  is the path of light passing through and refracted by the object, and  $DE$  is the path of the reference beam passing through the medium alone. The  $\text{OPD}_{NA}$  measured with this oblique illumination is given by

$$\text{OPD}_{NA} = (CB\mu_m + BE\mu_o) - DE\mu_m \quad (2)$$

It can be shown that

$$\text{OPD}_{NA} = T \left( \frac{\mu_o^2 - NA^2}{\mu_o \sqrt{1 - NA^2/\mu_o^2}} - \frac{\mu_m^2 - NA^2}{\mu_m \sqrt{1 - NA^2/\mu_m^2}} \right) \quad (3)$$

Designating the OPD measured with axial illumination as  $\text{OPD}_o$ , and combining equations (1) and (3), we obtain

$$\frac{\text{OPD}_o}{\text{OPD}_{NA}} = \frac{\frac{\mu_o}{\mu_o^2 - NA^2} - \frac{\mu_m}{\mu_m^2 - NA^2}}{\frac{\sqrt{1 - NA^2/\mu_o^2}}{\mu_o \sqrt{1 - NA^2/\mu_o^2}} - \frac{\sqrt{1 - NA^2/\mu_m^2}}{\mu_m \sqrt{1 - NA^2/\mu_m^2}}} \quad (4)$$

This expression is awkward algebraically, but knowing  $NA$  and  $\mu_m$  values of  $\text{OPD}_o/\text{OPD}_{NA}$  can be calculated for varying values of  $\mu_o$  and plotted graphically. From a measured value of the ratio  $\text{OPD}_o/\text{OPD}_{NA}$  the refractive index  $\mu_o$  of a microscopical object can be read off the graph and the thickness  $T$  obtained by substitution in equation (1).

Apart from the possibility of estimating both thickness and refractive index from measurements in a single medium, the two-aperture method has interesting theoretical implications.

Consider the case of a fixed biological object. This can be regarded as being composed of solid fibrils or lamellae, separated by interstices filled with immersion medium. With axial illumination the  $\text{OPD}_o = t(\mu_o - \mu_m)$ , where  $t$  is the effective thickness (that is, the thickness which the dry material would occupy if the interstices were eliminated), and  $\mu_o$  is the refractive index of the dry material. It is, however, equally valid to regard  $\text{OPD}_o$  as equal to

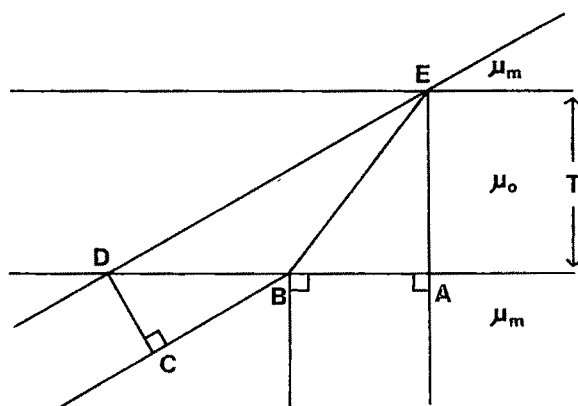


Fig. 1. Plane-sided object of refractive index  $\mu_o$ , mounted in a medium of refractive index  $\mu_m$  and illuminated by a parallel beam of light at an angle  $DEA$ .

$T(\mu_a - \mu_m)$ , where  $T$  is the true geometrical thickness of the object, and  $\mu_a$  is the "average" refractive index of the object plus any included immersion medium,  $\mu_a$  being defined by the expression

$$\mu_a = \frac{t\mu_o + (T - t)\mu_m}{T} \quad (5)$$

The two-medium method as conventionally used enables one to obtain the effective thickness  $t$  and the refractive index  $\mu_o$ , but not the geometrical thickness  $T$ . The significance of results obtained with the two-aperture method will depend on the physical state of the object. If the fibres and lamellae of which the object is composed are thick relative to the wavelength of the light used, and are separated by substantial amounts of medium, the  $OPD_{NA}$  measured with oblique illumination will be unaffected by the presence of the interstices, and the thickness and refractive index obtained by the two-aperture method will, as with the two-medium method, be  $\mu_o$  and  $t$ . If, however, the dry fibres of which the object is composed are of submicroscopic dimensions and the object appears microscopically homogeneous, the refractive index measured by the two-aperture method will be  $\mu_a$ , and the thickness will be the geometrical thickness  $T$ .

An important possibility which the two-aperture method offers is that of measuring the thickness and refractive index of a cell inclusion completely surrounded by cytoplasm. If  $T_1$  and  $T_2$  (Fig. 2) are the thickness of the cell and the inclusion, respectively, and  $\mu_m$ ,  $\mu_c$  and  $\mu_n$  are the refractive indices of the medium, cytoplasm and inclusion, respectively, the  $OPD_o$  of the cytoplasm relative to the medium is  $T_1(\mu_c - \mu_m)$ . By changing the immersion medium it is possible to estimate  $T_1$  and  $\mu_c$ , but the  $OPD_o$  of the nucleus (plus overlying cytoplasm) relative to cytoplasm alone always remains  $T_2(\mu_n - \mu_c)$ , and it is not possible to proceed further with the two-medium method<sup>2</sup>. Using the two-aperture method, it is, however, possible in principle to obtain  $T_1$ ,  $T_2$ ,  $\mu_c$  and  $\mu_n$  from measurements in a single medium provided both cell and inclusion can be taken to have plane-parallel sides (which will, regrettably, not often be the case). Measurements of the  $OPD_o$  and  $OPD_{NA}$  of cytoplasm relative to the medium will, as indicated previously, yield values for  $T_1$  and  $\mu_c$ . If one now measures the  $OPD_o$  and  $OPD_{NA}$  of the nucleus relative to the cytoplasm, one obtains an equation similar to equation (4), with  $\mu_n$  and  $\mu_c$  replacing  $\mu_o$  and  $\mu_m$ , respectively. From suitable tables one can as before read off the value of  $\mu_n$ , and obtain  $T_2$  by substitution in the equation  $OPD_o = T_2(\mu_n - \mu_c)$ .

Some calculated values of the ratio  $OPD_o/OPD_{NA}$  are given in Table 1, with various values of  $NA$  and assuming the medium to be either air ( $\mu_m = 1.0$ ) or water ( $\mu_m = 1.34$ ). It will be seen that the value of the ratio is very sensitive to change in  $NA$  and relatively insensitive to change in  $\mu_o$ , unless the value of  $NA$  is high. It is clearly necessary to determine the  $NA$  of the oblique illumination accurately and the condenser should preferably be well corrected, of high  $NA$ , and equipped with a movable pin-hole aperture precisely positioned at the first focal plane.

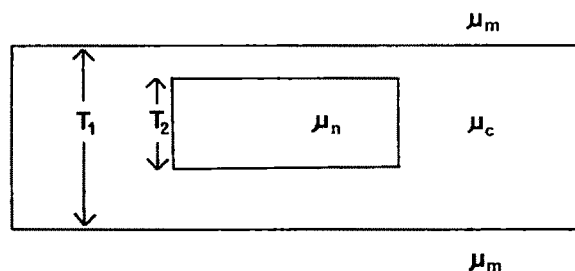


Fig. 2.  $T_1$  is thickness of the cell and  $T_2$  thickness of the inclusion, and  $\mu_m$ ,  $\mu_c$  and  $\mu_n$  are the refractive indices of medium, cytoplasm and inclusion respectively.

Table 1. NUMERICAL VALUES OF  $OPD_o/OPD_{NA}$  WITH VARYING  $NA$ ,  $\mu_n$  AND  $\mu_o$

$NA$	$\mu_m$	$\mu_o = 1.40$	$\mu_o = 1.50$	$\mu_o = 1.60$
0.3	1.34	0.9759	0.9774	0.9788
	1.0	0.9673	0.9695	0.9714
0.45	1.34	0.9445	0.9483	0.9515
	1.0	0.9245	0.9296	0.9340
0.90	1.34	0.7537	0.7721	0.7876
	1.0	0.6285	0.6544	0.6764
1.30	1.34	0.3082	0.3779	0.4278

In practice, various errors caused by non-ideal apparatus may be minimized by the application of what amounts to a null method, namely, the calibration of the  $NA$  of the oblique beam by determining the ratio  $OPD_o/OPD_{NA}$  with a standard object, the known refractive index of which is similar to that of the test specimen. Experiments with the two-aperture method will be reported elsewhere, but it is already clear that while with present equipment the two-aperture method is probably too insensitive to be usefully applied to most biological objects, the position is likely to change radically when interference microscopes of greatly increased precision<sup>4-6</sup> become available. The method may also prove useful in physics and industry.

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## BIOPHYSICS

### Synthesis of Nucleic Acids in Hair

A STUDY of the cells of the hair cortex of rats<sup>1</sup> showed that size changes of the nucleolus are not a reliable indication of changes in the rate at which cells are synthesizing proteins. The highest rate of protein synthesis was associated with the smallest nucleolar volume. It was not possible to demonstrate nucleoli in the cortical cells of the mid-keratogenous zone with specific stains for RNA.

Leblond *et al.* have obtained evidence that the nucleolus manufactures about 90 per cent of the RNA in a cell<sup>2</sup>. Biochemical investigations, especially those on the anucleolate Oxford mutation of *Xenopus*<sup>3</sup>, assign the synthesis of ribosomal RNA to the nucleolus. I have performed an experiment to discover if the anucleolate nuclei of the hair cortex synthesize RNA.

Twelve albino rats were injected subcutaneously with 0.4 mc./100 g body weight tritiated-cytidine (specific activity 990 mc./mmole). They were killed in pairs 1, 3, 12, 24, 48 and 72 h after the injection. Specimens of skin which bore actively growing hair were taken from the site of injection and the comparable site on the opposite side of the body. The specimens were fixed in Carnoy's fluid and autoradiographs prepared with 'Kodak AR 10' stripping film. Good autoradiographs were obtained after 6 days exposure of the sections from the site of injection. The observations reported here were made on a set of preparations from the injection sites.

One hour and three hours after the injection only one group of nuclei were not demonstrably radioactive. They were the cortical cells of the mid-keratogenous zone and higher levels of the follicles. The density of silver grains in the photographic emulsion over nuclei of the hair bulb was so high that it barely transmitted light. The pos-

sibility that this result was caused by diffusion of the  $^3\text{H}$ -cytidine away from the injection site is excluded by the radioactivity of the nuclei of the sheaths of the inner and outer roots. Evidently the synthesis of RNA by the anucleolate nuclei is depressed in these animals.

The cytoplasm was radioactive only in cells that had radioactive nuclei. After 1 h the density of silver grains over the cytoplasm was slight, after 3 h it was higher and after 12 h it was higher than that over most of the nuclei. After 12 h the cortical cells at the top of the keratogenous zone were radioactive.

The preparations that were killed 24 and 48 h after injection contained radioactive nuclei in the cortex and inner root sheath as far up the follicle as the upper bulb and mid-keratogenous zone respectively. These observations indicate that some  $^3\text{H}$ -cytidine was incorporated into nuclear DNA of matrix cells at the time of injection and was carried up the follicle as the cells migrated, in accordance with the time schedule proposed in earlier work<sup>4</sup>.

The cytoplasm of the cortical cells up to the end of the keratogenous zone was radioactive in the specimens killed after 12, 24 and 72 h. Because the cells continuously moved along the follicles from the matrix, RNA synthesized in the matrix was carried to higher levels. Cell division occurs only in the matrix, increase in cell size occurs only in the upper bulb<sup>5</sup>, and different proteins are manufactured at the top and bottom of the keratogenous zone<sup>6</sup>. We conclude that some RNA is associated with each of these different activities in turn and speculate that it may be non-specific ribosomal RNA.

These observations bear on the question of how the nucleolus and RNA participate in cell differentiation and metabolism.

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### Mechanism of Radiation-induced Granulocytosis

AN early and transient rise in the number of circulating neutrophilic granulocytes is known to occur in the blood of various mammals, including man, after exposure to radiation<sup>1-5</sup>. Neither the aetiology of this radiation effect, however, nor the source of the mature neutrophils has been established. The evidence discussed in the present communication suggests that the granulocytosis is caused by the release of one or more pharmacologically active biogenic amines, which mobilize large intravascular reservoirs of mature neutrophils.

The action of radiation appears to be indirect, releasing into plasma a humoral agent which mobilizes neutrophils and which may be a normal constituent of the body<sup>6</sup>. It is perhaps significant, therefore, that epinephrine, one of several biogenic amines (others are norepinephrine, serotonin, and histamine) released by radiation exposure<sup>6</sup>, has long been known to increase the number of circulating neutrophils in man<sup>7-9</sup>. This effect also appears to be shared by norepinephrine, as shown by experiments on rats<sup>10</sup> and human subjects<sup>9</sup>.

In investigations with rats, we observed that biogenic amines could, in fact, induce a neutrophilia comparable with that caused by X-rays (Fig. 1). These experiments confirmed the effect of epinephrine and norepinephrine previously found in humans or rats and demonstrated that

serotonin and, to some extent, histamine could also produce a neutrophilia. Circulation levels usually reached a peak about 3 or 4 h after treatment, and were increased by a factor of about 3 for histamine, 5 for X-rays, and 6 to 8 for serotonin, epinephrine and norepinephrine.

These observations do not agree with two other investigations, one of which has reported that epinephrine, serotonin or histamine had no effect on the circulating level of neutrophils in rats<sup>10</sup>, and the other of which showed that histamine causes a neutropenia in humans<sup>11</sup>. In the former investigation, the absence of response may have been caused by the low doses of the amines administered (10  $\mu\text{g}$ ); norepinephrine, which produced a neutrophilia in the same investigation, was given in a much higher dose (200  $\mu\text{g}$ ). In the case of histamine neutropenia in humans, it should be noted that the mode of injection differed; the intravenous route was used in man, causing a rapid effect of short duration, whereas a subcutaneous injection of histamine (in oil), producing a more protracted effect, was used in the present investigation.

The large intravascular reservoirs of mature neutrophilic granulocytes, which are found adhered to, or marginated along, the walls of small blood vessels<sup>12,13</sup>, are readily mobilized by epinephrine<sup>14</sup>, presumably by increasing the velocity of blood flowing through the vessels<sup>13,14</sup>. Although regions of margination occur throughout the body, one of the main sites appears to be the lungs from which neutrophils can be released by both epinephrine and norepinephrine<sup>6</sup>. This intravascular source and presumed mode of action may also explain the effects of serotonin, which, like the catecholamines, has marked vasoconstrictor properties, particularly on the lung vasculature<sup>15</sup>. Such mobilization would not account for the effect of histamine, a known vasodilator, which we observed, unless it is assumed that the protracted effect of histamine "shock" induced the release of one or more of the other amines. This might explain why the effect of histamine was comparatively small, despite the relatively high dose injected (Fig. 1), and suggests that epinephrine, norepinephrine, and serotonin were the

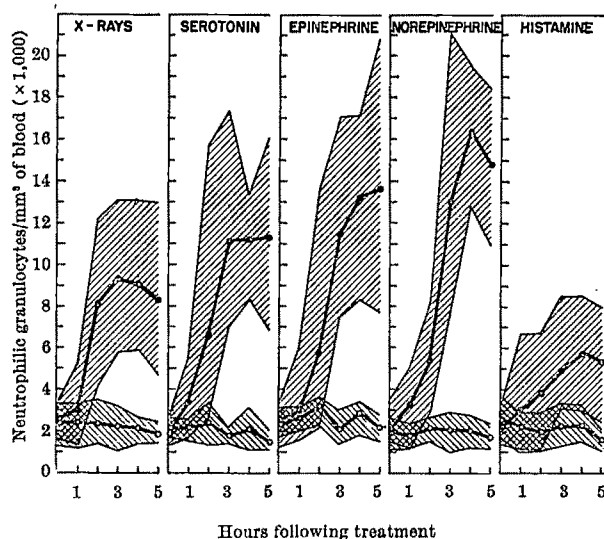


Fig. 1. The effect of whole body X-irradiation (800 r), serotonin creatinine sulphate ( $12.4-24.7 \times 10^{-3}$  mM/kg), epinephrine ( $5.5-18.4 \times 10^{-3}$  mM/kg), norepinephrine hydrochloride ( $4.9-14.6 \times 10^{-3}$  mM/kg) and histamine hydrochloride ( $27.2-54.3 \times 10^{-3}$  mM/kg) on the number of neutrophilic granulocytes circulating in the peripheral blood of rats at various times after treatment. The experimental (●) and control (○) curves represent the serial examination of tail blood from 8 to 15 animals. The hatched areas signify the absolute range of values obtained. All amines were injected subcutaneously as a suspension in 0.5 ml. of olive oil; controls were given only oil. The number of neutrophils was computed from the total white blood cell count and from the per cent neutrophils found in smears stained with May-Grünwald-Giemsa; a minimum of 500 cells was scored for both procedures.



more important factors in the radiation-induced granulocytosis.

That intravascular reservoirs are the chief source of granulocytes mobilized by radiation appears to be consistent with existing knowledge. Bone marrow has in the past been considered to be a likely source of the neutrophils<sup>3,4</sup>. Experiments in which developing neutrophils were labelled with tritiated thymidine *in vivo*, however, have indicated that regions of haemopoiesis may not be the origins of the cells mobilized by radiation<sup>16</sup>. The re-entry of cells into the circulation from other extracellular sources has also been mentioned<sup>4</sup>, but evidence suggests that re-entry from intercellular spaces is unlikely, particularly in the quantities required at one time<sup>17</sup>. These various facts, although not eliminating bone marrow or other extracellular regions as a source, point to the intravascular reservoirs as the main sites of the mature cells mobilized by radiation and to biogenic amines as the mobilizing agents.

The role of pharmacologically active amines in radiation-induced neutrophilia is yet to be established. In separate experiments, we have seen that adrenalectomy has no influence on the effect of radiation, indicating that the adrenals are not necessary for the mobilization of neutrophils. We have also investigated the effects of various pharmacological antagonists to the biogenic amines. The antagonists used were the sympatholytic drug, dibenzylamine (25–50 mg/kg), the serotonin antagonists, lysergic acid diethylamide (25 µg/kg), dibenzylamine, morphine (25 mg/kg), cocaine (50 mg/kg), and the antihistaminic, mepyramine maleate (20–35 mg/kg); intraperitoneal injections were given 10 min before X-radiation or the respective amine. These antagonists not only failed to alter the effect of X-rays, but also failed to interfere with the action of the corresponding amine.

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## PHYSIOLOGY

### Effect of Noradrenaline on Phosphatase Activity in Synaptic Membrane of the Rat Brain

THE classical experiments of Loewi<sup>1</sup> demonstrated the transmitter substance of the sympathetic nervous system to be adrenaline or noradrenaline<sup>2,3</sup>. Several further investigations have shown a transmitter substance to be present in the central nervous system (CNS), that is, the

hypothalamus, the central grey matter of mesencephalon and the area postrema<sup>4,7</sup>. Differential centrifugation of a CNS homogenate has demonstrated the presence of noradrenaline in the synaptic vesicles of the synaptic junction<sup>8–10</sup>. Electrophysiological investigation has revealed that electrical stimulation of amygdala or of terminals of bulbo-spinal neurones leads, in both instances, to a reduction of the noradrenaline content of certain areas of the brain<sup>11–13</sup>. The molecular basis for the action of noradrenaline in the CNS is, however, unknown. Previous investigations of the pharmacological action of noradrenaline, particularly on different enzyme systems<sup>14–16</sup>, have not provided the experimental evidence basic to a theoretical understanding of physiological events of depolarization and repolarization. The relationship of changes in the ion-flux across the synaptic membrane to the action of a transmitter substance has not been defined. Investigation (our report in preparation) of the distribution of phospholipids and glycolipids free from sialic acid in the sub-cellular fractions of hypothalamus revealed a preponderance of phosphatidyl-serine in the sub-mitochondrial fraction B–C<sup>19</sup> using the method of De Robertis *et al.*<sup>5,9</sup>. This discovery stimulated us to search for a possible function of phosphatidyl-serine in the synaptic endings. *In vitro* investigations revealed phosphatidyl-serine to be the only major lipid among phospholipids or glycolipids free from sialic acid of the CNS which binds the noradrenaline<sup>17</sup>.

Evidence has been presented for the presence in plasma membranes of liver of an alkaline phosphatase activated by magnesium and potassium ions and depressed by sodium ions<sup>18</sup>. This communication provides evidence for the presence of a similar phosphatase system in the CNS sub-cellular fraction which contains synaptic vesicles and nerve endings. The stimulatory effect of noradrenaline on the alkaline and neutral phosphatase in these sub-cellular fractions and the effect of several inorganic ions on this phosphatase-membrane system are shown.

Sub-cellular fractions were obtained by ultracentrifugation of homogenates of fresh rat brain from fourteen adult male Wistar rats of 200–225 g<sup>8</sup>. Electron microscopy of sub-cellular fractions was carried out as previously described<sup>19</sup>. Activities of neutral and alkaline phosphatase of the membrane fractions were estimated on the basis of the hydrolysis of *p*-nitro-phenylphosphate at pH 7.2 and pH 8.8 in *tris*-hydrochloric acid buffer (at 37° C<sup>18</sup>). The activities were expressed as mmoles of substrate hydrolysed/mg of membrane protein/min. Inorganic salts (sodium chloride, potassium chloride, caesium chloride, lithium acetate and magnesium sulphate) were of analytical grade and the noradrenaline (as bi-tartrate) was "a.m. pharmacopea Danica". To estimate the activity of phosphatase in the presence of only one inorganic ion (potassium, lithium) the corresponding salt of *p*-nitro-phenyl phosphate was prepared by ion exchange: 5 ml. of 0.1 molar disodium salt of *p*-nitrophenyl phosphate was added to 3 ml. of a suspension of 1.1 g of 'Dowex 50' (H<sup>+</sup> form). When the pH of the mixture appeared to be constant, the supernatant was isolated by centrifugation and 0.5 molar potassium hydroxide or 1 molar lithium acetate was added until the solution was at pH 7.0, yielding the potassium or lithium salts.

Tables 1, 1a and 1b demonstrate the activity of neutral and alkaline *p*-nitrophenyl phosphatase from different sub-cellular fractions of the CNS. Among the gradient fractions obtained by centrifugation of the crude mitochondrial fraction<sup>8,9</sup>, the fractions A, B and C which contain synaptic nerve ending and synaptic vesicles<sup>8,9</sup> had the highest specific activities. The activity at pH 8.8 is lower than at pH 7.2. In evaluating the effects of inorganic ions it is seen that potassium is the only ion which gives rise to activities higher than those of sodium, lithium, and caesium ions, and that activity is further increased by addition of potassium ions to a system containing sodium, lithium, and/or caesium ions. Further-

Table 1

Substrate addition of:	Na <sup>+</sup>	Na <sup>+</sup> K <sup>+</sup>	pH, 7.2 Na <sup>+</sup> Li <sup>+</sup>	Na <sup>+</sup> NA	Na <sup>+</sup> K <sup>+</sup> , NA	Na <sup>+</sup>	Na <sup>+</sup> K <sup>+</sup> pH, 8.8	Na <sup>+</sup> NA	Na <sup>+</sup> K <sup>+</sup> , NA
Subcellular fractions									
A	0.34 ± 0.02	0.78 ± 0.09	0.29 ± 0.00	1.82 ± 0.07	1.04 ± 0.06	0.49 ± 0.10	0.89 ± 0.07	1.82 ± 0.11	1.08 ± 0.05
B	0.38 ± 0.00	1.12 ± 0.09	0.48 ± 0.01	2.01 ± 0.11	1.09 ± 0.20	0.24 ± 0.07	0.89 ± 0.06	1.86 ± 0.13	1.00 ± 0.13
C	0.51 ± 0.00	0.86 ± 0.01	0.47 ± 0.01	0.54 ± 0.06	0.38 ± 0.01	0.25 ± 0.01	0.72 ± 0.05	0.59 ± 0.03	0.56 ± 0.06
D	0.22 ± 0.02	0.44 ± 0.02	0.31 ± 0.01	0.48 ± 0.00	0.31 ± 0.07	0.17 ± 0.02	0.33 ± 0.05	0.66 ± 0.03	0.40 ± 0.03
E	0.22 ± 0.09	0.34 ± 0.01	0.22 ± 0.00	0.57 ± 0.03	0.46 ± 0.07	0.13 ± 0.00	0.40 ± 0.05	0.72 ± 0.11	0.40 ± 0.04
F	0.64 ± 0.03	0.58 ± 0.02	0.54 ± 0.00	0.42 ± 0.01	0.30 ± 0.00	0.35 ± 0.04	1.02 ± 0.00	0.50 ± 0.02	0.55 ± 0.00
G	0.06 ± 0.00	0.07 ± 0.01	0.06 ± 0.00	0.19 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.15 ± 0.00	0.17 ± 0.00	0.10 ± 0.01

Table 1a

Substrate addition of:	K <sup>+</sup>	K <sup>+</sup> Na <sup>+</sup>	pH, 7.2 K <sup>+</sup> Li <sup>+</sup>	K <sup>+</sup> NA	K <sup>+</sup> Na <sup>+</sup> , NA	K <sup>+</sup>	K <sup>+</sup> Na <sup>+</sup> pH, 8.8	K <sup>+</sup> NA	K <sup>+</sup> Na <sup>+</sup> , NA
Subcellular fractions									
A	0.36 ± 0.06	0.26 ± 0.07	0.47 ± 0.00	1.08 ± 0.05	0.74 ± 0.05	0.71 ± 0.03	0.19 ± 0.05	1.22 ± 0.07	0.80 ± 0.08
B	0.57 ± 0.03	0.45 ± 0.07	0.65 ± 0.04	0.91 ± 0.09	0.61 ± 0.15	0.58 ± 0.05	0.19 ± 0.06	1.49 ± 0.14	0.56 ± 0.11
C	0.48 ± 0.02	0.37 ± 0.02	0.47 ± 0.04	0.55 ± 0.10	0.26 ± 0.01	0.52 ± 0.02	0.29 ± 0.01	0.67 ± 0.17	0.34 ± 0.11
D	0.36 ± 0.03	0.21 ± 0.05	0.37 ± 0.01	0.30 ± 0.03	0.20 ± 0.02	0.29 ± 0.02	0.11 ± 0.03	0.51 ± 0.00	0.23 ± 0.03
E	0.25 ± 0.02	0.19 ± 0.02	0.20 ± 0.01	0.48 ± 0.05	0.37 ± 0.03	0.46 ± 0.01	0.13 ± 0.01	0.67 ± 0.00	0.33 ± 0.06
F	0.83 ± 0.01	0.63 ± 0.01	0.61 ± 0.01	0.40 ± 0.02	0.35 ± 0.02	0.56 ± 0.01	0.44 ± 0.01	0.31 ± 0.04	0.29 ± 0.01
G	0.12 ± 0.01	0.06 ± 0.00	0.08 ± 0.00	0.15 ± 0.00	0.11 ± 0.00	—	—	0.24 ± 0.02	0.13 ± 0.01

Table 1b

Table 10						
Substrate addition of:		$pH, 7.2$ $Li^+$ $Na^+$	$Li^+$ $K^+$	$Li^+$ $NA$	$pH, 7.2$ $K^+$ $Cs^+$	$K^+$ $Cs^+, NA$
Subcellular fractions	$Li^+$					
<i>A</i>	$0.39 \pm 0.09$	$0.30 \pm 0.01$	$0.33 \pm 0.01$	$0.23 \pm 0.10$	$0.71 \pm 0.01$	$0.04 \pm 0.02$
<i>B</i>	$0.06 \pm 0.01$	$0.03 \pm 0.03$	$0.08 \pm 0.03$	$0.21 \pm 0.01$	$0.18 \pm 0.01$	$0.04 \pm 0.01$
<i>C</i>	$0.32 \pm 0.01$	$0.28 \pm 0.01$	$0.49 \pm 0.01$	$0.03 \pm 0.02$	$0.54 \pm 0.01$	$0.00 \pm 0.00$
<i>D</i>	$0.32 \pm 0.02$	$0.28 \pm 0.01$	$0.32 \pm 0.09$	$0.00 \pm 0.00$	$0.44 \pm 0.01$	$0.00 \pm 0.00$
<i>E</i>	$0.19 \pm 0.02$	$0.30 \pm 0.00$	$0.25 \pm 0.06$	$0.00 \pm 0.00$	$0.32 \pm \text{—}$	$0.00 \pm 0.00$
<i>F</i>	$0.57 \pm 0.03$	$0.47 \pm 0.02$	$\text{—}$	$0.50 \pm 0.02$	$\text{—}$	$\text{—}$
<i>G</i>	$0.06 \pm 0.00$	$0.05 \pm 0.00$	$\text{—}$	$0.25 \pm 0.00$	$\text{—}$	$\text{—}$

*p*-Nitrophenyl-phosphatase activities at pH 7.2 and 8.8 in subcellular fractions of homogenates of rat brain separated by ultracentrifugation<sup>21</sup>. Concentration of *p*-nitrophenyl-phosphate salts was 5 mmolar, and magnesium sulphate was 5.0 mmolar. Final concentration of additional salts was 48 mmolar (NA: 12 mM). Enzyme activity expressed as μmoles of *p*-nitrophenyl phosphate hydrolysed/mg of protein/min. All enzyme activities are results of four independent determinations. The activities are indicated with standard deviation of the mean. Content of sub-fractions of crude mitochondrial fraction (A-E): A, myelin, membranes of synaptic origin; B, synaptic vesicles and membranes and fragments of nerve endings; C, nerve endings; D, nerve endings; E, mitochondria, lysosomes, some nerve endings; F, microsomal fraction containing membraneous components with ribosomes; G, nuclear fraction with nuclei fragments some contaminating mitochondria, nerve endings. NA, Noradrenaline.

more, the presence of noradrenaline enhances the enzyme activities of sodium and/or potassium systems. This increased activity is maximally increased six times when noradrenaline is added to the pure system of sodium ions (B).

Previous experiments have demonstrated the involvement of phosphoprotein and probably phospholipids in the rapid exchange of phosphorus-32 induced by electrical stimulation of the CNS<sup>20,21</sup>. The exchange of phosphorus-32 provoked by electrical stimulation may be related to the activity of the membrane-bound ATPase which can be stimulated by magnesium ions<sup>23,24</sup>, because the activity of ATPase in the CNS contributes to the establishment of the uneven distribution of potassium and sodium ions in the CNS<sup>22,23</sup>. This may also be the chemical basis for the phenomena associated with electrical stimulation of the CNS. In contrast with the present findings of a *p*-nitrophenyl-phosphatase of sub-mitochondrial fraction A, B and C from the rat brain which are activated by potassium and magnesium ions and inhibited by sodium ions, the membrane-bound ATPase is activated by sodium ions<sup>23,24</sup>. The activity of ATPase should, therefore, be distinguished from the activity of *p*-nitrophenyl phosphatase of the CNS, which is activated by potassium and magnesium ions.

The present data which indicate activation of this phosphatase by noradrenaline imply that this hormone, liberated from the synaptic vesicles, may be a biological trigger mechanism leading to the exchange of potassium and sodium ions across the synaptic membrane, thus causing the electrical activity to continue in the post-synaptic dendrite.

The depressing effect of lithium ions on the activity of phosphatase, even in the presence of noradrenaline, could be part of the action of this drug on the CNS, which is used in the treatment of mania<sup>25</sup>. The effect caused by lithium ions seems to be opposite to that in the ATPase system<sup>24</sup>.

Finally, the high content of phosphatidyl-serine in the

biomembranes which contain the phosphatase which is activated by potassium and magnesium ions in CNS could control the action of noradrenaline, because this drug is specifically bound to phosphatidyl-serine<sup>20</sup>. Phospholipase D greatly enhances the activity of phosphatase apparently by degrading phosphatidyl-serine (our report in preparation).

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### Demonstration of Nerve Terminals containing Adrenaline by a New Histochemical Technique

PREVIOUS reports on the presence of high concentrations of adrenaline in the frog heart have been confirmed and extended<sup>1</sup>. Using the histochemical technique of Falck in whole mount preparations of the atrium and sinus venosus of the frog, it was shown that tissue catecholamines were confined within the nerve fibre terminals of the sympathetic plexus. These findings were in agreement with a previous report<sup>2</sup>.

In the histochemical method of Falck<sup>3</sup> distinction between noradrenaline and adrenaline is based on the difference in time required for development of the fluorescent products after exposure to paraformaldehyde<sup>4,5</sup>; however, the optimal time in each case has been found to vary considerably among different laboratories and it appears to depend on the source of paraformaldehyde, environmental humidity, and other unknown local laboratory factors. For these reasons, a more specific technique was sought for the histochemical differentiation of fibres containing adrenaline from fibres containing noradrenaline.

After considerable experimentation a technique based on the well known formation of trihydroxyindole fluorescent products was developed, and this can be used for the specific demonstration of fibres containing adrenaline. The technique is as follows.

Whole mount preparations are stretched and placed on a slide and allowed to dry at room temperature for 10–15 min. The slides are then placed in a closed Coplin jar containing 2 g of iodine and are exposed to iodine fumes for 5–10 min. Finally, they are transferred to another

Coplin jar and exposed to fumes of ammonia for 45–60 sec. The exact time required for proper drying and exposure to iodine and ammonia was critical and had to be worked out for each preparation which was investigated.

A "tissue blank" is prepared by reversal of the order of exposure to iodine and ammonia which prevents the formation of adrenaline fluorescence products and permits the detection of non-specific tissue fluorescence. This increases greatly the specificity of the method.

With this technique, preparations from frog tissues including atrium, sinus venosus, mesentery and urinary bladder showed fluorescent fibres which were morphologically similar to those seen with the formaldehyde condensation method (Fig. 1). Conventional wide band excitation filters (BC 12, Corning 5–58) used in conjunction with green-yellow barrier filters at the ocular (Kodak 15) were satisfactory for demonstration of the fluorescence. The use of interference filters showed optimal excitation to occur with the 405 m $\mu$  line of the mercury lamp and the fluorescence colour was green to blue-green (500–520 m $\mu$ ) when observed with wide band barrier filters (Kodak 2E or 3). These findings correspond closely to the known excitation and fluorescent spectra of adrenolutin<sup>6,7</sup>. Surprising, however, was the fact that although no reducing agents were used, the fluorescent product was stable for more than 24 h.

When the same technique was used in tissues known to contain noradrenergic fibres (for example, rat mesentery) no fluorescent structures were seen. Thus adrenergic fibres can be detected in the presence of noradrenergic fibres which is not possible with the formaldehyde condensation technique. Similarly, as would be expected, structures containing serotonin (for example, rat mast cells) showed no fluorescence in these conditions.

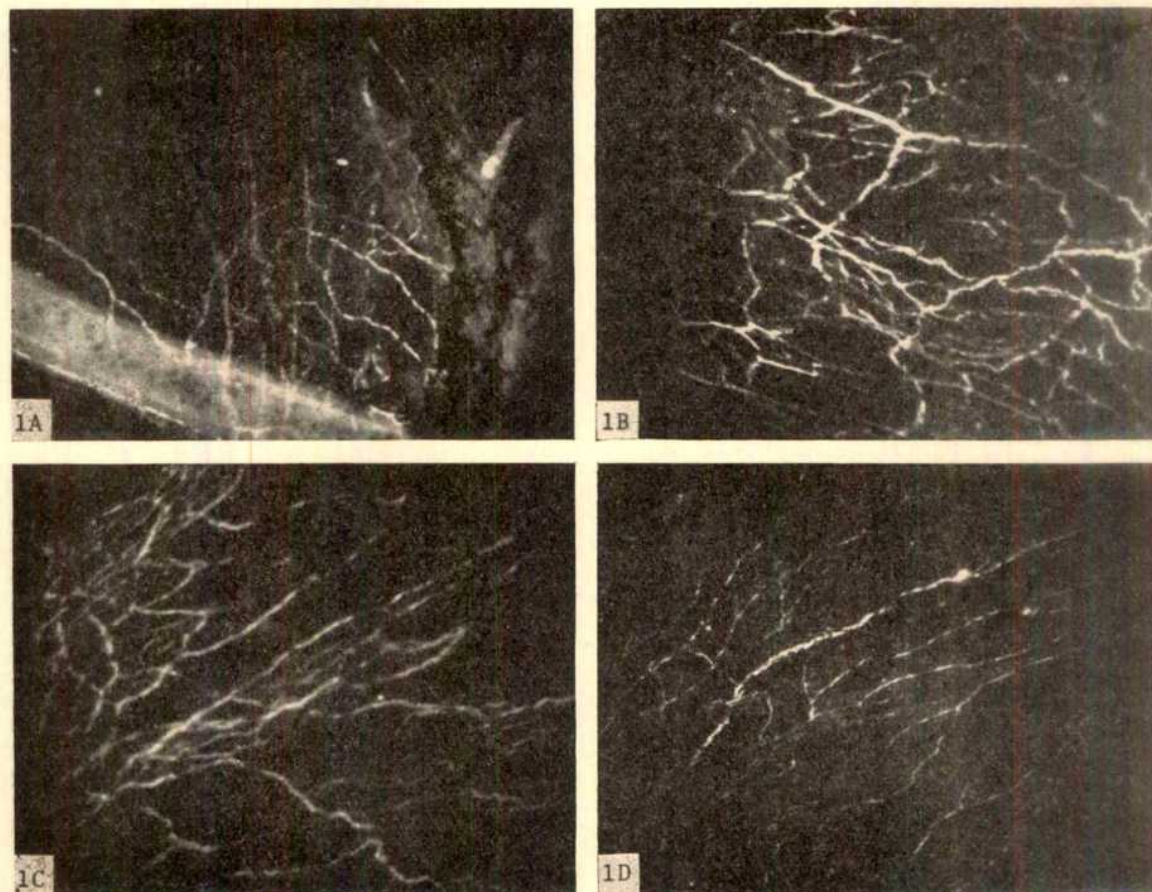


Fig. 1. Fibres containing adrenaline in tissues of frog demonstrated with the trihydroxyindole reaction; (A) mesentery; (B) urinary bladder; (C) sinus venosus; (D) atrium.



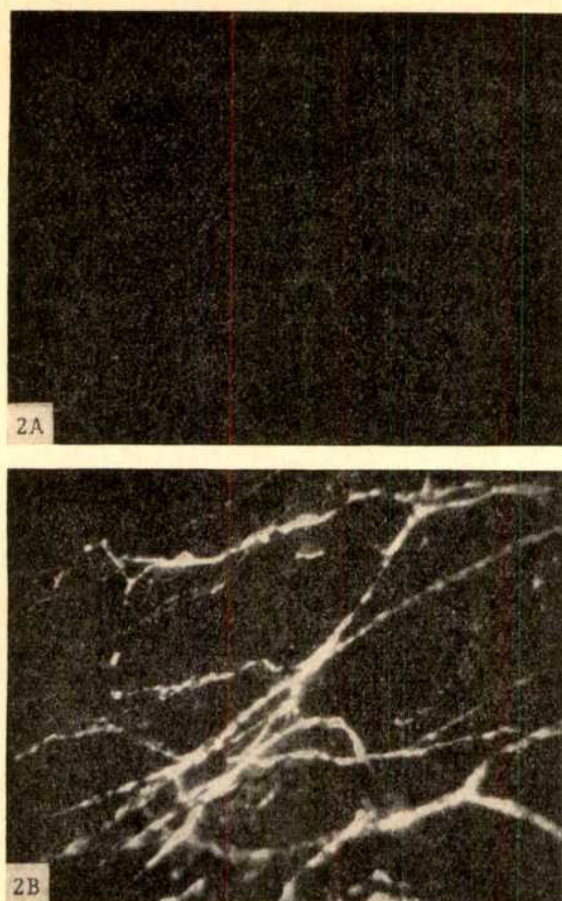


Fig. 2. Atrium of guinea-pig treated with the trihydroxyindole reaction before (A) and after (B) exposure to adrenaline.

When tissues containing noradrenergic fibres were exposed to exogenous adrenaline, considerable uptake of the amine by tissues was found by chemical analysis by the method of von Euler and Lishajko<sup>8,9</sup>. When such tissues were examined histochemically by the method described here characteristic green to blue-green fluorescent fibres were seen (Fig. 2). No such structures were present in control, untreated preparations. This indicated that adrenaline was taken up within the noradrenergic fibres and further demonstrated the specificity of the technique.

In the iris of the rat this technique revealed blue fluorescent structures associated with the endothelium of the blood vessel similar to those seen with the formaldehyde technique after exposure of the iris to dihydroxyphenylalanine. The structures involved are not nerve fibres but are similar to those said to occur along blood vessels of the brain<sup>10</sup>. This phenomenon has been studied in some detail and the results will be reported elsewhere<sup>11</sup>.

When applied to frozen sections of the adrenal medulla of the hamster, the present technique did not produce any fluorescence in the cells containing adrenaline (centre of the gland); instead, a blue to blue-white fluorescence was obtained with the cells containing noradrenaline (periphery of the gland). A fluorescent reaction with noradrenaline cells of the adrenal medulla and no reaction with noradrenergic fibres has been previously reported when an adaptation of the trihydroxyindole method was employed in a histochemical procedure proposed by Carlsson *et al.*<sup>12</sup>.

Investigations carried out in this laboratory indicate that blue to blue-white fluorescent products can be obtained in noradrenaline fibres with a similar method provided that the specimens have been previously prepared with

procedures which release noradrenaline from tissue binding<sup>13</sup>. Thus, the essential part of the proposed method may be dependent on the presence of adrenaline in a relatively free form. In this connexion, it is noteworthy that ultracentrifugation has demonstrated that the adrenaline found in frog tissues was present mostly in the non-particulate fraction<sup>1</sup>.

Previous observations<sup>1,2</sup> and the present results indicate that the sympathetic neurohumour in the heart of frog (and in most other tissues of this species) is adrenaline rather than noradrenaline. This is in agreement with other observations<sup>14,15</sup>. It is now appropriate that such fibres containing adrenaline be called adrenergic while the term noradrenergic should be used to describe the fibres which contain noradrenaline and are widely distributed in the tissues of all higher vertebrates that have been examined<sup>16</sup>.

A complete method for the specific histochemical demonstration and differentiation of adrenergic and noradrenergic fibres using the trihydroxyindole reaction is now under development in this laboratory.

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## Oculosympathetic Response to Circulatory Arrest

REPORTS of the role of the sympathetic nervous system in anoxic mydriasis are not clear. A generalized sympathetic discharge occurs during systemic anoxia<sup>1,2</sup>, but some published data suggest that the sympathetic nervous system plays no part in the pupillary dilatation which occurs during anoxia and ischaemia. Little or no dilatation of the parasympathectomized pupil was observed during anoxia in the presence of an intact sympathetic innervation<sup>3</sup>. The pupil which has had an interruption of sympathetic and parasympathetic innervation dilates during asphyxia<sup>4</sup>.

The right oculomotor nerve was sectioned intracranially in six adult cats. One to three weeks later under sodium pentobarbital anaesthesia a right lateral thoracotomy was carried out with complete occlusion of the ascending aorta and both venae cavae. After 6 min of circulatory arrest, a right preganglionic cervical sympathectomy was performed, and the great vessels were re-occluded for another 6 min. Transverse pupillary diameters were measured with a millimetre ruler before, during and after the vascular occlusions.

When an acute sympathectomy was superimposed on a chronic oculomotor section, the dilatation of the pupil



**Table 1. PUPILLARY RESPONSE OF CHRONICALLY PARASYMPATHETICALLY DENERVATED IRIS AND OF CONTRALATERAL NORMAL IRIS TO CIRCULATORY ARREST BEFORE AND AFTER ACUTE CERVICAL SYMPATHECTOMY**

Before acute cervical sympathectomy					After acute cervical sympathectomy				
Chronically parasympathetically denervated iris			Contralateral normal iris		Chronically parasympathetically denervated iris			Contralateral normal iris	
Initial size of pupil	Maximal size during occlusion	Maximal size after circulation restored (within 2-5 min)	Initial size	Maximal size during occlusion	Initial size of pupil	Maximal size during occlusion	Maximal size after circulation restored (within 2-5 min)	Initial size	Maximal size during occlusion
8	13	12	2	14	5	5	9	7	14
11	13	13	3	13	11	11	11	7	14
10	13	12	6	14	10	10	11	4	14
10	14	12	1	14	10	10	13	1	14
10	13	12	6	13	10	10	13	8	13
11	14	13	4	13	10	11	11	2	14

during ischaemia was abolished (Table 1). Dilatation still occurred when circulation was restored, presumably due to the effect of circulating neurohumours.

These data show that the chronically parasympathetically denervated iris was capable of further dilatation during circulatory arrest. This dilatation was not correlated with the changes in the arterial blood pressure (Fig. 1)

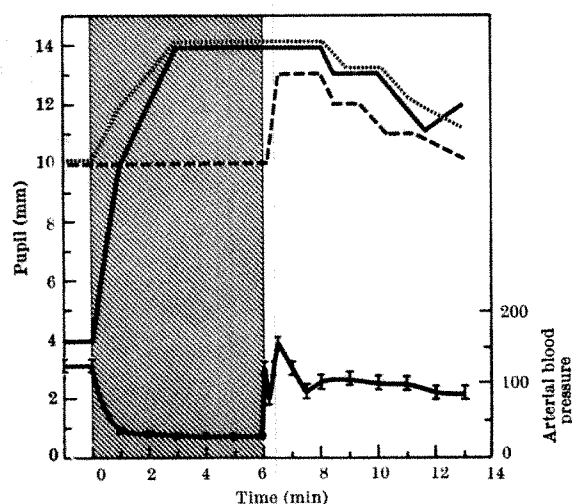


Fig. 1. Response of denervated pupil to circulatory arrest (cross-hatched area). ----, Parasympathetically denervated pupil; —, normal pupil; - · -, parasympathetically and sympathetically denervated pupil.

and occurred at a time when there was no flow of blood to the eye. This dilatation was abolished by cervical sympathectomy.

This investigation does not prove that the chief mediator of pupillary dilatation during circulatory arrest is the sympathetic nervous system, nor does it refute the idea of a balanced effect of both sympathetic and parasympathetic systems. Nevertheless, the sympathetic nervous system can produce extreme pupillary dilatation in an eye which has undergone chronic oculomotor nerve section, and such dilatation occurs at a time when no circulating neurohumours can reach the iris.

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## Respiratory Response to Carbon Dioxide in Man

In the course of our investigation on the respiratory regulation in man at high altitudes in the Himalayas<sup>1</sup> it was observed that the respiratory response of the Indian subject to carbon dioxide was lower than the European<sup>2</sup>. The unit of carbon dioxide response (pulmonary ventilation, l/min/mm mercury) is such that it should ultimately be related to the metabolic rate or the body weight. The body weight of the Indian subject was smaller (56 kg), and it was thought that his apparently low response to carbon dioxide may have been caused by his lower body weight.

Experiments aimed at investigating this relation were performed at sea level. No positive correlation between body weight and carbon dioxide response was obvious from the results, but the values for the response were consistently lower irrespective of the body weights in the Indian subjects.

The technique employed in this investigation was a simplified version of that developed at Oxford<sup>3</sup>. Briefly, the subject was seated comfortably in a chair and he breathed a mixture of oxygen, carbon dioxide and nitrogen from a large Douglas bag through a humidifier. The expirate was collected in a second bag. When a steady ventilation was reached the expirate was switched on to a third small bag and the collection was made for a timed period. During this period, the Rahn-Otis end tidal sample, which was drawn by water suction over a series of sampling tubes, was collected by mercury displacement. When the first bag was empty the subject was given the expirate bag to breathe from and his expired air was collected as before. Usually carbon dioxide was added to the second bag to raise the ventilation of the subject to about 40 l./min.

The volume of the expired air collected was measured in a spirometer and expressed as  $\dot{V}_{BTPS}$  (ventilation, body temperature pressure saturated), l./min. End tidal samples were analysed in a Lloyd-Gallenkamp analyser.

The relation between  $\dot{V}_{BTPS}$  and the atmospheric pressure of carbon dioxide ( $P_A\text{CO}_2$ ) (above threshold value) at any value of the atmospheric pressure of oxygen ( $P_A\text{O}_2$ ) is linear and the relation between the slope of these lines and  $P_A\text{O}_2$  is inverse. This respiratory response has been described<sup>4</sup> by the expression like  $\dot{V} = D(P_A\text{CO}_2 - B) [1 + A/(P_A\text{O}_2 - C)]$ , where  $B$  (mm of mercury  $P_A\text{CO}_2$ ) is the intercepts of these lines produced to  $P_A\text{CO}_2$  axis at zero ventilation, the asymptote  $D$  (l./min/mm of mercury  $P_A\text{CO}_2$ ) is the slope of the line in the complete absence of hypoxia, the parameter  $C$  gives a critical value of  $P_A\text{O}_2$  when the slope of the  $\dot{V} - P_A\text{CO}_2$  line approaches infinity and  $A$  (mm of mercury  $\text{O}_2$ ) describes the curvature of the hyperbola, that is, the hypoxia sensitivity.

With a few exceptions most of our results fit into this expression. Average values from six subjects for the parameters with their standard error are:  $B$ ,  $36.0 \pm 0.3$  mm of mercury  $\text{CO}_2$ ;  $A$ ,  $23.2 \pm 1.7$  mm of mercury  $\text{O}_2$ ;  $C$ ,  $30.9 \pm 9.0$  mm of mercury  $\text{O}_2$ ;  $D$ ,  $1.60 \pm 0.16$  l./min/mm of mercury  $\text{CO}_2$ . All these values fall within the range given by Cunningham, Patrick and Lloyd<sup>2</sup> except for  $D$ . Their value for the European subjects is on average  $3.98 \pm 1.35$  l./min/mm of mercury  $\text{CO}_2$ , which is significantly different



from the mean value obtained in our experiments on the Indian subjects.

In another series of experiments on sixteen subjects  $\dot{V}_E P_A\text{CO}_2$  lines were determined by breathing carbon dioxide in pure oxygen. When the upper linear part of the response was taken into account the mean slope of the lines was  $1.97 \pm 0.13$  l./min/mm of mercury  $\text{CO}_2$  and the extended intercept on the  $P\text{CO}_2$  axis was  $35.2 \pm 0.6$ . These values are in agreement with that obtained from the other series of full experiments. The value of  $D$ , however, is somewhat smaller than the slope of the  $\dot{V}_E P_A\text{CO}_2$  line on oxygen at 1 atmosphere. This is perhaps not unexpected, because the value of  $D$  corresponds to infinitely large oxygen pressure.

The body weights of these subjects ranged from 41 to 82 kg, and the range of oxie slope was 1.10 to 3.92. There was no correlation between the body weights and the response to carbon dioxide. The values for the parameter  $D$  and for the slope of the response to carbon dioxide line in high oxygen/kg body weight were  $30.1 \pm 4.4$  and  $36.4 \pm 3.0$  ml./min/mm of mercury, respectively.

The lower value of the oxie slope was not due to any greater resistance in the breathing system. The main difference between the Oxford experiments and our present experiments is in the groups of subjects. In our studies the subjects were Indians (Bengalis), while Cunningham *et al.*<sup>2</sup> used European subjects only. Thus these results demonstrate a significant difference of carbon dioxide response in different groups of subjects, if not a racial effect.

This conclusion appears to be supported by the observation on one Sherpa (Himalayan highlander) and one European subject at sea level whose oxie slopes of  $\dot{V}_E P_A\text{CO}_2$  lines were 1.30 and 3.72, respectively.

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### Effect of Botulinum Toxin on Autonomic Nerves in a Dually Innervated Tissue

A HYPOTHESIS has suggested the existence of a cholinergic link in adrenergic transmission of postganglionic sympathetic nerve endings<sup>1</sup>. Much evidence has been presented in support of this hypothesis<sup>2,3</sup>. Botulinum toxin has been said to block sympathetic nerve transmission<sup>4</sup>. Botulinum toxin is known to block the release of acetylcholine from classically cholinergic nerve fibres<sup>5,6</sup>, and so it was suggested that sympathetic impairment by botulinum toxin reflects an action on the hypothetical cholinergic link in the sympathetic nerve fibre ending. Rather large concentrations of the toxin (20,000 to 50,000  $L.D_{50}$ /ml.) and long times (2–5 h) were, however, necessary to produce sympathetic blockage. It was therefore desirable to test the action of botulinum toxin on a dually innervated tissue so that effects on both classically adrenergic sympathetic fibres and classically cholinergic parasympathetic fibres could be observed in the same preparation.

The spontaneously beating rabbit sino-atrial node was isolated, perfused and prepared for stimulation of the intranodal autonomic nerve fibres and recording of spontaneous beat interval as previously described<sup>7</sup>.

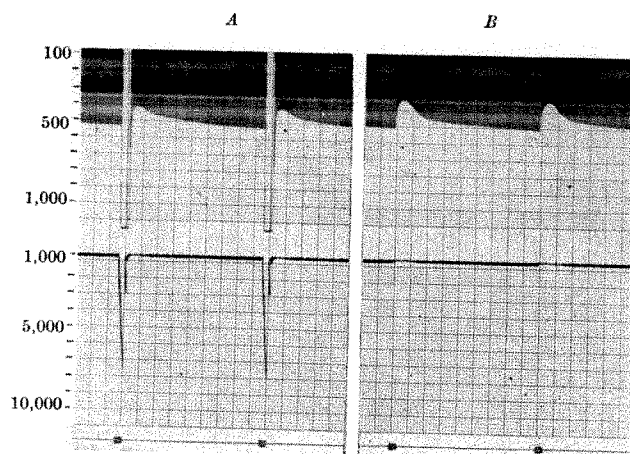


Fig. 1. Influence of botulinum toxin on the biphasic chronotropic response of the sino-atrial node to excitation of intranodal autonomic nerve fibres. Beat interval records of A, control responses; B, responses after 90 min of perfusion with a solution containing 850  $L.D_{50}$ /ml. of botulinum toxin. The toxin produced complete blockage of the cholinergic response but did not reduce the adrenergic response. The inverted, two range scale indicates spontaneous beat interval in msec. The marker channel at the bottom of the records denotes periods of nerve stimulation.

Intranodal autonomic nerve fibres were stimulated every 180 sec throughout each experiment by a 10 sec burst of 10 V (+), 100/sec (rectangular wave pulses) with a pulse duration of 0.1 msec. Such stimulation produces a biphasic (negative, then positive) chronotropic response, which is mediated through the release of acetylcholine and nor-adrenaline<sup>8</sup>. In control conditions the response is stable for at least 4–6 h. Magnitudes of the cholinergic (negative) and adrenergic (positive) responses were quantified as previously described<sup>7</sup>, by measurement of the difference between the beat interval just before nerve stimulation and the maximal and minimal beat intervals, respectively, resulting from intranodal nerve stimulation.

Botulinum toxin was produced by anaerobic incubation of *Clostridium botulinum*, type E, strain Beluga. The toxin employed was present in the supernatant of the incubation medium after removal of the cells by centrifugation. The incubation medium was composed of 5 per cent tryptocase, 0.5 per cent peptone and 0.4 per cent glucose. To activate the toxin, 0.1 per cent trypsin was added and the mixture was incubated at 37° C for 45 min. This activated toxin solution contained 170,000 intraperitoneal mouse  $L.D_{50}$ /ml. The dosage of the toxin is expressed in these units. Except when diluting the toxin, it was stored in the cold, but not frozen. The activated toxin solution was incorporated in buffered Ringer-Locke solution in a known concentration and applied by continuous perfusion (approximately 3 ml./min).

Botulinum toxin was applied to seven preparations in concentrations of 170–17,000  $L.D_{50}$ /ml. In each preparation there was nearly complete, or complete, blockage of the cholinergic response to intranodal nerve stimulation. Characteristic of the action of the toxin was the slow progressive and irreversible nature of the cholinergic blockage produced. The greater the concentration of the toxin, the shorter was the time before complete blockage ensued. A complete cholinergic block occurred in 80 min with 850  $L.D_{50}$ /ml., and in 24 min with 17,000  $L.D_{50}$ /ml. Fig. 1 presents interval-meter records of chronotropic responses of a preparation before, and after, 90 min of perfusion with 850  $L.D_{50}$ /ml. As can be seen, there was complete blockage of the cholinergic response to intranodal nerve stimulation. Perfusion with 170  $L.D_{50}$ /ml. produced a slowly progressive cholinergic blockage. After 4 h of perfusion with this concentration the magnitude of the cholinergic response was reduced to 4.9 per cent of the control response. Thus, a 95 per cent blockage was produced with as little as 170  $L.D_{50}$ /ml. On the other hand, no adrenergic blockage

was observed in any preparation with up to 4 h perfusion with doses up to 3,400 *L.D.*<sub>50</sub>/ml. In one preparation perfused with 17,000 *L.D.*<sub>50</sub>/ml., there was an immediate reduction of the adrenergic response to 28 per cent of the control response, but this effect was not progressive and was immediately reversible when, after 30 min, the solution containing toxin was replaced with normal Ringer-Locke solution. This is in contrast with the complete, progressive and irreversible cholinergic blockage which had occurred in this preparation at this time. At 17,000 *L.D.*<sub>50</sub>/ml., the solution containing toxin constituted 10 per cent of the perfusate, and so it seems likely that this reversible effect is non-specific and is unrelated to the usual irreversible action of botulinum toxin. Specificity of the cholinergic blockage seen at lower concentrations was substantiated by treating two preparations with toxin solution which had been heated to 100° C for 15 min. This heat-inactivated toxin solution in a concentration of 0.5 per cent or 1.0 per cent (equivalent to 850 and 1,700 *L.D.*<sub>50</sub>/ml., respectively, of active solution) produced no cholinergic or adrenergic blockage.

These results do not represent a comprehensive examination of the effects of botulinum toxin, but they serve to emphasize a very important point which is already documented<sup>9-11</sup>; botulinum toxin is very effective in blocking parasympathetic nerve endings, but has little blocking action on postganglionic sympathetic fibres. It should be noted that the chronotropic response of the sino-atrial node to intranodal nerve stimulation depends on the excitation of postganglionic nerve fibres<sup>12</sup>, so that the present results are not related to any possible ganglion blocking action of botulinum toxin<sup>13</sup>.

These results are not easily compared with previous ones<sup>4</sup> because different conditions, tissues and toxin types were employed. Nevertheless, in this study botulinum toxin produced an apparently specific cholinergic blockage at much smaller concentrations than was used to block sympathetic function<sup>4</sup>. The great difference in the potency of botulinum toxin on parasympathetic and sympathetic nerves supports the classical concepts of cholinergic and adrenergic transmission.

The results presented here are exactly analogous to those obtained using hemicholinium in the same system<sup>7</sup>. Both hemicholinium, which blocks the synthesis of acetylcholine<sup>14</sup>, and botulinum toxin, which blocks the release of acetylcholine<sup>5,6</sup>, are effective in blocking the cholinergic response of the dually innervated tissue. On the other hand, even large concentrations of these agents do not produce adrenergic blockage. The results, therefore, do not support the earlier hypothesis<sup>1</sup>.

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## MICROBIOLOGY

### Stimulating Effect of Xylose on the Utilization of Galactose in some Fungi

THE wild type of the ascomycete *Ophiostoma multiannulatum* does not grow on the hexose D-galactose as a sole source of carbon although the fungus can respire on it<sup>1</sup>. In an investigation<sup>2</sup> of the ability of *Ophiostoma* to utilize galactose in the presence of glucose it was found that in such mixtures a certain amount of galactose, which depended on the concentration of glucose, was utilized for synthesis of cell material. Similar results were obtained when galactose was mixed with fructose, mannose or glycerol. It therefore seems that cells of the wild type can utilize galactose for growth only if another source of utilizable carbon or some metabolic intermediate of such a compound is present in the cells.

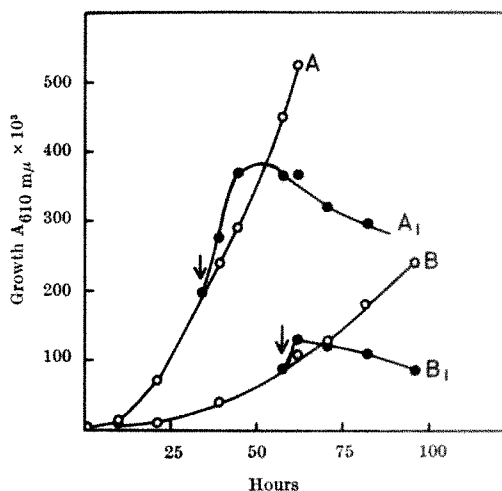


Fig. 1. Growth of *Ophiostoma multiannulatum*, strain 51 (mating type +), on 0.4 per cent arabinose (curve A) and 0.4 per cent rhamnose (curve B). At the time indicated by the arrows the cultures were divided into two halves and to one of these 0.4 per cent galactose was added (curves A<sub>1</sub> and B<sub>1</sub>). Growth was measured photometrically at 610 mμ.

In a later study of the growth of the wild type strain 51 (mating type +) of *Ophiostoma multiannulatum* on various pentoses it was found that an addition of galactose to cultures growing on L-arabinose or L-rhamnose resulted immediately in an increased rate of growth which was shortly followed by complete cessation (Fig. 1). A culture on 0.4 per cent D-xylose also grew more rapidly when galactose was added (Fig. 2), but in this case the growth continued until both sugars were used up. In further growth experiments with mixed sugars the concentration of galactose was kept constant while the concentration of xylose was varied. A concentration of 0.004 per cent xylose, although too low to support measurable growth, nevertheless made it possible for the culture to grow on galactose (Fig. 3, curve C). The xylose is used simultaneously and the growth stops when the concentration of this sugar becomes limiting. As illustrated in Fig. 3, curve D, the addition of more xylose to a culture which had grown on 0.4 per cent galactose + 0.004 per cent xylose resulted in immediate resumption of growth. To obtain complete utilization of galactose it was necessary to add xylose at such a concentration that the ratio between galactose and xylose was approximately 40 to 1.

A number of different mutant strains of *Ophiostoma multiannulatum*, which use galactose as a sole source of carbon, have been isolated<sup>2</sup>. One of these strains (No. 5121) grows very slowly on galactose after a long lag phase. The addition of xylose at a concentration of 20 mg/l. to a

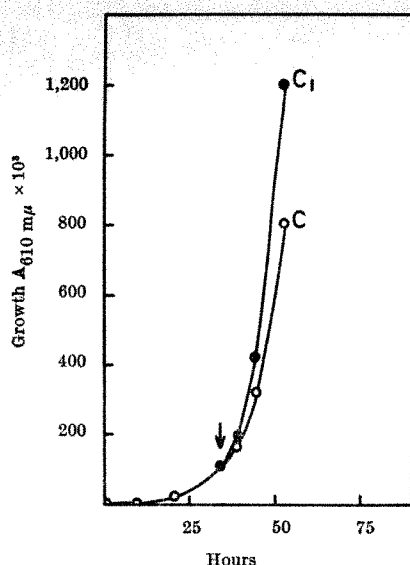


Fig. 2. Growth of strain 51 (mating type +) on 0.4 per cent xylose (curve C). At the time indicated by the arrow the culture was divided into two halves and to one of these 0.4 per cent galactose was added (curve C<sub>1</sub>).

medium containing 0.4 per cent galactose increased the growth rate and shortened the lag phase.

The promoting effect of xylose on the utilization of galactose was also tested in some other fungi. *Ophiostoma ulmi* and *Aspergillus niger*, which both grow rather slowly on galactose, responded with a marked increase in the rate of growth when the galactose was supplemented with traces of xylose. *Ophiostoma piceae* was found to grow faster on a mixture of 0.4 per cent galactose + 0.002 per cent xylose than on 0.4 per cent glucose. On the other hand, the growth on galactose of four *Coprinus* species was unaffected by xylose.

Relatively little is known about the metabolism of galactose in fungi, with the exception of yeasts<sup>3,4</sup>. It is

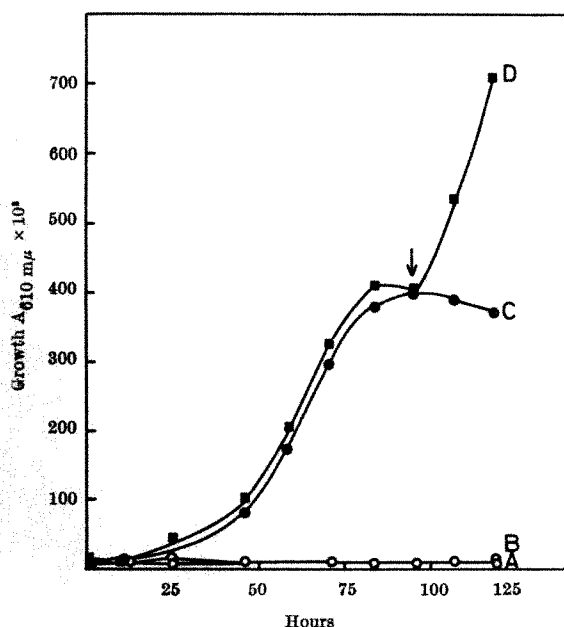


Fig. 3. Growth of strain 51 (mating type +) on 0.004 per cent xylose (curve A), 0.4 per cent galactose (curve B), 0.4 per cent galactose + 0.004 per cent xylose (curve C) and 0.4 per cent galactose + 0.004 per cent xylose with another addition of 0.004 per cent xylose at the time indicated by the arrow (curve D).

therefore difficult to interpret the observed xylose effects in biochemical terms. In *Ophiostoma multiannulatum*, galactose is readily taken up by all strains tested<sup>2</sup>, but conversion to glucose intermediates appears to be blocked in the wild type strains. Yet even in these wild type strains some metabolism of galactose undoubtedly occurs since galactose is respired<sup>1</sup>. It is possible that a metabolic product of xylose may function as a cofactor for the conversion of galactose to glucose intermediates. Another possibility is that xylose can help the cells to overcome a block in the metabolism of galactose by preventing the accumulation of some growth inhibiting compound.

Enzyme studies on the metabolism of galactose in *Ophiostoma multiannulatum* are in progress.

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### Synthesis of Seleno-amino-acids in Cell Free Extracts of *Candida albicans*

SELENIUM, long considered an element toxic to the majority of living organisms, may be of physiological importance in various animals as an indispensable part of the diet<sup>1-5</sup>. It may replace the sulphur atom in sulphur amino-acids<sup>6-14</sup> in the proteins of plants, animals and micro-organisms<sup>15-18</sup>, and could stimulate cell division in a filamentous mutant of *Candida albicans*<sup>19</sup>. This stimulation has been related to the replacement of sulphhydryl groups in the composition of the cell wall with —SeH groups, the greater stability of which can overcome in the filamentous mutant the deficiency in protein-disulphuro-reductase<sup>20</sup>, an enzyme indispensable to plastification of the cell wall<sup>21</sup>.

Identification of selenocysteic acid and cysteine in the enzyme hydrolysate of *Candida albicans* cells cultivated in the presence of sodium selenite labelled with selenium-75 (ref. 14) would therefore be of special physiological significance.

The routes by which seleno-amino-acids are synthesized are still unknown, and there is close structural and chemical analogy between selenium and sulphur compounds, so we decided to investigate whether the reactions involved in the synthesis of sulphurated amino-acids could lead to the synthesis of analogues containing selenium.

Two routes are at present known for the incorporation of inorganic sulphur into organic bonds: these are the reversibility of the desulphydration of cysteine<sup>22-24</sup> and the reversibility of the desulphination of cysteine-sulphinic acid<sup>25,26</sup>. The synthesis of cysteine-sulphinic acid from sulphite, pyruvic acid and glutamic acid occurs in *Proteus*<sup>27</sup> through direct enzyme condensation of the first two compounds, with formation of sulphinylpyruvic acid which is then transaminated with glutamate. (The efficiency of this metabolic route has been established by Shepherd in *Aspergillus nidulans*, using parathiotrophic mutants<sup>28</sup>.)

The present investigations have been carried out to demonstrate the efficiency of this metabolic route in *C. albicans* and the possibility of replacing the sulphite with selenite in the direct condensation reaction with pyruvic acid, and consequent synthesis of seleno-amino-acids.

Cells of *Candida albicans*, strain RM 806, resistant to high concentrations of selenite, capable of reducing it to

amorphous metallic selenium<sup>19</sup> and of incorporating it into their own proteins<sup>14</sup>, have been cultivated in medium GGY<sup>19</sup>, collected after 48 h incubation at 28° C by centrifugation and disintegration in a 'Nike X-press' (Eskilstuna, Sweden) and cooled in acetone-dry ice. The material which was collected was suspended in an equal volume (w/v) of 0.066 molar phosphate buffer, pH 7.2, and centrifuged at 16,000g for 10 min at +2° C. The supernatant liquid was used as an enzyme in the test.

The incubation mixture used for the enzyme assays contained 10 mmolar sodium glutamate and sodium pyruvate, 1 mmolar pyridoxalphosphate, 10 mmolar sodium sulphite or labelled sodium selenite, and 5.0 ml. of cell-free extract in a final volume of 10.0 ml. Incubation was carried out at 37° C for 5 h, and then blocked by the addition of 1 ml. of 15 per cent trichloroacetic acid. After centrifugation at 16,000g for 10 min at +2° C, the trichloroacetic acid was extracted from the supernatant liquid with ethyl ether.

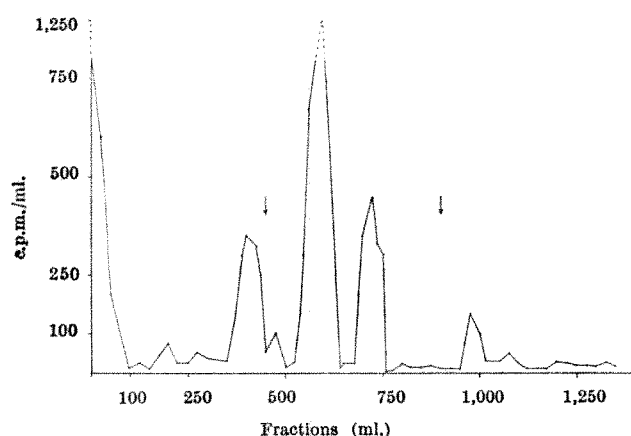


Fig. 1. Chromatographic analysis of deproteinized incubation mixtures on a column of 'Dowex 50W × 4', 200–400 mesh, H<sup>+</sup> form. Speed of elution was 2.0 ml./min; 10.0 ml. fractions were collected. Radioactivity was measured on 1.0 ml. samples with a well scintillation counter radioanalyser. Arrows show change in molarity of hydrochloric acid (1.1 molar/2.5 molar/4.0 molar).

After assays of efficiency of the enzyme preparation for the synthesis of cysteine and cystine in the presence of sodium sulphite had been carried out by means of the chemical determination of these amino-acids<sup>20</sup>, the mixture incubated with labelled sodium selenite was fractionated by chromatography on columns of 'Dowex 50W × 4', 200–400 mesh, in the H<sup>+</sup> form (15.0 cm high and 2.5 cm in diameter). Water (250 ml.), followed by a solution of hydrochloric acid of increasing molarity (1.1, 2.5, and 4.0 molar, 450 ml. of each), were used as the eluents. The radioactivity of each fraction was determined, and the radioactive fractions were concentrated under vacuum and chromatographed on Whatman No. 1 paper.

With the water the labelled selenite was eluted along with other salts. With 1.1 molar hydrochloric acid a peak in the fractions was obtained towards 400 ml., in the area which corresponded to selenomethionine<sup>16</sup>. Two radioactive peaks were eluted with 2.5 molar hydrochloric acid, one in the first fractions, for which no references are known, and another in the central fractions where, according to Blau<sup>13</sup>, selenocystine appears. Finally, 4.0 molar hydrochloric acid gave a rapid appearance of a fourth radioactive peak (Fig. 1). Paper chromatography of the concentrated radioactive fractions revealed the presence of ninhydrin-positive substances, radioactive on scintillometric analysis, the  $R_F$  values of which are given in Table 1. The close similarities between the  $R_F$  values of methionine and of the compound eluted with 1.1 molar hydrochloric acid are obvious, while the compounds eluted

with 2.5 molar hydrochloric acid have  $R_F$  values near to but not identical with those of cysteine acid, and the compound eluted with 4.0 molar hydrochloric acid has an  $R_F$  which corresponds to that of cystine.

The  $R_F$  of the compound eluted with 1.1 normal hydrochloric acid is almost identical to the  $R_F$  of synthetic selenomethionine, obtained with the same solvents by Tuve and Williams<sup>12</sup>.

Table 1.  $R_F$  VALUES OF SOME SULPHUR CONTAINING AMINO-ACIDS AND OF RADIOACTIVE SELENIUM COMPOUNDS ISOLATED ON 'DOWEX 50'

Amino-acids	$R_F$	Eluted radiocompounds	$R_F$
Methionine	75.5	1 (1.1)	74.5
Cysteine	58.0	2 (2.5)	50.3
Methionine sulphoxide	56.7	3 (2.5)	50.2
Cysteic acid	48.0	4 (4.0)	34.0
Cystathionine	37.0		
Cystine	35.0		

Paper was Whatman No. 1, solvent was tertiary butanol, normal hydrochloric acid and water (70.0 : 6.7 : 23.3); spots were developed with 1 per cent ninhydrin in water saturated *n*-butanol. Figures in brackets are molarities of hydrochloric acid.

Direct paper chromatographic analysis of the deproteinized incubation mixture (Fig. 2) has revealed four ninhydrin-positive spots with  $R_F$ s identical to those of methionine, glutamate or alanine, methionine sulphoxide and cysteic acid, respectively.

These spots were always found to be radioactive, with a higher intensity in the case of spot B caused by glutamic acid and alanine (Fig. 2). Autoradiography of chromatograms on which labelled sodium selenite was placed to migrate beside the sample has permitted confirmation that the radioactivity of this spot is caused by the selenite, which has an  $R_F$  identical to those of glutamic acid or alanine.

In conclusion, the *in vitro* enzyme synthesis of selenomethionine seems to be certain, while the synthesis of selenocysteine is likely, although this compound has not been isolated, probably because of its extreme lability.

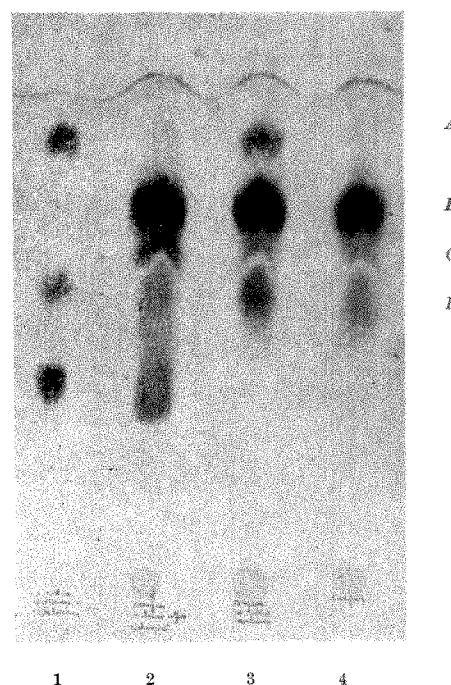


Fig. 2. Chromatography on Whatman No. 3 paper of a deproteinized incubation mixture. (1) Standards used were methionine, cysteic acid and cystathionine (from top to bottom); (4) A, B, C and D were samples of the incubation mixtures; (2) sample of incubation mixture plus methionine sulphoxide and cystine standards; (3) sample of incubation mixture plus methionine and cysteic acid standards. Solvent and ninhydrin solutions were as in Table 1.

A radioactive compound with  $R_F$  identical to that of cysteine acid has been identified by direct paper chromatography, which suggests that spontaneous oxidation of the seleno-amino-acids of the test sample may occur, partially in the case of selenomethionine and almost completely in the case of selenocysteine. This is also confirmed by the demonstration of a radioactive compound with an  $R_F$  identical to that of cysteine, in the eluate of 4.0 molar hydrochloric acid. Finally, another indirect proof of the presumed synthesis of selenocysteine is given by the fact that in the system used the synthesis of selenomethionine is constant, which necessarily presupposes that of selenocysteine.

Our results are important for the understanding of the mechanism of synthesis of organic selenium compounds, although all the steps involved are not fully explained. Furthermore, they clearly show that sulphite could be replaced by selenite in the condensation with pyruvic acid, which could, among the extensive biochemical relationships between sulphur and selenium compounds, be either an antagonism at the membrane level<sup>30,31</sup>, or possibly replacement of the sulphur atom by an atom of selenium in organic compounds<sup>6-18</sup>.

The incubation mixture used apparently permitted the formation of cysteine and cystine but not of methionine, because a complex enzyme methylating system is generally necessary for synthesis of the latter<sup>32-34</sup>. Thus, although it is possible that all the factors necessary for synthesis of methionine were present in the "crude" enzyme preparation used, it is necessary to investigate its mechanism more thoroughly, and research is now in progress.

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## IMMUNOLOGY

### Thymectomy and Anaphylactic Antibody in Rats infected with *Nippostrongylus brasiliensis*

It has been reported that neonatal thymectomy causes a defect in the production of "IgA" antibody in the rat. Arnason *et al.*<sup>1</sup> have shown that thymectomized rats immunized with bovine serum albumin (BSA), diphtheria toxoid, or heat-killed *Salmonella typhosa* produce comparatively small amounts of anti-BSA precipitating antibody and anti-diphtheria toxin (both largely IgA antibodies) but produce average amounts of anti-typhoid "O" agglutinin (IgM antibody) and of anti-BSA haemagglutinin (IgG antibody). In the present investigation, the production of anaphylactic antibody was compared in neonatally thymectomized and sham-operated rats infected with the intestinal nematode, *Nippostrongylus brasiliensis*. This infection normally results in high titres of reagin-like antibody to worm antigen<sup>2</sup>. The object of the present experiments was to determine whether the immunological deficit produced by neonatal thymectomy affects the production of anaphylactic antibody and the acquisition of immunity to infection with *N. brasiliensis*.

Sprague-Dawley rats were thymectomized or sham-operated within 24 h of birth<sup>3</sup>. All animals were weaned at 31 days, separated by sex, and kept under standard conditions. Thirteen thymectomized and ten sham-operated rats, 6-10 weeks of age, were inoculated subcutaneously with 1,000 larvae of *N. brasiliensis* (NIH strain). The infective larvae were cultured as described by Weinstein and Jones<sup>4</sup>. To control possible bacterial infection, all rats were given oxytetracycline hydrochloride in their drinking water (50 mg/l.); the drug was replaced three times a week. One month after the primary infection each rat was reinfected with 2,500 larvae. Egg-counts were done on the faeces of infected animals<sup>5</sup> between the sixth and eleventh days of infection. All animals were autopsied at the end of the experiment and examined grossly or histologically for thoracic thymus tissue.

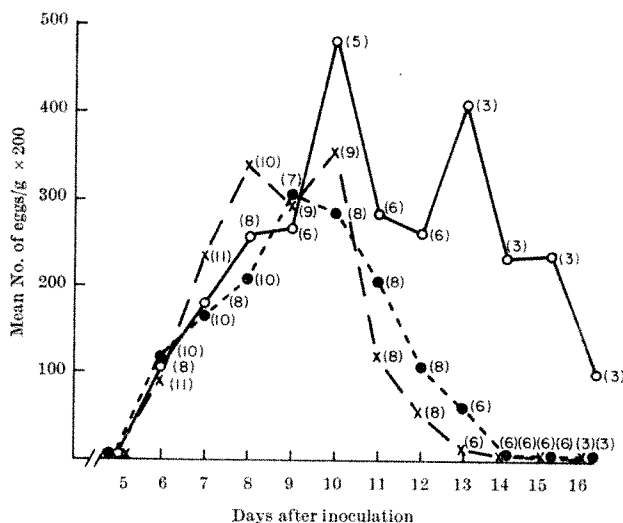


Fig. 1. Counts of the number of eggs/g of the faeces of groups of thymectomized (○), sham-operated (●) and normal rats (×) after a primary infection with 3,000 larvae of *N. brasiliensis*. The number of rats per group is shown in brackets.

Rats were bled from the retro-orbital sinus or from the heart before infection, 1 month after the first infection and 6 days after the second infection. Sera were stored at  $-20^{\circ}\text{C}$ . Anaphylactic antibody titres of the sera were determined by passive cutaneous anaphylaxis (PCA) in



normal Sprague-Dawley rats (150–200 g). Antigen for the PCA tests was prepared from adult worms collected from the small intestine of rats 10 days after subcutaneous infection with 3,000 larvae of *N. brasiliensis*. The worms were washed by repeated sedimentation in saline, and then homogenized in buffered saline (pH 7.2) in a Potter-Elvehjem glass homogenizer packed in ice. The homogenate was centrifuged at 1,000g for 10 min and the supernatant fluid (2 mg protein/ml.) used as antigen. PCA tests were carried out as follows. Rats were injected intradermally with 0.1 ml. of serum diluted in buffered saline 1/2, 1/3, or 1/5 to 1/2,000. Forty-eight hours later, 0.1 ml. of worm antigen mixed with 0.9 ml. 1 per cent Evans blue dye was injected intravenously. The rats were killed with ether 30 min later, skinned, and the reflected surface of the skin examined for extravasated dye.

Before evaluating the antibody response of thymectomized rats infected with *N. brasiliensis*, a preliminary experiment was done to test the effect of thymectomy on the course of primary infection. It was found that thymectomy did not inhibit establishment of the infection or egg production by the worms (Fig. 1). The typical pattern of spontaneous termination of egg production and elimination of worms from the small intestine was delayed, however, by a few days in thymectomized as compared with sham-operated and control rats.

The anaphylactic antibody response of thymectomized and sham-operated rats following a primary infection with *N. brasiliensis*, and a challenge infection 1 month later, was determined by PCA tests as already described. Anaphylactic antibody was not demonstrable in the sera of uninfected rats. Four weeks after primary infection only two out of ten thymectomized rats had PCA titres as high as 1/80, whereas seven out of ten sham-operated rats had PCA titres of 1/80 to 1/320 (Table 1). Six days after the second infection, five out of eight thymectomized rats showed no increase in PCA titre, while the three others showed increases in titre ranging from 1/30 to 1/450; residual thymus was found in two of the latter three animals. In contrast, seven out of eight sham-operated rats bled 6 days after the second infection had increased PCA titres (maximum 1/1,000). Thus, the PCA titres of the thymectomized rats were lower than those of the sham-operated rats both before and especially after a second infection.

Table 1. RECIPROCAL OF PCA TITRES OF SERA FROM THYMECTOMIZED AND SHAM-OPERATED RATS AFTER A FIRST AND SECOND INFECTION WITH *N. brasiliensis*

Rat No.	Treatment	Titres after		State of thymus
		First infection*	Second infection†	
9	Thymectomized	<2	<2	None
1		<3	<5	None; rat wasted
14		<3	Not done	None; rat wasted
3		<3	10	None
4		<3	75	Some present
8		3	30	Some present
10		27	30	None
6		64	Not done	None
7		80	<3	None
21		80	450	Not done
15	Sham-operated	3	320	Intact
18		9	300	Intact
19		10	81	Intact
22		80	Not done	Intact
20		80	320	Intact
2		100	320	Intact
17		250	450	Intact
16		>250	1,000	Intact
23		>250	Not done	Intact
		320	320	Intact

\* Four weeks after first infection.

† Six days after second infection.

Observations were also made on the output of eggs in the faeces of thymectomized and sham-operated rats after a second infection with *N. brasiliensis* (Fig. 2). Thymectomized rats infected for the second time passed few eggs, and sham-operated rats passed none; the output of eggs

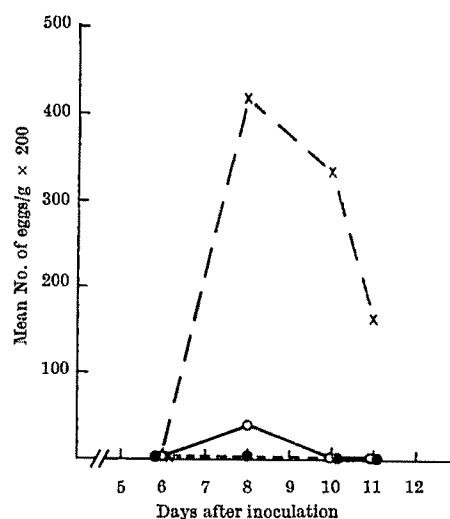


Fig. 2. Counts of the number of eggs/g of the faeces of groups of thymectomized (○) and sham-operated (●) rats following a second infection with 2,500 larvae of *N. brasiliensis*. Normal rats (×) were given a primary infection as a control.

was normal in rats given a primary infection with larvae from the same batch. The low output of eggs in the thymectomized rats was taken to indicate strong resistance to the second infection because, as already demonstrated (Fig. 1), thymectomy does not inhibit the production of eggs in a primary infection. Thus, despite relatively low titres of circulating anaphylactic antibody, thymectomized rats resisted a second infection almost as well as did sham-operated rats which had relatively high titres of such antibody. These results do not exclude the possibility that anaphylactic antibody bound to tissue plays a part in resistance to reinfection with *N. brasiliensis*.

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## HAEMATOLOGY

### Reduction of Methaemoglobin in Haemoglobin Samples using Gel Filtration for Continuous Removal of Reaction Products

EVEN a small degree of oxidation of haemoglobin to methaemoglobin, such as occurs in the transport and storage of frozen red cells, interferes with the study of its oxygen dissociating properties. When abnormal are separated from normal haemoglobins, even more methaemoglobin accumulates during the manipulations involved. The methaemoglobin may be efficiently reduced by passing a haemolysate of such a partly oxidized

sample by means of gel filtration through a band of reducing agent. Advantages of this method are that the methaemoglobin constantly encounters fresh reducing agent and that the continued elution quickly removes the haemoglobin formed from excess reagent. These factors are important when reduction is by dithionite, as side reactions can then destroy the haemoglobin. A similar principle can be applied to the oxidation of cytochromes<sup>1</sup>, and to the conversion of the aldimine form of aspartate aminotransferase (*E.C.* 2.6.1.1) into the amino form by transamination with an amino-acid<sup>2</sup>. The continuous removal of the  $\alpha$ -oxoacid formed is particularly important in the latter case because the position of equilibrium is unfavourable to formation of the amino form.

The efficacy of the method can be demonstrated with the methaemoglobin formed by oxidizing a fresh haemolysate. A column of 25 x 1.5 cm of cross-linked dextran gel particles ('Sephadex G-25', fine) was equilibrated with 20 mM phosphate buffer of pH 7.0. A solution of 12 ml. sodium dithionite in 1 ml. of this buffer was run on to the column, followed by 0.2 ml. of buffer. A sample of 2 ml. haemolysate (about 5 per cent haemoglobin) was oxidized to methaemoglobin with about 3 mg of sodium ferricyanide and was added in turn to the column. The haemoglobin was eluted with buffer. The course of the reactions was easily followed by observing the colour changes. The methaemoglobin separated from the ferricyanide which it left behind as a more slowly moving yellow band. As the protein entered the dithionite zone the brown methaemoglobin was reduced to purple haemoglobin. It then turned orange-red as it moved out of the dithionite and bound the oxygen dissolved in the buffer farther down the column. As the volume of buffer thus depleted of oxygen increased, the change of colour from purple to orange-red moved away from the dithionite band. Oxygenation was completed before the haemoglobin was collected from the bottom of the column. The whole procedure took less than 20 min.

Haemoglobin treated in this way showed an absorption spectrum from 450 to 700 m $\mu$  indistinguishable from that of the untreated haemoglobin. Three determinations of the degree of oxygenation of the treated haemolysate at each of two fixed oxygen partial pressures, at 38° C, showed that this property was unaffected by the treatment. The rate of formation of methaemoglobin at 38° C and pH 7.0 was similar in treated and untreated samples. These experiments showed that haemoglobin suitable for study of its properties had been simply regenerated from methaemoglobin.

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### Platelet Electrophoretic Behaviour in Thrombasthenia

Blood platelets from patients with thrombasthenia do not adhere to glass in either a whole blood system or in a platelet-rich plasma system to which adenosine diphosphate (ADP) has been added, and they fail to adhere to each other in the presence of ADP, noradrenaline, thrombin, or connective tissue extract<sup>1,2</sup>. The electrophoretic mobility of thrombasthenic platelets has been shown to be normal<sup>3</sup>, but hitherto the changes in mobility which normally occur in the presence of aggregating agents<sup>4</sup> have not been studied in thrombasthenia.

We have investigated two thrombasthenic patients, a boy aged 12 years and a girl aged 16, using the technique

described by Hampton and Mitchell<sup>4</sup>. (The boy was Case 1 in a previous report<sup>1</sup>; we thank Dr. Katharine Dormandy for permission to study the other patient.) Platelet electrophoretic mobility was measured on samples of platelet-rich citrated plasma (PRCP) diluted ten times with platelet-poor plasma (PPP).

The platelet electrophoretic mobility of the two patients was 1.23 and 1.24  $\mu$ /sec/V/cm, respectively, and in each patient the mobility was the same before and after contact with a non-siliconized glass surface<sup>5</sup>. In neither case was there any change in mobility after incubation with ADP (added to give final concentrations of 0.05–5.0  $\mu$ g/ml.), noradrenaline (final concentrations 0.05–0.5  $\mu$ g/ml.) or a connective tissue extract.

These are the first subjects we have investigated in whom changes in platelet mobility could not be induced by glass contact or by these agents; the changes in the mobility of normal platelets can, however, be abolished by compounds which interfere with sulphhydryl groups<sup>6</sup>. In the presence of sulphhydryl blocking agents, normal platelets behave like those from the two patients with thrombasthenia, and when additional SH groups are provided by adding *L*-cysteine the normal electrokinetic behaviour of the platelets with aggregating agents is restored. Extra sulphhydryl groups were therefore made available to the thrombasthenic platelets by incubation of the PRCP–PPP mixture with 10<sup>-4</sup> molar *L*-cysteine at room temperature for 15 min. This had no effect either on the basic platelet mobility or on the response to ADP.

The PRCP from one of the patients was incubated with fresh PPP from a normal subject for 30 min at room temperature: the platelets remained unresponsive to ADP.

It has been suggested<sup>4</sup> that the increase in surface charge which is normally induced by low concentrations of aggregating agents results from the adsorption of ADP by the platelets: this can be exogenous ADP which has been added to the suspending medium or endogenous ADP released from within the platelet by noradrenaline, thrombin, or collagen. As we found no electrokinetic response to either exogenous ADP or to noradrenaline in the two patients, and as the release of ADP from thrombasthenic platelets has been shown to occur normally<sup>2</sup>, we conclude that in thrombasthenia there is a defect in the binding of ADP to the platelet surface. Because the thrombasthenic platelets had the same mobility before and after glass contact, this conclusion is also consistent with the previous suggestion<sup>5</sup> that in normal subjects the reduction of platelet mobility on contact with a non-siliconized glass surface arises from the removal of adsorbed ADP. Our results also provide further evidence that the change in electrophoretic mobility induced by aggregating agents is due to a change in surface charge and not to a change in shape or size of platelets, for shape changes in response to ADP have been shown to be normal in thrombasthenia<sup>7</sup>.

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## PATHOLOGY

Effects of Oestrone-17 $\beta$ -thiol on the Endocrine Organs of the Castrated Female Rat

THE biological properties of oestrone-17 $\beta$ -thiol (3-hydroxy 1,3,5 (10) oestratriene-17 $\beta$ -thiol) were compared with those of oestradiol-17 $\beta$ . Oestrone-17 $\beta$ -thiol has already been synthesized<sup>1-3</sup>, but no report of its biological properties has been published. We have investigated the effect of this compound on the weight of the endocrine organs, vaginal smear, and on adeno-hypophyseal histology in the castrated female rat. Adult animals were ovariectomized 15 days before the start of the experiments. Details of the investigation are given in Table 1a and b. All doses were compared with the same amounts of oestradiol-17 $\beta$ .

At a dose of 10  $\mu$ g/100 g of body weight (twenty to fifty times the necessary dose of oestradiol-17 $\beta$  for maximal uterine growth), no significant effect on the uterus or

vaginal smear could be demonstrated. A greater dose, of 40  $\mu$ g/100 g of body weight, caused an increase in uterine weight and an oestrous vaginal smear. The hypophysis, however, showed only slight change in weight and no effect on the thymus was visible. With the highest dose tested, 200  $\mu$ g/100 g of body weight, practically maximal uterine weight was obtained, but again the thymus maintained the same weight as the control animals, and the difference between the two drugs is highly significant at this level.

On the other hand, the smallest dose (10  $\mu$ g/100 g of body weight) which was ineffective in the production of vaginal epithelium or uterine changes caused the disappearance of castration cells from the adeno-hypophysis.

The results indicate that the sulphhydryl group at the  $\beta$  position of the seventeenth carbon atom of the oestrone molecule has not only reduced the oestrogenic potency, but has also affected other properties generally associated with the oestrogenic molecule such as the ability to

Table 1a. EFFECT OF OESTRONE-17 $\beta$ -THIOL AND OESTRADIOL-17 $\beta$  ON THE WEIGHT OF ORGANS OF CASTRATED FEMALE RAT

	Initial weight (g)	Final weight (g)	Pituitary	Thyroid	Thymus* (mg/100 g of final body weight)	Adrenal*	Uterus*
(1) Control	141	167	4.2	15.5	143	27	36
	137	159	6.2	16.9	125	27	43
	165	186	5.3	17.2	178	33	48
	160	165	5.4	18.0	141	23	39
	157	177	5.0	11.8	257	26	49
	178	204	5.6	12.7	197	28	56
Mean $\pm$ S.E.	156 $\pm$ 7.5	176 $\pm$ 6.8	5.2 $\pm$ 0.3	15.3 $\pm$ 1.0	173.5 $\pm$ 19.8	27.3 $\pm$ 1.3	45.1 $\pm$ 2.9
(2) Oestradiol-17 $\beta$ (10 $\mu$ g)	146	149	6.7	12.7	142	23	158
	160	162	7.1	13.2	104	27	132
	180	178	6.4	12.9	125	29	173
	160	164	6.4	13.9	175	31	188
	173	179	6.4	11.1	139	31	157
	160	163	7.6	12.8	82	30	119
153	162	8.3	12.3	109	31	158	
Mean $\pm$ S.E.	161 $\pm$ 4.3	165 $\pm$ 3.9	6.9 $\pm$ 0.3	12.7 $\pm$ 1.0	125.1 $\pm$ 11.5	29.5 $\pm$ 0.7	155.0 $\pm$ 8.8
(3) Oestrone-17 $\beta$ -thiol (10 $\mu$ g)	147	159	6.3	9.5	229	30	53
	175	200	5.5	9.0	168	24	50
	172	178	5.5	14.0	144	26	48
	159	183	5.4	12.0	252	30	49
	170	178	5.0	11.0	140	29	52
	165	163	6.1	14.0	144	32	52
153	153	6.5	14.0	114	27	58	
Mean $\pm$ S.E.	163 $\pm$ 3.9	173 $\pm$ 6.0	5.7 $\pm$ 0.2	11.9 $\pm$ 2.5	170.1 $\pm$ 19.2	28.2 $\pm$ 1.0	51.7 $\pm$ 1.3

(a) Daily injections of 10  $\mu$ g/100 g of body weight as subcutaneous injections in propylene glycol. Data were recorded 15 days after castration.

\* Student test between (2) and (3) gave  $t=1.69$ ,  $P<0.2$ .

Table 1b

	Initial weight (g)	Final weight (g)	Pituitary	Thyroid (mg/100 g of final body weight)	Thymus*	Adrenal	Uterus
(1) Control	164	163.5	5.7	12	116	32	60.5
	175	180	4.6	19	233	37	54
	147	154	6.4	14	252	34	58
	147	144	4.5	12	153	24	70
	179	186	4.9	13	169	26	52
Mean $\pm$ S.E.	162 $\pm$ 6.7	165 $\pm$ 6.2	5.2 $\pm$ 0.36	14.0 $\pm$ 1.3	185.6 $\pm$ 25	30.6 $\pm$ 2.4	58.8 $\pm$ 3.1
(2) Oestradiol-17 $\beta$ (40 $\mu$ g)	180	141	8.5	16	145	43	145
	150	140	7.5	12	76	40	200
	149	138	7.2	16	177	43	129
	153	135	7.8	11	152	35	183
	167	154	8.8	12	160	32	147
Mean $\pm$ S.E.	156 $\pm$ 3.3	141 $\pm$ 3.2	7.9 $\pm$ 0.30	13.4 $\pm$ 1.0	142.0 $\pm$ 17	38.6 $\pm$ 2.2	160.8 $\pm$ 13.1
(3) Oestradiol-17 $\beta$ (200 $\mu$ g)	181	164	9.1	17	134	37	195
	159	145	8.6	14	123	37	150
	169	156	6.1	18	139	38	99
	177	166	8.0	11	69	34	176
	171	161	8.6	12	106	31	143
Mean $\pm$ S.E.	171 $\pm$ 3.7	158 $\pm$ 3.7	8.0 $\pm$ 0.52	14.4 $\pm$ 1.3	114.2 $\pm$ 12	35.4 $\pm$ 1.3	153.2 $\pm$ 16
(4) Oestrone-17 $\beta$ -thiol (40 $\mu$ g)	159	155	5.4	15	212	35	95
	163	161	6.4	12	166	81	79
	170	164.5	5.7	21	176	28	73
	168	162	6.3	—	148	43	100
	176	173	5.5	14	173	36	88
Mean $\pm$ S.E.	167 $\pm$ 2.9	163 $\pm$ 2.9	5.8 $\pm$ 0.20	15.5 $\pm$ 1.9	175.0 $\pm$ 10	34.6 $\pm$ 2.3	87.0 $\pm$ 4.9
(5) Oestrone-17 $\beta$ -thiol (200 $\mu$ g)	168	156.5	7.6	13	201	36	149
	170	159	7.2	9.4	209	33	158
	163	154	5.8	10	198	37	142
	181	172	6.9	13	199	28	141
	164	153	6.6	14	227	35	115
Mean $\pm$ S.E.	168 $\pm$ 2.5	158 $\pm$ 3.4	6.8 $\pm$ 0.30	11.8 $\pm$ 0.94	206.8 $\pm$ 53	33.8 $\pm$ 1.2	141.0 $\pm$ 7.1

(b) Doses in propylene glycol were of 40  $\mu$ g and 200  $\mu$ g/100 g of body weight.

\* Student test between (2) and (4) gave  $t=1.64$ ,  $P<0.2$ ; between (3) and (5) gave  $t=6.8$ ,  $P<0.01$ .

promote growth of the adenohypophysis and atrophy of the thymus. It is clear that, even when a definite oestrogenic effect was elicited, the weight of the thymus was not affected. The atrophy of the thymus after administration of oestrogen is caused through more than one mechanism<sup>4</sup>; experiments to determine the action of oestrone-17 $\beta$ -thiol on adrenal function and release of ACTH are necessary, even considering that the adrenal gland weight was not affected. The fact that even without showing any oestrogenic effect the drug was able, in small doses, to block the appearance of castration cells in the adenohypophysis, can be of some importance. We have not determined whether this histological picture corresponds to low concentrations of pituitary gonadotrophin.

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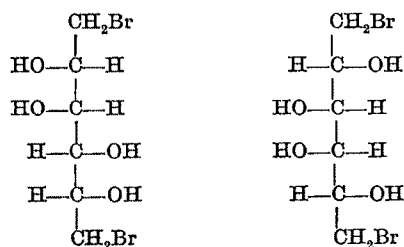
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### 1,6-Dibromo-1,6-dideoxy-dulcitol: a New Antitumoral Agent

AMONG the  $\alpha,\omega$ -substituted sugar-alcohols the dibromo derivatives represent a special group because of their biological properties<sup>1</sup>. The first dibromo-hexitol introduced into clinical use, the dibromo-mannitol ('Myelobromol'<sup>®</sup>), has myelotoxic effects<sup>2</sup>. Its diastereoisomer dibromo-dulcitol (DBD) revealed, in pharmacological experiments, remarkable qualities.

DBD was prepared by treating dulcitol with aqueous hydrobromic acid saturated with gaseous hydrogen bromide at a temperature less than 0° C. On dilution of the reaction mixture with water, crude DBD precipitated to give a yield of about 70 per cent. Recrystallization from methanol gave colourless plates, of melting point 186°–190° C (decomp., corr.). Analysis showed 23.8 per cent carbon, 4.1 per cent hydrogen, and 51.85 per cent bromine. The formula  $C_6H_{12}O_4Br_2$  requires 23.4 per cent carbon, 3.9 per cent hydrogen, and 51.9 per cent bromine.



1,6-Dibromo-1,6-dideoxy-D-mannitol, 'Myelobromol'<sup>®</sup> (DBM)

1,6-Dibromo-1,6-dideoxy-dulcitol (DBD)

In acute toxicity experiments the  $LD_{50}$  is 685 mg/kg for mice, intraperitoneal, 470 mg/kg for rats, intraperitoneal, whereas it is 1,400 mg/kg for rats and 200 mg/kg for rabbits when given perorally. (Because of its poor solubility in water, 1:2,500, the DBD was administered in solution prepared with 'Tween 80'.) A single dose of 10–30 mg/kg markedly inhibits growth of more sensitive tumours (Yoshida, Walker, Shay tumours). A pronounced inhibition of more resistant tumours was brought about by six to ten repeated doses of 100–200 mg/kg. The peroral equivalent of these doses was about twice as much

DBD. Both the individual doses and the total quantity administrable could be raised considerably by giving the dose every second or third day; the side-effects became much milder.

The administration of 200 mg/kg to normal rats resulted in a transient increase of about 30 per cent in the leucocyte count of the peripheral blood which lasted for 12 h, together with the appearance of some immature forms. By 24 h a distinct leucopenia had set in, and reached its maximum, 50 per cent, within 72–96 h. The leucopenia ceased within a fortnight. There was no change in the red blood cells, even after larger doses, while the platelets decreased in number slightly. Optimal treatment did not cause a loss of weight; there was some gain in weight. Post-mortem examination revealed that the spleen was of normal size, the duodenum free of any microscopic lesion, and the inner organs appeared normal. The larger dose of 400 mg resulted in a severe depression of bone marrow: at the time of greatest effect the myeloid elements disappeared almost completely, and the nuclei of megakaryocytes showed lytic and pyknotic changes. Regeneration of myeloid elements commenced 120–144 h after damage. A fairly extensive destruction of white blood cells began in the spleen and lymph nodes after 3–6 h. There was also a large number of disintegrating cells, with

Table 1. GROWTH-INHIBITING EFFECT OF DBD ON VARIOUS TUMOURS

Tumour	Dose, treatment schedule (mg/kg)*	Duration of observation (days)	Tumour weight(g) Controls	Percentage inhibition	No. of animals dead/total†
<b>Mice</b>					
NK/Ly ascites lymphoma	6 × 150/24 h i.p.	8	4.8 11.0	56	0/10 0/10
Ehrlich ascites carcinoma	6 × 150/24 h i.p.	8	2.2 3.2	32	0/10 0/10
Crocker sarcoma	8 × 200/24 h i.p.	10	0.29 0.64	55	1/10 0/10
<b>Rats</b>					
Walker carcinoma	7 × 100/24 h** i.p.	8	1.19 31.25	96	0/11 0/11
Yoshida sarcoma	7 × 200/24 h** oral	8	0.99 31.25	96	1/11 0/11
Yoshida sarcoma	6 × 50/24 h i.p.	9	1.03 15.75	93	0/6
	6 × 25/24 h i.p.	9	0.85 15.75	94	0/6
	6 × 12.5/24 h i.p.	9	1.60 15.75	89	0/6
Yoshida ascites	6 × 6.25/24 h i.p.	9	11.43 15.75	27	0/6 0/6
	7 × 200/48 h i.p.	60		100‡	
Guérin carcinoma	7 × 200/48 h i.p.	14	1.54 13.47	98	0/10 0/10
	7 × 200/48 h oral	21	0.54 22.80	98	0/10 0/10
	5 × 200/72 h i.p.	16	7.24 22.12	67	0/10 0/10
Rhabdomyosarcoma	9 × 200/24 h i.p.	12	0.97 16.15	93	0/10 0/10
Shay acute leukaemia	5 × 150/24 h i.p.	60		75§	

\* Treatment commenced 24 h after inoculation.

\*\* Treatment commenced 72 h after inoculation.

† Weight loss not more than 10 per cent.

‡ Free of tumour after 60 days. Cured 10/10.

§ 75 per cent, with 25 per cent average life span of 28 days (controls 11 days).

pycnotic nuclei, in the duodenum. This effect, however, regressed within 48–72 h and after a further 1 or 2 days the tissue damage seemed to be repaired. In the parenchymatous organs histological investigation showed no damage. Data concerning the effects of DBD on tumour inhibition are given in Table 1.

With the Guérin tumour large doses of DBD resulted in visible cytological alterations by 1–3 h. The chromatin substance showed, under the electron microscope, a rough bundle-like arrangement<sup>3</sup>. Pycnotic derangement soon resulted in necrosis of the tumour (within 72–96 h). By this time there were multinuclear giant cells scattered around the vessels and in the connective tissue at the periphery of the tumour. Mitoses were scarce and occasionally deformed, even in the early stage. Yoshida ascites cells were enlarged in diameter to multiple size. *NK/Ly* ascitic lymphoma cells also showed less enlargement<sup>4</sup>. The same changes also occurred in tissue cultures of HeLa cells, especially with chronic treatment. The nuclei and nucleoli appeared puffy<sup>5</sup>.

The biological and tumour inhibiting properties of DBD differ from those of any other drug which we have so far tested: its cytological effects are obvious very soon and last for a long time, especially in tumours, and it therefore seems advisable to have long intervals between doses. DBD has a wide therapeutic range. The effect on the lymphoid organs and the duodenum fades much sooner than that on the myeloid elements and tumours. Relatively large doses are tolerated when given perorally and the effect seems even greater than that following intraperitoneal administration.

DBD in therapeutic doses provokes leucopenia and minor organic lesions of short duration. Of the cytological findings, cellular, nuclear and nucleolar enlargement is noteworthy, together with the early and long lived reduction of mitosis and a few deformed mitoses.

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### Effect of Histones, Other Basic Proteins and Some Antibiotics on the Transplantability of Mouse Mammary Tumours

UNFRACTIONATED preparations of histones from normal mammalian tissues have been reported to inhibit the growth of transplantable tumours in rats<sup>1</sup>. Incubation of mucous cancer cells of liver with rat liver histone resulted in the inhibition of the growth of tumour by as much as 95.5 per cent. No cell or species specificity was found and there was a similar inhibition with rat thymus or calf thymus histones. Histones have been reported to play an important part in the regulation of RNA synthesis through the inhibition of the DNA-dependent RNA polymerase<sup>2,3</sup>. The ability to suppress DNA-primed RNA synthesis varies with different fractions of histones. Interest, therefore, now centres on histone fractions rather than on whole histone as regulators of gene action. We have examined the effects of whole histone, its two chief fractions and other chemicals on the growth of transplantable mouse mammary tumours.

Histone was prepared from calf thymus according to the method of Laurence *et al.*<sup>4</sup>. The fractions rich in lysine and arginine were isolated according to the methods of Johns *et al.*<sup>5</sup> and Johns and Butler<sup>6</sup>. The lysine-rich fraction was extracted in 5 per cent perchloric acid.

Mouse mammary tumour was induced in Swiss albino mice and maintained in our laboratory. Solid tumours were passed through a tissue press. The material was then suspended in Hanks solution and forced several times through a 20 gauge needle in a 20 ml. syringe into a sterile serum bottle. It was thus possible to obtain a satisfactory homogeneous cell suspension. The chemicals were dissolved in Hanks solution and the pH was adjusted to 7. They were mixed with the cell suspension, in an appropriate concentration and incubated for 2 h at 37°C. A sample (0.1 ml.) of this suspension containing approximately  $2 \times 10^6$  cells was injected into the left hind leg of a mouse. Tumours of 10–12 days growth were collected and weighed. A total of more than 400 Swiss albino mice of 5–7 weeks of age were used in these experiments.

The results of the effects of the basic proteins and other chemicals on the growth of the transplantable tumours are shown in Table 1. It was noticed that the lysine-rich histone, when used in a concentration greater than 5 mg/ml., inhibited tumour growth significantly. For example, at a concentration of 10 mg/ml. it showed 69 per cent inhibition. Whole histone or the arginine-rich fraction produced little or no inhibition. Unfractionated histone at a concentration of 7 mg/ml. showed 34–35 per cent stimulation in tumour growth. Spermine in a concentration of 10 mg/ml. resulted in 35–36 per cent inhibition while protamine and spermidine were ineffective. Polylysine when used in concentrations greater than 7 mg/ml. completely blocked the growth of transplanting tumours. Actinomycin had some effect, as 25 µg/ml. resulted in 59 per cent inhibition, but puromycin showed only 20–21 per cent inhibition at a concentration of 100 µg/ml.

In contrast with earlier observations<sup>1</sup> we found that unfractionated histone had no inhibitory effect on the growth of tumour. On the other hand, some stimulatory effect was observed with total histone (Table 1). Only the lysine-rich fraction reduced growth significantly. It

Table 1. EFFECT OF HISTONES, OTHER BASIC PROTEINS AND ANTIBIOTICS ON GROWTH OF TRANSPLANTING TUMOURS

S. No.	Compound	Concentration (mg/ml.)	Total weight, average of tumours in five mice (g)	Percentage inhibition or stimulation
(1)	Control Hanks solution	—	32.58	0
(2)	Unfractionated histone	3	28.86	–11.39
		5	36.88	+13.22
		7	43.95	+34.92
(3)	Arginine-rich histone	3	26.97	–17.19
		5	29.63	–8.89
		10	28.38	–12.89
(4)	Lysine-rich histone	3	26.66	–18.14
		5	24.79	–23.90
		10	10.09	–69.00
(5)	Protamine sulphate	3	27.85	–14.49
		5	26.49	–18.67
		10	32.60	+00.07
		20	35.54	+09.09
(6)	Polylysine	3	13.94	–57.19
		4	7.18	–77.96
		5	3.32	–89.78
		7	0.75	–97.68
(7)	Spermine	10	0.00	100.00
		3	25.48	–21.77
		5	21.54	–33.88
		10	20.87	–35.93
(8)	Spermidine	3	36.73	+12.76
		5	32.60	00.00
		10	30.91	–5.12
(9)	Actinomycin-D	(µg/ml.) 10	24.13	–25.03
		25	13.35	–59.00
(10)	Puromycin	25	31.50	–3.31
		100	25.84	–20.67



is likely that inhibition of growth of transplanted tumours in this case is due to the ability of lysine-rich fraction to repress the synthesis of DNA-primed RNA synthesis. This is supported by the fact that actinomycin-*D* and polylysine, well known suppressors of RNA synthesis, were also found to inhibit tumour growth. Our results are consistent with the report that the lysine-rich fraction of histone is most effective in repressing RNA synthesis while other fractions do so to a lesser degree<sup>2</sup>. Our results, however, are at variance with those which suggest that the arginine-rich fraction is a more effective inhibitor of RNA synthesis than the lysine-rich fraction in isolated calf thymus nuclei<sup>3</sup>. We also found that polylysine at concentrations more than 7 mg/ml. totally blocked the growth of transplanted tumours while protamine was not effective even at a concentration of 20 mg/ml. It is still difficult to suggest a mechanism for the striking difference between the effect of the lysine-rich fraction on the one hand, and the arginine-rich fraction and total histone on the other, in repression of tumour growth. It could be caused, as was already suggested<sup>2</sup>, by conformation variations of DNA-histone complexes which result from different fractions.

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### Quantitative Determination of Conjugated Bile Acids in Serum in Acute Hepatitis

LITTLE information is available about the variation in concentration of bile acid in serum in health and disease. This is mainly a result of methodological difficulties which result from the solubility of bile acids which closely resembles that of other lipids. Serum extracts prepared according to previous techniques often contain small quantities of lipid material which cause errors in the assay of bile acids<sup>1</sup>. Attempts to separate these interfering substances are often associated with considerable losses of bile acids. These difficulties explain discrepancies in values for the concentration of normal bile acid in serum which have been reported to range between 0 and 60 mg/ml.<sup>2</sup>

We therefore developed a method for quantitative determination of bile acids in serum which permits their assay in their biological forms of taurine and glycine conjugates<sup>3,4</sup>. After the extraction of serum with hot ethanol the bulk of lipids is removed by distribution in ether, ethanol, heptane and water. The conjugated bile acids are separated by thin layer chromatography as shown in Fig. 1. This procedure removes simultaneously bilirubin and the remaining lipids from the spots of bile acid. Reactions with sulphuric acid, salicylic aldehyde, sulphuric acid and acetic acid, and ethyl acetate, acetic acid anhydride and sulphuric acid, respectively, which are performed in the presence of the silica gel then permit the quantitative determination of the different conjugates.

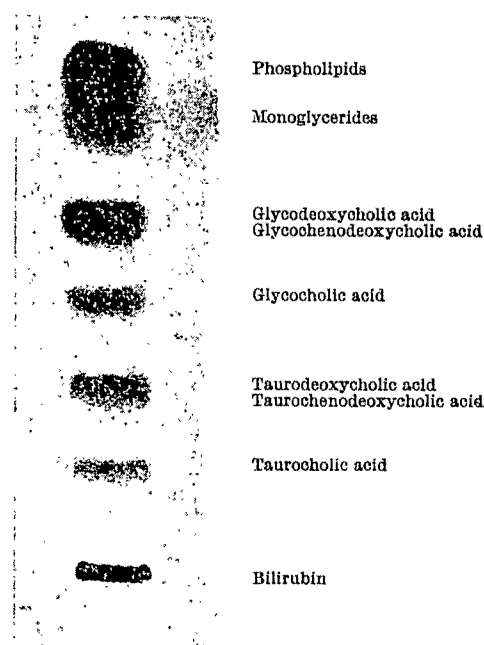


Fig. 1. Thin layer chromatographic separation of serum conjugated bile acids. Silica gel *G* provided the layer; butanol-acetic acid-water (10:1:1, v/v) was the solvent and 5 per cent ethanolic phosphomolybdic acid was the spray.

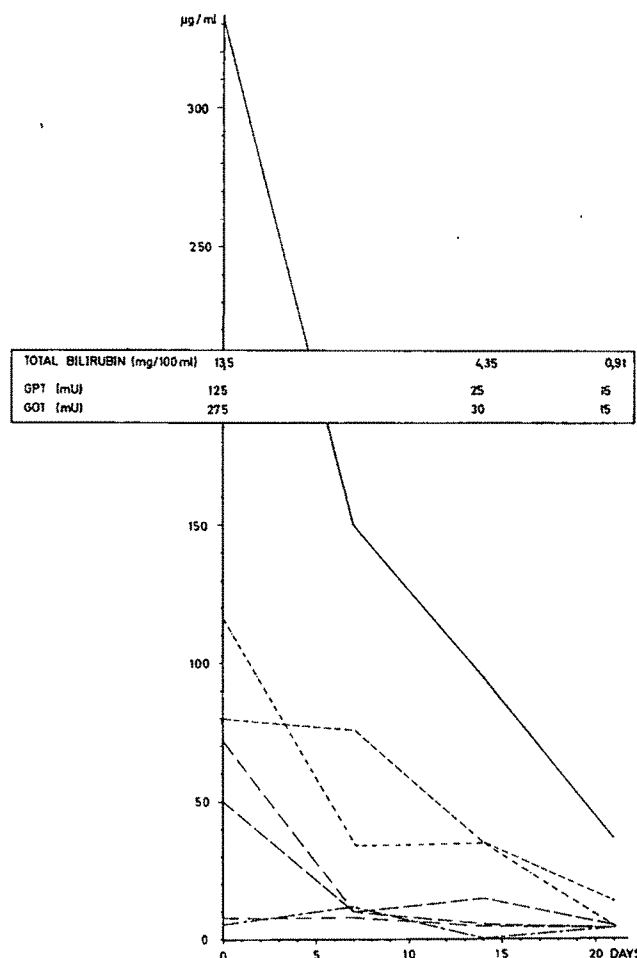


Fig. 2. Values of total and individual conjugated bile acids, bilirubin, and transaminases in serum during the course of acute hepatitis for patient K.R.E., aged 57. —, Total conjugated bile acids; ---, taurocholic acid; ····, taurochenodeoxycholic acid; - · - ·, glycocholic acid; - - - -, glycodeoxycholic acid.

Added pure conjugated bile acids were recovered to nearly 100 per cent. The sensitivity is 0.5 µg/ml. of serum. By this procedure serum from normal individuals contains no conjugated bile acids or only traces which cannot be measured.

Fig. 2 shows the alterations in serum conjugated bile acids in a case of acute hepatitis. There was an interval of 8 days between onset of icterus and the first determination of bile acids. Initially there was a considerable increase in most conjugates mainly caused by increased concentrations of taurochenodeoxycholic and glycochenodeoxycholic acids. The starting value of total conjugated bile acids was 330 µg/ml. of serum. During the period of observation in this patient with a clinically uncomplicated course of acute hepatitis the total conjugated bile acids decreased to 40 µg/ml. of serum. These alterations in concentrations of bile acid in serum were accompanied by similar deviations of concentration of bilirubin and enzyme in serum. Although the latter parameters were normalized at the end of the period of observation, however, bile acids still showed increased values.

This observation could be confirmed in twenty-eight additional patients with acute hepatitis, and in some cases increased concentrations of bile acid in serum on the one hand, and normal bilirubin and enzyme values on the other hand, persisted over several months. Concentrations of total serum conjugated bile acid after normalization of bilirubin and transaminases were 12–130 µg/ml. of serum. Liver biopsy specimens of such cases still showed histological abnormalities.

It is therefore concluded that determinations of conjugated bile acids in serum in the course of liver diseases are superior to other tests in the demonstration of abnormal liver cell function.

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## BIOCHEMISTRY

### Multiple Specificity of Thrombin for Synthetic Substrates

THE original work on the activity of thrombin on synthetic substrates<sup>1</sup> indicated that its hydrolytic action was confined to the arginine esters and amides, but more recent investigations<sup>2</sup> have shown that the specificity of thrombin is very similar to that of trypsin, which hydrolyses both arginine and lysine esters. We have recently reported that thrombin also hydrolyses phenylalanine and tyrosine ester substrates (unpublished results), and we have therefore investigated the enzyme activity of thrombin towards a variety of amino-acid esters, to define more precisely the specificity of the enzyme.

Thrombin was obtained by activation of purified bovine prothrombin in 25 per cent sodium citrate, followed by purification of thrombin and removal of autoprothrombin C by chromatography on DEAE-cellulose columns<sup>3</sup>. The thrombin peak was concentrated as described<sup>4</sup>, dissolved in a 50 per cent solution of glycerol and saline, and the fibrinogen clotting activity was measured<sup>4</sup>. Amino-acid esters were obtained commercially as the hydrochlorides, dissolved in distilled water, and the pH was adjusted to 7.6 before it was made up to volume. The assay mixture consisted of 1 ml. of 0.2 molar *tris* hydrochloric acid buffer, pH 7.6; 0.2 ml. of 0.2 molar substrate; 0–0.2 ml. of thrombin; and 0.9 per cent saline to adjust the volume

to 2.0 ml. Hydrolysis was followed by a modification of the Hestrin technique<sup>5</sup>. Incubation was at 37° C. Control assays were run in the absence of enzyme to measure non-enzyme hydrolysis, and the values were subtracted from those obtained in tubes containing the enzyme.

Table 1 shows the results of the action of a thrombin preparation on a number of amino-acid esters. The results indicate that the specificity of thrombin is not as restricted as formerly believed. Not only are phenylalanine and tyrosine esters hydrolysed, but also tryptophan ethyl ester. These esters are typical chymotrypsin substrates. Their rate of hydrolysis, however, is quite slow compared with that at which substituted arginine and lysine esters are hydrolysed. Methionine ester, which can supply to some degree the necessary structural element for chymotrypsin catalysis, was not hydrolysed by thrombin. Histidine methyl ester is of intermediate activity as a thrombin substrate, and we have found that not only thrombin, but also trypsin and  $\alpha$ -chymotrypsin, catalyse the hydrolysis of histidine methyl ester. With crystalline preparations of trypsin and  $\alpha$ -chymotrypsin, approximately 38 µg of trypsin and 140 µg of chymotrypsin/ml. will catalyse 40 per cent of hydrolysis of histidine methyl ester in 1 h at 37° C at a substrate concentration of 0.02 molar, but 25 µg of thrombin are required. When thrombin is boiled its ability to catalyse the hydrolysis of histidine methyl ester is destroyed, and there is no measurable hydrolysis when 1 mg/ml. of crystalline serum albumin replaces thrombin in the assay mixture.

Current theory holds that the interaction of thrombin (and trypsin) with substrates containing arginine or lysine is facilitated by a positive charge on the guanido- or  $\epsilon$ -amino group of arginine and lysine, respectively, and so it is not surprising that those esters in Table 1 which meet these requirements are the most sensitive to thrombin catalysis. Histidine methyl ester would also belong to this category of positively charged amino-acid substrates, although the number of charged species would be lower at pH 7.6 than with arginine or lysine, because of the lower  $pK_a$  of the ionizable hydrogen of the imidazole group of histidine. It was recently demonstrated that a binding site for aromatic compounds exists at the active site of trypsin<sup>6</sup>, so that thrombin may also possess this capacity for binding aromatic compounds. This could explain how tyrosine, phenylalanine and tryptophan esters show some sensitivity to thrombin catalysis. The other esters in Table 1 apparently do not fulfil either a charge or structural requirement for binding to the active site of thrombin.

It is obvious from the data in Table 1 that substitution on the  $\alpha$ -amino group greatly increases the susceptibility of amino-acid esters to thrombin catalysis. The most

Table 1. HYDROLYTIC ACTIVITY OF BOVINE THROMBIN ON SYNTHETIC SUBSTRATES

Substrate*	Clotting (u/ml.)†	Percentage of hydrolysis (60 min)
Tosyl-lysine methyl ester	3	43
Tosyl-arginine methyl ester	3	41
Benzoyl-arginine methyl ester	3	22
Benzoyl-arginine ethyl ester	3	22
Arginine methyl ester	45	37
Benzoyl-histidine methyl ester	60	(44)‡
Histidine methyl ester	120	45 (29)‡
Lysine methyl ester	150	38
Tryptophan ethyl ester	650	21
Tyrosine ethyl ester	650	18
Phenylalanine methyl ester	650	16
Methionine methyl ester	650	0
Proline methyl ester	650	0
Serine methyl ester	650	0
Aspartic acid $\beta$ -methyl ester	650	0
Cysteine ethyl ester	650	0
Glycine methyl ester	650	0
Leucine ethyl ester	650	0

\* All substrates were tested at a final concentration of 0.02 molar. Except for DL-methionine methyl ester, the substrates were L isomer compounds.

† Clotting u/ml. refers to the amount of thrombin used/ml. of assay media. One clotting unit is that amount of thrombin which converts fibrinogen to fibrin in 15 sec under certain specified conditions<sup>4</sup>.

‡ Figures in parentheses indicate percentage of hydrolysis in 60 min where the concentration of ethanol in the assay system was 10 per cent.

notable example is the effect of the introduction of a *p*-toluenesulphonyl group on the  $\alpha$ -amino group of lysine, which increases the susceptibility to hydrolysis by about a factor of fifty, in these conditions of assay. Benzoyl-L-histidine methyl ester is also more susceptible to hydrolysis than histidine methyl ester, but because of the insolubility of benzoyl-L-histidine methyl ester in completely aqueous solutions, comparisons had to be made in a medium containing 10 per cent ethanol.

Certain amino-acid esters inhibit aggregation which is induced by adenosine diphosphate in blood platelets in an apparently competitive manner<sup>7</sup>. There is striking qualitative and quantitative similarity between those amino-acid esters which inhibited aggregation induced by ADP in platelets and the esters which we have found to be hydrolysed by thrombin preparations. The only exceptions are cysteine ethyl ester and aspartic acid- $\beta$ -methyl ester. The inhibition exhibited against ADP-induced aggregation of platelets by cysteine ester is probably due to its free sulphhydryl group rather than its ester linkage, since cysteine also inhibited, and inhibitory activity disappeared if the free sulphhydryl group was blocked. We have not detected hydrolysis of aspartic acid- $\beta$ -methyl ester by thrombin, while Salzman and Chambers found the ester to be of moderate activity as an inhibitor of ADP-induced platelet aggregation.

It is not yet known whether thrombin is the enzyme involved in the aggregation of platelets by ADP. It may be another enzyme similar in specificity to thrombin, but there is much evidence to indicate a relationship between prothrombin activation and degradation of ATP to ADP in haemostatic plug formation<sup>8</sup>, and others have shown that thrombin can liberate ADP from platelets<sup>9,10</sup>, resulting in platelet aggregation, although the mechanism by which this occurs is not known. There is similarity between the amino-acid esters which are hydrolysed by thrombin and those which inhibit ADP-induced aggregation of platelets, and so investigation of compounds which inhibit thrombin esterase activity might also indicate some which inhibit this aggregation as well. We have examined the inhibition of histidine methyl ester hydrolysis by thrombin, and our results are shown in Table 2. Both histamine and adenosine inhibit this reaction, although the effect of the latter is weak compared with those shown by phenethylamine, tyramine and tryptamine. Both histamine<sup>11</sup> and adenosine<sup>12</sup> have been reported as inhibitors of the ADP-induced aggregation of platelets. Agmatine and cadaverine, the decarboxylated derivatives of arginine and lysine, are also effective inhibitors of the hydrolysis of histidine methyl ester which is catalysed by thrombin.

The presence of carboxyl groups completely destroys the inhibitory action of the compounds as shown by comparison of the analogous compounds histamine and histidine. Reduction of the carboxyl group of histidine to histidinol changes it from an inactive to an active inhibitor. Another analogous pair of compounds are

$\epsilon$ -amino caproic acid and cadaverine. There is evidence that a primary amine is not necessary for inhibition in that choline and imidazole are inhibitory, although it has been reported that imidazole did not inhibit the aggregation of platelets in the same system that indicated histamine to be an inhibitor<sup>11</sup>.

Cross-reactivity of proteolytic enzymes to certain substrates is not unique in enzymology, as shown by the fact that  $\alpha$ -chymotrypsin can catalyse the hydrolysis of benzoyl arginine ethyl ester and trypsin can hydrolyse acetyl-L-tyrosine ethyl ester, although the rates at which these reactions occur are considerably lower than with the enzyme for which the substrate is usually considered to be specific. It is not surprising, therefore, that thrombin also demonstrates this cross-reactivity. Thrombin catalyses the hydrolysis of only arginyl-glycine bonds in fibrinogen, but in view of the several reactions in which thrombin is considered to play a part, such as activation of fibrin stabilizing factor, aggregation of blood platelets and release of contractile protein formation of prothrombin derivative and perhaps for the activation of prothrombin itself, thrombin may possibly exhibit multiple specificity depending on the presence of an inhibitor or accelerator and the availability of suitable hydrolysable bonds.

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## Protein Synthesis and Enzyme Response to Contractile Activity in Skeletal Muscle

It was reported earlier<sup>1</sup> that increases in the specific creatine phosphokinase activity in the isolated frog sartorius muscle could be demonstrated in response to repeated isotonic contraction *in vitro*. A similar enzyme response has now been shown to take place when the frog sartorius muscle is made to contract isometrically once every 5 sec for 6 h at 18° C by stimuli of supramaximal intensity from a multi-electrode assembly of the type described by Hill<sup>2</sup>. It is of interest that under conditions in which the creatine phosphokinase activity increased by 30 per cent to 40 per cent, no significant increase in the ATPase of the whole homogenate could be demonstrated when it was measured in 2.5 mmolar ATP, 2.5 mmolar calcium chloride, 100 mmolar potassium chloride, and 100 mmolar *tris*-hydrochloric acid, pH 9.1. In these conditions of assay myosin is probably responsible for most of the ATPase activity, and so it can be concluded that the activity of this myofibrillar enzyme does not respond so readily to repeated contraction as do creatine phosphokinase and other sarcoplasmic enzymes. Preliminary experiments in which the frog sartorius was stimulated isometrically for up to 90 h at 2° C gave evidence of slight increases in the specific ATPase activity in the whole homogenate.

Table 2. INHIBITORS OF THE THROMBIN CATALYSED HYDROLYSIS OF HISTIDINE METHYL ESTER

	0.02 molar	Per cent inhibition* 0.04 molar	0.06 molar
Phenethylamine	42	48	60
Tyramine	33	64	70
Tryptamine	32	—	—
Agmatine	31	50	53
Cadaverine	17	25	38
Imidazole	10	16	—
Histamine	6	11	20
Choline	5	14	22
L-Histidinol	6	7	12
Adenosine	1	5	10

\* Inhibitor compounds were studied in the same assay system as that used for determining the specificity of thrombin toward amino-acid esters. The concentration of histidine methyl ester was 0.02 molar, and inhibition figures represent that after 60 min of incubation at 37° C, compared with the hydrolysis of histidine methyl ester by 120 U/ml. of thrombin in the absence of inhibitor. Inhibitors were adjusted to pH 7.6 before adding it to the assay system.

Related structures which are not inhibitors are  $\epsilon$ -amino caproic acid;  $\gamma$ -histidine; *N*-acetyl-L-histidine; imidazole acetic acid; urocanic acid; and  $\beta$ -phenyl propionic acid.

Table 1. EFFECT OF INHIBITORS OF PROTEIN SYNTHESIS ON THE INCREASE IN CREATINE PHOSPHOKINASE ACTIVITY INDUCED BY CONTINUED ISOMETRIC CONTRACTION IN THE ISOLATED FROG SARTORIUS MUSCLE

Inhibitor	Concentration	No. of muscle pairs	Mean creatine phosphokinase activities (mg of creatine/mg of nitrogen/min)			S.E. of difference	t	P
			Untreated stimulated	Treated stimulated	Difference			
Puromycin	200 mg/ml.	8	0.766	0.538	0.228	± 0.0122	18.73	< 0.001
p-Fluorophenyl alanine	10 mmolar	6	0.652	0.469	0.183	± 0.0079	23.2	< 0.001
Ethionine	20 mmolar	5	1.022	0.676	0.346	± 0.0136	25.4	< 0.001
Actinomycin D	20 µg/ml.	8	0.544	0.537	0.007	± 0.0123	0.833	< 0.44
8-Azaguanine	2 mmolar	6	0.800	0.591	0.209	± 0.0069	1.22	< 0.3
5-Bromouracil	2 mmolar	6	0.635	0.645	-0.010	± 0.0063	1.58	< 0.25

Creatine phosphokinase was assayed<sup>1</sup> on aliquots of whole homogenates of treated and untreated paired sartorius muscles after stimulation once every 5 sec for 6 h. Muscles were maintained at 18° C in oxygenated bicarbonate Ringer, pH 7.4 (ref. 4), containing 0.4 per cent (w/v) glucose. Variations in the concentrations of creatine phosphokinase in untreated unstimulated muscles in the different series are not significant as they are due to seasonal variations and differences in the batches of *Rana temporaria* used.

The time scale of the increase in creatine phosphokinase activity and the general nature of the adaptive response suggested that new enzyme had been synthesized as a result of the continued contractile activity. To test this hypothesis, the effects of various inhibitors of protein synthesis on the increase in the creatine phosphokinase activity observed after repeated isometric contraction were investigated. The summary of the results presented in Table 1 indicates that the amino-acid analogues ethionine and p-fluorophenylalanine, and the antibiotic puromycin, prevented the increase in creatine phosphokinase activity which was observed in stimulated but untreated control muscles. On the other hand, no significant effect on the increase in enzyme activity could be observed when the muscles were treated with actinomycin D, 5'-bromouracil and 8'-azaguanine. In all cases the tensions developed in the treated and untreated paired muscles were not significantly different as judged by the kymograph records. The heat produced<sup>2</sup> and presumably the metabolic activity were therefore similar in the two muscles. It was concluded that under those conditions in which creatine phosphokinase increases were inhibited, the effects could not be ascribed to impaired contractile or metabolic activity as a result of treatment by the inhibitor.

These results are compatible with the view that the increase in creatine phosphokinase observed in normal muscles is caused by the synthesis of new enzyme protein controlled by messenger RNA, which is stable during the 6 h experiment. It is possible, however, that the lack of effect obtained with the inhibitors of RNA synthesis is the result of their inability to penetrate the muscle cell membrane. In view of the experience of other investigators with these inhibitors in different systems and the results presented here with puromycin and amino-acid analogues, this explanation seems unlikely. Control experiments, in which the unstimulated frog sartorius muscles were treated with the inhibitors in otherwise identical conditions to those used during the experiments presented in Table 1, indicated that no significant changes in enzyme concentration were produced by the inhibitors *per se*.

Attempts have been made to demonstrate directly the synthesis of new protein in response to contractile activity by comparing the incorporation of leucine labelled with carbon-14 in the myofibrillar and the combined sarcoplasm and granular fractions of stimulated and resting sartorius muscles. After 1-2 h the level of incorporation of labelled leucine was similar in the myofibrillar and sarcoplasmic fractions, but after more prolonged periods of contractile activity, both fractions, and particularly the sarcoplasmic-granular fraction, contained significantly more counts in the stimulated muscle than in the resting control (Fig. 1). In view of the progress of incorporation with time it seems unlikely that these differences merely reflect a speeding up of the equilibration between the added leucine with the amino-acid pool and proteins in the stimulated muscle. The results illustrated in Fig. 1 suggest that complete equilibration is reached in both muscles within 2 h and that the increased rate of incorporation which is apparent in

prolonged stimulation is a consequence of an increase in protein synthesis which is induced by the repeated contractile activity.

It is possible that the increased creatine phosphokinase activity and the rise in incorporation of leucine which occur during continued contractile activity of the isolated frog sartorius may be related to changes in the non-collagen protein fraction which have been reported by Gutmann and collaborators<sup>6-8</sup> to accompany contractile activity *in vivo*. It is difficult, however, to relate the latter findings to our own, for after tetanic stimulation for some minutes increases in total protein continue for several hours<sup>6-8</sup>. Despite this difference in experimental design it could be significant that the effects observed were maximal 4-6 h after the start of the contractile activity in both cases.

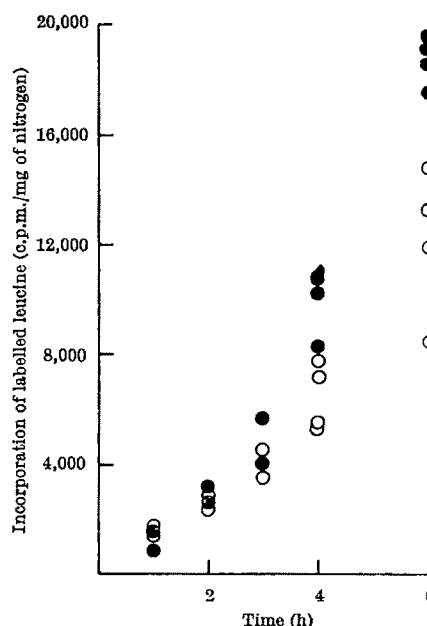


Fig. 1. Comparison of the incorporation of labelled leucine in the total protein of the combined sarcoplasm and granular fraction of stimulated and resting frog sartorius muscles. ●, Stimulated muscle; ○, resting muscle. Paired frog sartorius muscles were maintained in Ringer as described in Table 1. In addition 2 µc. (0.01 µmole) of labelled leucine and 20 µmoles of valine were added to 12 ml. of Ringer solution. Right muscle was stimulated isometrically once every 5 sec for 6 h, and left muscle was unstimulated control. Muscles were immediately homogenized in 10 mmolar potassium chloride, and centrifuged for 15 min at 500 × g. Supernatant was precipitated with 15 per cent trichloroacetic acid and prepared for determination of protein bound radioactivity by a procedure similar to that of Manchester and Young<sup>4</sup>. Total nitrogen was determined by Nesslerization after conversion to ammonium sulphate.

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### A Stain for Sweat Pores

ALTHOUGH the hair follicle is clearly visible on simple inspection of the skin, the sweat pore remains hidden. In the course of chromatographic studies with *o*-phthalaldehyde, we have found a distinctive prolonged surface staining of the sweat pores of ourselves and our technicians<sup>1</sup>. As a result of subsequent study and observation a simple method was developed for selectively staining the sweat pore in light skinned individuals.

A 5 per cent solution of *o*-phthalaldehyde<sup>2</sup> in xylene was applied locally to the skin. Within 2–3 min black puncta appeared at each of the sweat gland orifices (Fig. 1). These stained pores remained in evidence until surface desquamation occurred 5–8 days later.

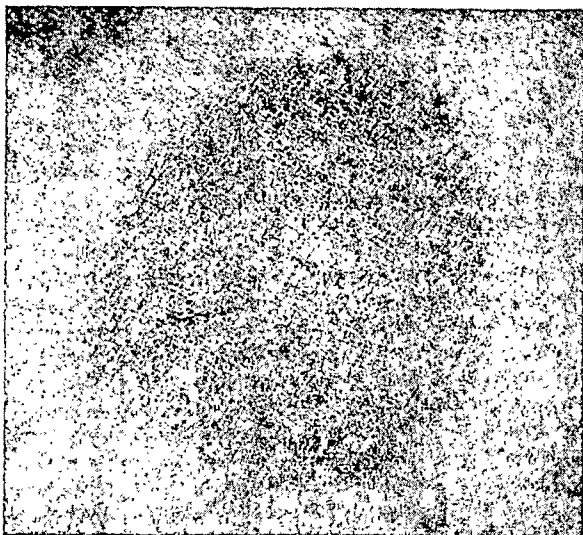


Fig. 1. Black puncta are sweat pores stained selectively by topical application of 5 per cent *o*-phthalaldehyde in xylene on human skin. Staining persists for several days.

Staining does not occur if sweating is completely absent. In the presence of gross sweating, diffuse staining of the stratum corneum develops. Thus optimal poral stains appear in association with low or minimal sweating rates. Although xylene is recommended as the vehicle for the *o*-phthalaldehyde, a variety of volatile anhydrous solvents can be used. Thus, ethyl ether is equally satisfactory. The concentration of *o*-phthalaldehyde may be varied from 1 to 10 per cent without greatly altering the stain. Highly purified *o*-phthalaldehyde has been found to be the most satisfactory of four grades.

Our investigation of the nature of this poral staining has suggested that the black non-fluorescent pigment results from the reaction of *o*-phthalaldehyde with a sweat constituent bound by the sweat pore.

Using paper chromatography, it was found that the addition of *o*-phthalaldehyde to a sweat droplet produced a black stain. Urine and saliva were similarly reactive. Testing known constituents of these secretions revealed that only ammonia produced a black colour in the presence of *o*-phthalaldehyde. It could be detected in dilutions of 1/50,000, a dilution appreciably greater than that found in sweat<sup>3,4</sup>. Further evidence that ammonia was responsible for the stain was furnished by the fact that ammonia shows the same electrophoretic mobility as the constituent in sweat, urine and saliva which is stained black by *o*-phthalaldehyde.

The specific poral staining observed seems to reflect the localizing effect of the sweat, as water is required for an ammonia-*o*-phthalaldehyde condensation to occur. It probably also reflects a greater concentration of ammonia in the keratin of the sweat pores.

This simple method of staining pores has been found useful as a site marker, as the black puncta persist even when the skin is washed. Furthermore, the puncta afford a means of measuring the rate of shedding of the poral horny layer. There seems to be no available method for bleaching or dissolving this black pigment, but 'Scotch' tape strippings or abrasive rubbing will remove it mechanically. Finally, this stain technique has been found useful in the study of disease. As an example, the healed plaques of psoriasis fail to show any staining, again demonstrating that no functional sweat pores exist in these areas.

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### Bioassay of the Red Chromatophore Concentrating Hormone of the Crayfish

THE colour of many crustaceans changes in response to variations in the illumination and colour of their surroundings. These changes are brought about by the movement of pigment granules within special epidermal cells or chromatophores, and are controlled by substances liberated by neuro-secretory cells<sup>1–3</sup>. So far, however, no substance or substances responsible for the movement has been isolated in a pure form, although the preparation of extracts of enhanced activity has been reported by many authors<sup>4,5</sup>.

Crustacean eyestalks, generally considered to be rich in red concentrating hormone, have been used as a source of these extracts. We have found, however, that the activity, in units per weight of tissue, of the eyestalks of the sea water crayfish, *Jasus lalandei*, is not more than twice the activity of the ventral thorax, consisting of the thoracic exoskeleton, the legs, gills and the thoracic nervous cords. This portion of the animal, available commercially in a frozen state, has been used as our source of extracts of red concentrating hormone.

In the fresh water crayfish, the highest concentrations of red concentrating hormone are in the circumoesophageal connectives and tritocerebral commissures, while the thoracic cords also show substantial activity<sup>6</sup>. This may also be the case in the sea water crayfish. The portion of the animal containing the tritocerebral nervous tissues, however, is not well preserved by freezing and thus large scale extractions so far have had to be limited to thoracic material.



To measure the red concentrating activity of eyestalk extracts Östlund and Fänge<sup>6</sup> introduced the "Leander unit". They defined this as the smallest amount of hormone that will cause a distinct blanching of eyestalkless specimens of *Leander adspersus* within 20 min. Such a unit is difficult to measure precisely. We have studied the effect of highly purified *Jasus* red concentrating hormone at various concentrations on the red chromatophores of several crustaceans in an attempt to formulate a better activity unit and to find a convenient test animal. To determine the degree of concentration, the chromatophores of the upper tail fan were examined microscopically ( $\times 80$ ). The chromatophores were examined using the scale of Hogben and Slome<sup>7</sup>, in which stage 1 corresponds to complete concentration and stage 5 to full dispersion of pigment. The response of Australian fresh water crayfish or yabbie, *Cherax destructor*, is shown in Fig. 1.

The chromatophore stage reached at 15 or 30 min could not be accurately correlated with the concentration of the extract, but the plot of the logarithm of the area between control and experimental curves against the logarithm of extract concentration is approximately a straight line (Fig. 2). As the chromatophore stage value of control animals, injected with saline, remains reasonably constant, the area or activity value ( $A$ ) may be calculated from the following expression

$$A = 20[nV_0 - (V_1 + V_2 + \dots V_n)]$$

where ( $V_0$ ) is the control stage value and ( $V_1, V_2, \dots V_n$ ) are the values measured at equal intervals of time ( $t$ ) in hours after injection of the test solution and ( $n$ ) the number of intervals of time required for the chromatophores of the test animals to return to the stage value of the controls. For most animals, accurate values for ( $A$ ) are obtained when ( $t$ ) is equal to 15 min.

The value of twenty was chosen so that the amount of hormone which will just effect complete concentration of the red pigment in 15–30 min (see lowest curve, Fig. 1) has an activity value of approximately 100. The correlation of the activity value with hormone concentration enables the concentration of the red concentrating hormone to be determined and expressed in arbitrary units/ml. of dissolved substance. A convenient unit is the amount of hormone dissolved in 20  $\mu$ l. of Van Harreveld's saline<sup>8</sup> which on injection into a specimen of *Cherax destructor* of approximately 5 cm body length and adapted to a dark background ( $V_0 = 4.5-5$ ) has an activity value of 100. As 0.1 of this *Cherax* unit with an activity of 40 produces distinct blanching, 0.1 *Cherax* units probably approximates to one Leander unit.

As *Cherax destructor*, common in Australia, is not universally available, Table 1 tabulates the effect of

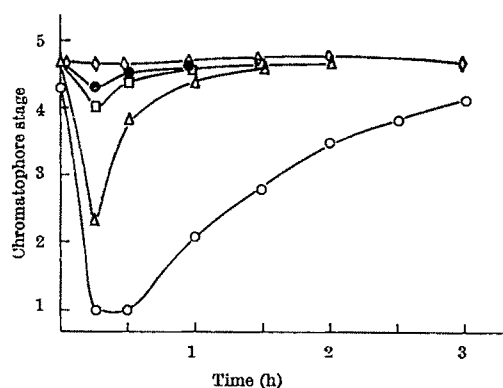


Fig. 1. Effect of *Jasus* extract of various concentrations on red chromatophores of *Cherax destructor*. Units of *Jasus* extract per dose and activity values:  $\circ$ , 1.0, 115;  $\Delta$ , 0.1, 23;  $\square$ , 0.01, 8;  $\bullet$ , 0.001, 4;  $\diamond$ , control, 0.

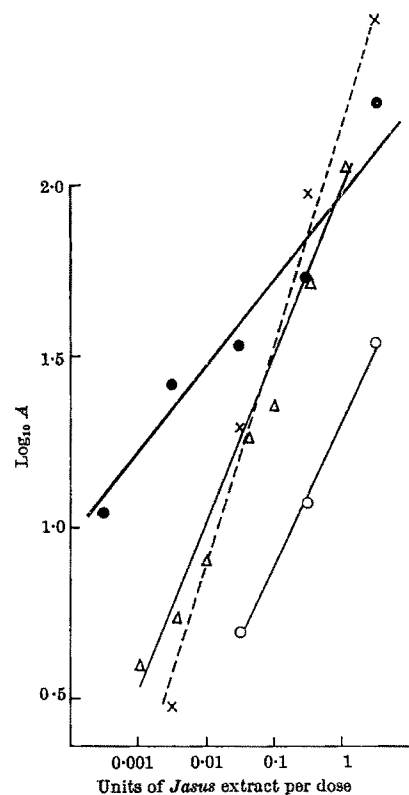


Fig. 2. Effect of *Jasus* thorax extract on chromatophores of various crustaceans.  $\bullet$ — $\bullet$ , *Uca pugnator*;  $\times$ — $\times$ , *Procamburus simulans*;  $\Delta$ — $\Delta$ , *Cherax destructor*;  $\circ$ — $\circ$ , *Pandalus danae*.

standard *Jasus lalandei* extract of 4 *Cherax* units per 20  $\mu$ l., activity value 180, on some crustaceans available in the United States. In all cases normal intact animals were used for assays. Strong responses were produced in two other Reptantia. The crab, *Uca pugnator*, showed a response equal to that of *Cherax*. Only the red pigment concentrated, the black pigment not being affected. The crayfish, *Procamburus simulans*, responded even more strongly than did the other two animals. With *Procamburus* the slope of the log-log plot (Fig. 2) is a little steeper than that obtained for *Cherax*, but with *Uca* the slope is less and allowances for this will be necessary to convert activity values to *Cherax* units when these animals are used for assay work. When a crude extract of the excised circumoesophageal connectives and trito-

Table 1. COMPARISON OF EFFECT OF *Jasus* THORAX EXTRACT WITH EFFECT OF CRUDE EXTRACTS OF EACH CRUSTACEAN'S NERVOUS SYSTEM

Crustacean tested	Average weight of animal (g)	<i>Jasus</i> extract †	Activity value		
			Eye-stalk§	Brain§	Connectives and commissures
Isopoda					
<i>Ligia exotica</i> *		0*			
Decapoda, Natantia					
<i>Penaeus setiferus</i>	3–4	2			
<i>Pandalopsis dispar</i>	2.5	20			
<i>Pandalus danae</i>	2.0	40	70		210
<i>Palaeomonetes</i> spp.	0.5	20	100		10
<i>Eualus suckleyi</i>	1.0	10			
<i>Crangon franciscorum</i>	1.0	20, 30*			30, 30*
Decapoda, Reptantia, Macrura					
<i>Cherax destructor</i>	6–8	180	10	50	220 (conn.) 280 (comm.)
<i>Procamburus simulans</i>	1.5	240	2	10	150
Brachyura					
<i>Hemigrapsus nudus</i>	1.5	0*			
<i>Uca pugnator</i>	2.0	170, 0†			100

\* Black pigment of body (tail not affected).

† Black pigment.

‡ Extract concentration 4 *Cherax* units per dose (20  $\mu$ l.).

§ Extract concentration of one organ per dose (20  $\mu$ l.).

cerebral commissures of *Cherax* was tested in *Cherax* itself, a log-log plot with a much flatter gradient was obtained (Fig. 3). This suggests that the active factors of *Cherax* and *Jasus* may be different, or that the crude *Cherax* extract contains substances that inhibit the catabolism of the hormone.

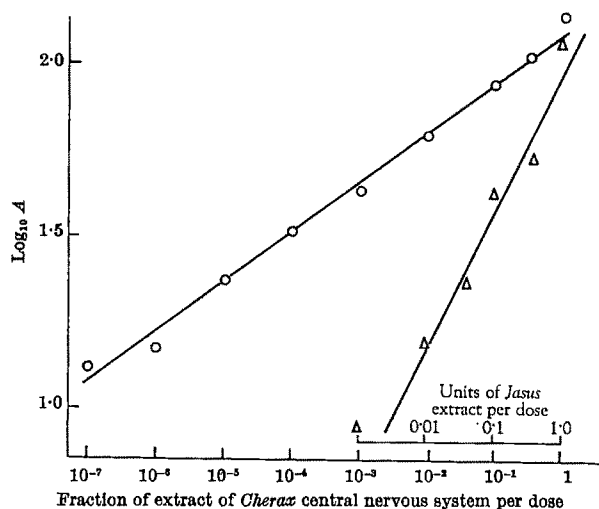


Fig. 3. Comparison of effects of *Cherax* central nervous system extract and *Jasus* thorax extract on *Cherax* red chromatophores. O, *Cherax* into *Cherax*; Δ, *Jasus* into *Cherax*.

In all Natantia (shrimp and prawns) tested, the response was considerably less than that of animals classified as Reptantia. This suggests a distinct difference in chromatophore control between the two sub-orders of the Decapoda. Also, both the black and red pigments of the Natantia tested responded to the *Jasus* extract.

The hormone is present in the source material in extremely small amounts and is unstable, especially in alkaline solution. Large amounts of tissue must therefore be extracted to obtain workable quantities of extract of high activity. In a series of purification steps which commence with the alcoholic extraction of about 1,000 lb. of crayfish and use countercurrent distributions, partition chromatography, and chromatography on 'Sephadex G-25', we have obtained a 5 mg fraction with an activity of 50,000 *Cherax* units per mg, or 0.02 μg = 1 unit. Although only 0.002 μg of this material is required to produce a distinct colour change, thin layer chromatography shows that it is still a mixture of several components. Work is in progress, starting with larger quantities of extract to isolate the hormone in sufficient amount for further purification and structural investigation.

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## Perfusion Fluid for the Scorpion, *Heterometrus fulvipes*

In his work on the cardiac physiology of the scorpion, *Palamnaeus bengalensis* C. Koch, Kanungo<sup>1,2</sup> used a saline solution containing 0.65 g sodium chloride, 0.03 g potassium chloride and 0.03 g calcium chloride in 100 ml. of distilled water and adjusted the pH of the saline to 6.3 with phosphate buffer, according to Maluf<sup>3</sup>. He also studied the effect of pH and temperature on the heart beat of the scorpion and he has shown that the heart remained active between pH 6.1 and 6.5 and the maximum rate of heart beat was observed at 42° C.

The saline prepared and used by Kanungo<sup>1,2</sup> was not, however, satisfactory for use with the scorpion, *Heterometrus fulvipes*. It was found that the constituents and the pH of the saline medium used by Kanungo<sup>1,2</sup> caused the heart to stop beating in this species and it was therefore essential to arrive at a more satisfactory formula.

I have investigated various inorganic and organic constituents of the blood of the scorpion, *Heterometrus fulvipes*<sup>4,5</sup>, and prepared a perfusion fluid on the basis of a biochemical analysis of scorpion blood. The formula for the perfusion fluid was arrived at by converting the concentration of the various constituents of the blood (in mmoles/l. or mg/100 ml.) into g ions/l. The average value of the pH of the blood of the scorpion *Heterometrus fulvipes* investigated is 7.316 ± 0.089 (ref. 5). The quantity and relative proportions of sodium, potassium, calcium, magnesium, chloride, sulphate and glucose present in the blood and also the pH of the blood were considered while making the formula.

Using this method, the perfusion fluid for the scorpion, *Heterometrus fulvipes*, contains 8.596 g sodium chloride, 0.144 g potassium sulphate, 0.532 g calcium chloride, 0.464 g magnesium chloride and 0.500 g glucose in 1 l. of redistilled water. 'AnalaR' grade salts, except glucose, were dissolved in 1 l. of redistilled water and the pH of this solution was adjusted to pH 7.3 by adding 10–15 ml. of pH 7.3 *tris* buffer. The solution was tested and finally adjusted to pH 7.3. This solution (without glucose) was kept in the cold and could be used for 15 days. Before using this perfusion fluid, suitable amounts of glucose were added to it<sup>6</sup>.

The perfusion fluid prepared in this way was found to be more satisfactory for the scorpion, *Heterometrus fulvipes*. The heart, both *in situ* and isolated, maintained a constant beat for considerable lengths of time (that is, for 10–12 h). The duration of activity of the heart was further enhanced by changing the perfusion fluid at intervals. The period of normal heart activity of the scorpion was quite satisfactorily maintained for about 24 h. Normal heart activity was found at pH 7.3, and this value agrees with the normal blood pH of the scorpion, *Heterometrus fulvipes*<sup>6</sup>.

This perfusion fluid is being used to study the effect of various factors on the heart beat of the scorpion, *Heterometrus fulvipes*.

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### Amino-acid Composition of Ascitic Fluid and Blood Plasma from Mice bearing Ehrlich-Lettre Tumour

WHEN an *in vitro* incubation system for amino-acid incorporation and protein synthesis by Ehrlich ascites tumour cells (unpublished results) was in development there was a need to find a natural environmental supply of amino-acids on which a synthetic medium could be based. For this reason the amino-acids of the ascitic fluid around the cells, the plasma of mice bearing ascitic tumour and the plasma of normal controls were analysed. The results give an added insight into the protein nutritional state of the tumour-bearing mouse.

Weanling male Webster Swiss white mice were fed on 'Purina' laboratory chow. Two days after arrival half of the mice were injected intraperitoneally with 0.1 ml. of the Ehrlich-Lettre strain of mouse ascites carcinoma. Seven days later both tumour-bearing and control groups were deprived of food for 4.5 h. Blood was then taken from the subclavian vein of each anaesthetized mouse with a heparinized Pasteur pipette and all the samples from each group were pooled in a heparinized 40 ml. centrifuge tube until 25 ml. of blood had been collected (thirty to forty mice). From this volume of blood 10 ml. of plasma could be obtained. The mice were decapitated, and a suspension of tumour was withdrawn from each animal and placed in separate 12 ml. centrifuge tubes containing heparin. In one experiment tumour was collected at room temperature during a period of 2.5 h. Haemorrhagic samples or those of insufficient quantity were discarded, and of the remaining seventeen samples 1.6 ml. portions of each were pooled in a 40 ml. centrifuge tube. To avoid any loss of amino-acids from the fluid for analysis as a result of cellular uptake during the long period of contact between cells and fluid, a second experiment was carried out with ascitic fluid obtained with the following precautions observed: tumour was collected into ice-cold tubes, centrifuged within 15 min at 0° C and the fluid was immediately decanted. From eighteen samples portions of 0.7 ml. were pooled to provide the 10 ml. of fluid required.

The plasma and ascitic fluid were prepared for quantitative analysis of the amino-acids essentially according to the instructions for preparation of blood samples given in the Beckman Instruction Manual. 'Dowex' 2 × 10 (200–400 mesh) was used for the column. Analysis for free amino-acids was performed in a Beckman Spinco automatic amino-acid analyser. The procedure for blood samples was also found satisfactory for tumour fluid samples.

The results of the analyses of amino-acids of mouse plasma are given in Table 1. The amino-acid composition of normal mouse plasma resembles that of human<sup>1</sup> and rat<sup>2</sup> plasma in general profile. Concentrations of amino-acids in plasma are in most cases lower in mouse than in the human or the rat, with the exception of lysine and arginine, which are higher in the mouse. In the plasma of tumour-bearing mice (compared with normal) there are decreased concentrations of practically all amino-acids. The decrease is particularly noticeable in concentrations of the essential amino-acids, whereas for the non-essentials most decreases are smaller, although aspartic and glutamic acids are even higher than normal.

Table 2 shows the amino-acid composition of ascitic fluid to be entirely different in profile from that of plasma. Many essential amino-acids are in even smaller concentrations than in the tumour-bearing plasma, with glycine, proline, glutamic and aspartic acids in particular greater than in normal plasma. On the other hand, glutamine plus asparagine is extremely low in ascitic fluid, which is of interest in view of the suggestion<sup>3</sup> that the stimulated incorporation of amino-acids into Ehrlich cells in the presence of ascitic fluid is largely a result of its content of glutamine. Incubation of cells in their fluid at room

temperature further increases the concentration of non-essential amino-acids in the fluid, especially alanine, glycine, proline, hydroxyproline and glutamic acid. Concentrations of essential amino-acids in the incubated sample are usually greater than those for ascitic fluid collected at 0° C. The relative constancy of the values for other substances measured in the analysis, apart from the amino-acids required for protein synthesis, contradicted any appreciable discrepancy between two different groups of source animals. It is, however, possible that the cells extrude amino-acids in addition to taking them up, and that this overshadows the preferential uptake (or retention) of essential amino-acids. In the presence of a greater total content of amino-acids, the balance between essential and non-essential amino-acids must be noted, that is, the increase in non-essentials is greater than the increase in essentials.

Table 1. COMPOSITION OF FREE AMINO-ACIDS AND OTHER NITROGENOUS SUBSTANCES IN PLASMA OF NORMAL AND TUMOUR-BEARING MICE

Ninhydrin reacting substance	Normal (μmoles/100 ml.)	Tumour-bearing (μmoles/100 ml.)
Arginine	16.8	4.52
Histidine	6.14	1.14
Isoleucine	6.68	0.98
Leucine	11.6	4.97
Lysine	39.5	23.2
Methionine	3.62	2.93
Phenylalanine	5.27	3.26
Threonine	9.91	6.66
Tryptophan	1.86	0.45
Valine	14.8	8.35
Total essential amino-acids	116.18	62.66
Alanine	38.9	20.4
Asparagine	49.4	41.9
Glutamine		
Aspartic acid	0.78	1.41
1/2 Cystine	4.55	1.50
Glycine	27.6	26.0
Glutamic acid	4.16	7.75
Hydroxyproline	4.13	3.11
Proline	8.74	8.56
Serine	11.5	3.35
Tyrosine	5.0	4.31
Total non-essential amino-acids	154.76	118.29
Total "protein" amino-acids	270.94	180.95
Ornithine	7.06	9.04
Ethanolamine	1.65	2.57
Ammonia	26.0	45.8
3-Methyl-histidine	0.27	0.80
Phosphoethanolamine	1.11	1.77
Taurine	52.4	49.4
Urea	772.0	686.0
Citrulline	7.57	5.18
α-Amino-n-butyric	0.45	0.72
Cystathionine	0.12	0.18

In interpreting the results, it appears on one hand that the tumour draws on its immediate environment, the ascitic fluid, for total amino-acids at a rate that taxes the ability of the tumour-bearing mouse to supply them by diffusion from the plasma. In turn, the plasma itself is deficient in its normal complement of amino-acids as a result of the presence of the tumour. On the other hand, the essential amino-acids are in particular demand, and the normal reaction of the body in such a situation, the breakdown of tissue protein, must take place to replenish the concentrations in the plasma. Since essential amino-acids are removed again at a greater rate than non-essentials, the plasma and in turn the ascitic fluid become more unbalanced. Swendseid *et al.* have used the molar ratio of essential to non-essential amino-acids (*E/N* ratio) in the plasma of humans<sup>4</sup> and rats<sup>5</sup> as an indication of protein nutritional status. An effect similar to the decrease in this ratio associated with insufficient protein diet and an attempt by the body to furnish essential amino-acids by breakdown of protein is observed in comparisons between normal mouse plasma (*E/N* ratio = 0.75), plasma of tumour-bearing mice (0.53) and ascitic fluid (0.36). Similarly, the contact of the ascitic fluid with cells *in vitro* suggests that the tumour cells also break down protein and replenish the medium so as to cause further imbalance of amino-acid composition (*E/N*

Table 2. COMPOSITION OF FREE AMINO-ACIDS AND OTHER NITROGENOUS SUBSTANCES IN ASCITIC FLUID OF EHRlich TUMOUR

Ninhydrin reacting substance	Fluid collected at 0° C (μmoles/100 ml.)	Fluid after being in contact with cells (μmoles/100 ml.)
Arginine	1.86	2.69
Histidine	5.03	7.22
Isoleucine	1.29	4.52
Leucine	2.07	7.49
Lysine	22.1	34.1
Methionine	1.89	3.05
Phenylalanine	2.57	5.60
Threonine	11.6	15.7
Tryptophan	1.53	0.90
Valine	5.39	11.5
Total essential amino-acids	55.33	92.77
Alanine	30.5	60.2
Asparagine	2.43	3.77
Glutamine	2.90	3.08
Aspartic acid	5.60	9.25
1/2 Cystine	58.7	133.5
Glycine	20.6	55.4
Glutamic acid	5.99	12.5
Hydroxyproline	22.2	46.1
Proline	3.71	3.23
Serine	2.84	4.61
Tyrosine		
Total non-essential amino-acids	155.47	331.64
Total "protein" amino-acids	210.80	424.41
Ornithine	4.07	7.10
Ethanolamine	1.50	4.25
Ammonia	33.7	77.2
3-Methyl-histidine	0.54	1.17
Phosphoethanolamine	6.41	8.47
Taurine	85.0	87.1
Urea	829.0	802.0
Citrulline	1.77	2.07
α-Amino-n-butyric	1.47	1.74
Cystathionine	0.12	0.18

ratio = 0.28 after incubation). An alternative explanation could be that loss of amino-acids to the medium causes the cells to lose essential ones to a lesser extent.

The stepwise depletion of concentrations of amino-acid both in plasma and further in ascitic fluid of tumour-bearing mice, and the similar increasing imbalance in the amino-acid "profile", are indicative of the great nutritional demands of the tumour on the plasma and the unsuccessful attempt of the host to replenish the amino-acids of the plasma. It is well known that the tumour grows by obtaining its amino-acids for protein synthesis not only from the diet but from breakdown of proteins in the normal tissues of the host<sup>6</sup>. The selective advantage given to tumours by their great capacity to take up and concentrate amino-acids from the blood, in view of the dynamic turnover of protein and subsequent release of its structural units to the blood, has been pointed out<sup>7</sup>. The data presented here fit in with the known syndrome whereby tumour gains protein at the expense of the host, and further elucidate the relationship between the tumour's uptake of amino-acids from the host and the host's stimulated breakdown of protein.

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## Topochemical Approach in Studies of the Structure-Activity Relation: Enantio-enniatin B

THE interaction of a biologically active substance with a selective receptor (for instance, substrate and enzyme) to cause a certain physiological effect is chemically complex, requiring not only a certain minimum of steric congruity of the molecules but also a given electron density distribution in the interacting functional centres. If either of these requirements is not satisfied, the reaction will either not take place at all or its effectiveness will be diminished by several orders of magnitude. It is thus important to discover the limits of steric and electronic matching, both in the rational search for substances with similar, modified or antagonistic action, and to understand the underlying mechanism.

In order to define these limits it is not always necessary to use the usual technique of changing restricted areas of the molecule step by step. In the case of cyclic peptides and depsipeptides it is possible to change the whole molecule in such a way that the resultant analogues are still very similar to the parent compound, both in the overall spatial arrangements, that is topologically, and also with respect to the electronic nature of the functional groups. The topochemical approach to the structure-activity relation then becomes particularly fruitful.

We began to study the structure-activity problem from the topochemical standpoint with the enniatin antibiotics, in which the essential part played by steric factors (size of the depsipeptide ring, configuration of the hydroxy- and amino-acid residues, etc.) in biological activity had already been shown<sup>1</sup>. The antipode of enniatin B—enantio-enniatin B with the configurations of the amino- and hydroxy-acid residues opposite to those of enniatin B—was chosen as one of the first objects and was therefore synthesized.

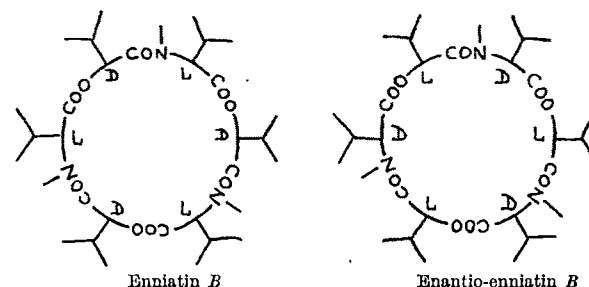


Fig. 1.

Since enantio-enniatin B is the mirror image of the natural product, the stereoselective receptor should behave quite differently towards these two stereochemically non-equivalent molecules. One would not, therefore, have expected enantio-enniatin B to show biological activity; it would have been as groundless as looking for it in antipodes of such naturally occurring peptides as bradykinin<sup>2</sup>, angiotensin<sup>3</sup>, oxytocin<sup>4</sup>, etc., compounds that one may consider to be biologically inactive, provided, of course, no fundamentally different mechanism exists which could render them active (as, for instance, in the case of the *l* isomer of cycloserine<sup>5</sup>). We considered it very likely, however, that enantio-enniatin B would possess biological activity equal or close to that of enniatin B. Indeed, if one turns one of the formulas depicted in Fig. 1 by 60° in the plane of the figure, all the like asymmetric centres coincide, while each ester group will take the place of the *N*-methyl amide group and vice versa. Consequently, relative to the receptor there is only a structural difference between these two antipodes. At the same time the ester group models the amide group both spatially and in the electron density distribution. There should therefore be a close matching of both these topochemically similar antipodes to the same stereoselective receptor and they should possess similar activities. Another ground

Table 1. ANTIMICROBIAL ACTIVITY OF ENNIATIN B AND ENANTIO-ENNIATIN B  
Minimal growth inhibiting concentration ( $\gamma$ /ml.)

Compound	<i>Staph. aureus</i> 209 P	<i>Staph. aureus</i> UV-8	<i>Sarcina lutea</i>	<i>Bac. mycoides</i>	<i>Bac. subtilis</i>	<i>E. coli</i>	<i>Mycob. phlei</i>	<i>Mycob. tuberc.</i>	<i>Cand. albicans</i>	<i>Sacch. cerevisiae</i>	<i>Botrytis cinerea</i>	<i>Nigrospora oryzae</i>
Enniatin B	18	9	18	25-37	35-50	> 50	9-12	4-5-6	9-12	9-12	9	9
Enantio-enniatin B	18	9	18	25-37	35-50	> 50	9-12	4-5-6	9-12	9-12	9	9

for such an assumption was that in a number of cases<sup>6,7</sup> an exchange of amide and ester groups in naturally occurring peptides and depsipeptides without affecting their biological activity had been found.

We synthesized enantio-enniatin B by a scheme analogous to that for the total synthesis of enniatin B<sup>8</sup>. The product was found to be identical to the natural one in all physical and chemical properties except the optical properties (the rotatory dispersion curves were of the same shape but of opposite signs). A test of the antimicrobial behaviour of enantio-enniatin B showed it to possess exactly the same activity both qualitatively and quantitatively towards the organisms investigated as enniatin B (Table 1).

It is noteworthy that this is the first case when a naturally occurring compound and its antipode display absolutely the same biological properties along the entire range of the antimicrobial spectrum, which virtually excludes the possibility of a different mechanism of action.

Our discovery of the biological activity of enantio-enniatin B led to the conclusion that the mechanism of action of enniatin antibiotics depends on the correspondence of their entire molecules to the receptor, which according to the latest data<sup>9</sup> seems to belong to the group of mitochondrial lipoproteins, controlling the active cation transport in the mitochondria.

It should be mentioned that enniatin B and its antipode are an example in which two molecules are very similar, both topologically and in the character of the functional centres. If the latter is retained and the effective volume of the radicals in the depsipeptide molecule is changed, one may easily determine the limits of its topological correspondence to the receptor. At the same time, for a number of biologically active cyclopeptides one may obtain analogues which are identical topologically, but differ considerably from them in the nature or position of the atoms in the functional centres. The topochemical investigation of such analogues, which we are at present engaged in, might shed light not only on the nature of the interaction between the biologically active substance and receptor, but also on the structure of the receptor itself which is of particular importance in studies of enzyme systems. The authors are very grateful to Dr. I. D. Ryabova (Laboratory of Microbiology of our Institute) for carrying out the determination of the antimicrobial activity of enantio-enniatin B.

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## Measurement of Gas Exchange in Woody Plants

METHODS of controlling Dutch elm disease have been investigated at this laboratory for the past four years. Emphasis has been placed on two approaches to the problem of control. One approach has been the search for a suitable chemotherapeutant to control the causal agent, the fungus *Ceratocystis ulmi* (Buism) C. Moreau. The other approach has been the screening of systemic insecticides to control its vectors, the European elm bark beetle, *Scolytus multistriatus* (Marsham), and the native elm bark beetle, *Hylurgopinus rufipes* (Eich). A difficulty was found in methods of measuring the effects of chemotherapeutants and pesticides on diseased and healthy American elms, *Ulmus americana* L., in the laboratory under controlled conditions. A careful survey of existing literature showed that no device existed for measuring the effects of chemotherapeutants, pesticides and disease. Indeed, other workers in these fields have expressed and emphasized the lack of methods of measuring disease in woody plants<sup>1,2</sup>. This led us to investigate respiration as a means of studying the effects of chemotherapeutants and pesticides on woody plants.

It has long been known that injured and diseased higher plants show an increase in respiratory rate<sup>3-5</sup>. Thus, it was assumed that if a diseased plant shows an increase in respiratory rate, then surely, once cured with a chemotherapeutant, it should respond with a decrease in its respiratory rate. Further, any injurious pesticide applied to a healthy plant should cause an increase in respiratory rate. With these assumptions in mind, a preliminary experiment was carried out with a simple device for measuring carbon dioxide, to determine whether the apparatus could readily distinguish healthy, injured and infected branch sections of woody plants.

References to diseased material in what follows are in all cases to branch sections of American elm, *Ulmus americana* L., infected with the Dutch elm disease fungus *Ceratocystis ulmi* (Buism) C. Moreau.

Various methods have been suggested and elaborate equipment constructed for observing gas exchange in plants<sup>6-8</sup>. But none is so simple and inexpensive to set up as the Pettenkoffer method<sup>9</sup>.

The apparatus used in our experiments consisted of twelve gas washing bottles and solenoid valves, an absorption tower, a respiration chamber, a multiple timer and a suction pump. The twelve gas washing bottles and solenoid valves were connected in parallel and then connected in series with the absorption tower, respiration chamber and suction pump. The entire apparatus was operated in total darkness in a growth chamber set at a temperature of 72° F.

Air was passed through an absorption tower containing a strong alkaline solution to remove the carbon dioxide. The air then entered the respiration chamber and, by means of solenoid valves activated by a multiple timer, each washing bottle was in turn switched into the air stream for 2 h. Each gas washing bottle contained 50 c.c. of 0.1 normal sodium hydroxide solution.

Titration was usually carried out after the end of a 24 h run. A 10 c.c. sample was pipetted from each gas bottle and 2 c.c. of a saturated solution of barium chloride was added to the sample to precipitate the carbonates. The solution was then titrated to the phenolphthalein end point.

Live branches of American elm were cut into 15 cm sections and the ends immediately sealed with paraffin.



The sealed branch sections were then sorted into three sets of samples, each set consisting of approximately 625 cm<sup>2</sup> of bark surface.

In each experiment the first sample set was a control. All branch sections in the second sample set were wounded with a girdling cut. The branch sections of the third sample set were wounded with a girdling cut and also inoculated with mycelium taken from a pure malt agar culture of *C. ulmi*. All branch sample sets were "conditioned" in respiration chambers in which air free from carbon dioxide was circulated for 48 h before the experiment. A 24 h run was made of each of the three sample sets before and after treatment as already described. All three sets were then maintained in respiration chambers into which a stream of air free from carbon dioxide was passed continuously. Periodically, during the next 14 days, the respiration level was determined for all three sets. The results for the first 72 h are plotted in Fig. 1.

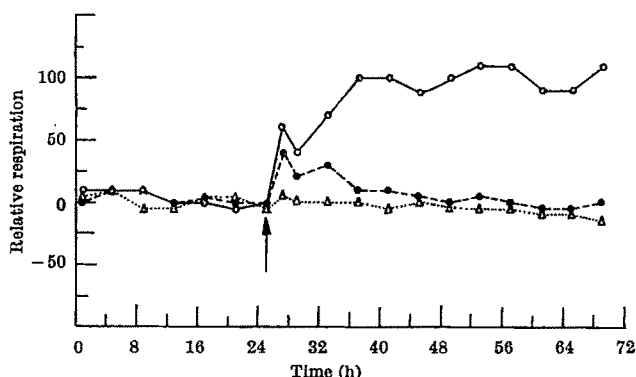


Fig. 1. Relative respiration levels for control (...△...), injured (---●---) and inoculated (—○—) branch section for a 72 h period. Time of treatment is shown by an arrow. The recorded differences could be observed for periods up to 21 days.

It can readily be seen from the figure that the respiration rate for the first 24 h after "conditioning" for all three sets is approximately the same. After wounding however, the respiration rate increases sharply and then slowly falls to its previous level in about 8 h. In the wounded and inoculated set, the respiration rate increased sharply at first and then gradually increased to a maximum and maintained this level, indicating a diseased condition.

Branch sections which were wounded with a girdling cut only developed extensive wound tissue within 1 week. Those branch sections which were wounded and inoculated with mycelium of *C. ulmi* did not, however, develop any wound tissue, but exhibited the typical browning of the sapwood associated with vascular disease of elm.

The rate of respiration of the wounded and inoculated branch sections increased sharply at first and then gradually reached a maximum in approximately 8 h. The initial sharp rise in respiration was apparently caused by wounding, while the secondary increase was due to the presence of the fungus mycelium. It is difficult to believe that hyphae are able to penetrate the sapwood and cause such an extensive reaction in such a short time, and the reaction is therefore taken to indicate the presence of a toxin produced by the fungus. Other workers have shown that toxins are formed by *C. ulmi* in culture<sup>10,11</sup>.

This latter experiment was repeated several times, and a spread of 10.5–40.9 per cent in total respiration was found after inoculation of live branch sample sets from various elm trees. This is to be expected, as not all American elm show the same degree of susceptibility to the Dutch elm disease<sup>12</sup>. Elm branch sections from different trees should thus exhibit varying degrees of reaction when inoculated with *C. ulmi* mycelium.

The prolonged higher level of respiration of diseased branch sections can be thought of as due to prolonged injury. Just as the respiration rate in a wounded branch section returns to approximately its level before wounding, a diseased branch section can be expected to react with a lowering of respiration if the source of the prolonged injury is eliminated or reduced. Chemotherapeutants can therefore be screened in the laboratory by gas-exchange measurements on treated diseased branch sections. Systemic pesticides can be tested in the laboratory for phytotoxicity to woody plants, using respiration changes as a measure of the degree of injury. This approach may eliminate the costly and extensive field tests with chemotherapeutants and pesticides which have not yet proved their value. It should be emphasized, however, that this method of screening chemotherapeutants and pesticides is only a preliminary test which can help to establish desirable concentrations. Positive results in the laboratory will need confirmation under field conditions.

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## Artificial Diet for the Adult Froghopper

THE adult froghopper, *Aeneolamia varia saccharina* (Distant), has been reared in the insectary on pieces of sugar cane leaf<sup>1</sup>, but the use of an artificial diet for the culture of this insect has not been reported. During investigations of the site of feeding and nutrition of *A. varia saccharina* a chemically defined diet (Table 1) was developed, and on this the insect could be successfully reared.

For preparation of the diet the required amount of cholesterol was dissolved in 1.5 ml. of acetone, the solu-

Table 1. COMPOSITION OF AN ARTIFICIAL DIET FOR REARING ADULT *Aeneolamia varia saccharina*\*

L-Amino-acids (mg)†	Vitamins (mg)†	Lipids
Alanine (15)	Biotin (0.01)	Linoleic acid 0.03%
Aminobutyric acid (15)	Calcium pantothenate (0.5)	(v/v)
Arginine (30)	Choline chloride (5.0)	Cholesterol, 2 mg
Asparagine (50)	Folic acid (0.05)	
Aspartic acid (15)	†-Inositol (5.0)	
Cystine (2)	Nicotinic acid (1.0)	
Glutamic acid (35)	Pyridoxine (0.5)	
Glutamine (30)	Riboflavin (0.25)	
Glycine (5)	Thiamine (0.25)	
Histidine (5)	Ascorbic acid (100)	
Isoleucine (2)	Cyanocobalamin (B <sub>12</sub> )	
	0.015% (v/v)	
Leucine (5)		
Lysine (15)	Carbohydrate (g)	
Methionine (1)	Sucrose (4)	
Phenylalanine (1)		
Proline (5)	Mineral salts (mg)	
Serine (5)	Salt-mix (Wesson's) (100)	
Threonine (10)	MgCl <sub>2</sub> ·6H <sub>2</sub> O (50)	
Tyrosine (15)	KH <sub>2</sub> PO <sub>4</sub> (50)	
Tryptophan (5)		
Valine (5)		

\* Quantities of chemicals used/100 ml. of diet.

† Quantities used were based on analyses of extracts of sugar cane stem and leaf tissue<sup>2-4</sup>.

Table 2. LONGEVITY AND FECUNDITY OF ADULT *Aeneolamia varia saccharina* REARED ON PIECES OF SUGAR CANE LEAF AND AN ARTIFICIAL DIET

Medium	Longevity (days)		Female		Average No. of eggs/female	Maximum No. of eggs/female	Oviposition (days) of oviposition period	Average No. of eggs/female/day	Percentage hatch of eggs
	Average	Male Maximum	Average	Maximum					
Pieces of sugar cane leaf (ref. 1)	4.0	7.0	4.9	9.0	52	168	2.8	24	99.6
Artificial diet	5.8	14.0	7.2	24.0	77	295	4.8	16	88.0
			6.7	16.0	90	205	4.3	23	96.7

tion was added to 300 ml. of distilled water and boiled to remove the solvent<sup>6</sup>. The suspension was then remade to the original volume and the required quantities of all the other nutrients were added. The mixture was swirled for 20 min with one drop of 'Tween 20'<sup>6</sup>, boiled for a further 5 min, and the pH was adjusted to neutrality with 0.8 normal potassium hydroxide. The prepared diet was stored in a refrigerator at 4°–6° C and used when required.

Pairs of newly emerged adult insects which were held in cylindrical glass cages 1.5 in. long and 1.2 in. in diameter were given the diet which was impregnated on circular pieces of Whatman No. 1 filter paper placed between two pieces of parafilm stretched over one end of the cage<sup>7</sup>. The diet was renewed, and observations on longevity and fecundity were made every 24 h.

Comparisons of data from twenty-seven adult pairs reared on pieces of sugar cane leaf and the artificial diet are summarized in Table 2. Data previously presented<sup>1</sup> on the longevity and fecundity of thirty-seven female insects collected in the field and reared on pieces of sugar cane leaf are also included for comparison.

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## ANTHROPOLOGY

### Radiocarbon Dating of Bone

SELLSTEDT *et al.*<sup>1</sup> have described a method for the removal of inorganic carbonates from bone so that the residual collagen can be used as a source material for radiocarbon dating, and they conclude that this "offers the archaeologist an elegant and versatile tool which is likely to find wide application".

The statement is not in accord with previous work in this field of which the authors appear to be unaware, and which has indicated that the problem of dating bone protein is, in fact, much more complex than Sellstedt *et al.* would seem to imply. Thus, Münnich<sup>2</sup>, using a method similar to that of Sellstedt *et al.*, was led to the conclusion that the mere removal of inorganic carbonates from buried bone (and also antler) was not in itself sufficient to guarantee a reliable result, because the strongly absorbent nature of the protein in these materials causes it to absorb "younger" organic material from its surroundings, and this cannot be completely removed. Also, Olson and Broecker<sup>3</sup> have described a method for the removal of both carbonates and humic acids from bone and have compared the dates obtained from this treated bone with those obtained from contemporaneous charcoal from the same sites. They obtained good agreement in some cases but not in others<sup>4</sup>. Tamers and Pearson<sup>5</sup> have compared dates obtained from bones which have been digested in strong acid (that is hydrolysed bone protein free from carbonates) with those from associated charcoal

and have also found serious discrepancies in many cases. Finally, Berger and Libby<sup>6</sup> have shown that a simple extraction with alkali will remove a considerable amount of more "recent" humic contamination from bone collagen, but their results do not indicate whether this treatment removes completely all non-contemporaneous contamination derived from humic material or other sources.

These results clearly indicate, therefore, that no general conclusions can safely be drawn about the suitability of bone as a source material for radiocarbon dating. Until a completely satisfactory method is developed for the removal of all non-contemporaneous contamination from bone protein, we must continue to rely on the establishment of criteria by which it is possible to assess the reliability of bone as dating material in any particular situation; as, for example, by direct comparison with more "reliable" organic materials such as wood charcoal<sup>7,8</sup> or bone charcoal<sup>9</sup> from the same archaeological context or even by exploiting the non-homogeneity of bone itself to provide a test for contamination as has been done for antler<sup>10</sup>.

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## BIOLOGY

### Party Game Model of Biological Replication

THE analogy between the party game of Michie and Longuet-Higgins<sup>1</sup> and the reproduction of *E. coli* may not be exact, but it can, I think, be taken a little further.

All biosynthetic reactions need a source of energy, and the processes of cell reproduction involve much biosynthesis. In the same way, the party game requires a source of energy, and this is provided by the guests, who therefore have a far from passive role. They are the immediate sources of energy without which the printed instructions (enzymes) could not cause the assembly of ink and paper (substrates) to produce new forms (cells).

Those forms (cells) which are not reproduced merely fail to utilize a potential source of energy (uncooperative guests).

The limit to the final population can be corrected in the analogy if the previous paragraph is taken into account. There appears to be no limit to the number of forms a guest may copy, and therefore they need not be saturated with forms. What will be saturated as the number of forms increases, however, is this capacity to reproduce new forms, so that the rate of production becomes constant. This is comparable with linear growth on a restricted energy source, for example, of photosynthetic bacteria in a constant light source. Reproduction, of

course, stops when substrate or energy is no longer available.

Alterations can be made to the statements in French (that is, DNA). If they were minor (for example, the omission in Form 1, direction (2) of "un" or "de") the information would still be intelligible. But omission of such words as "papier" or "vingt" would form a nonsense statement. This would correspond to non-lethal and lethal mutations of DNA, respectively.

The statements in English could be wrongly printed. If the errors were small (for example, in Form 1, instruction II, omission or modification of "you" or "a") they would still allow the instruction to be obeyed. Omission of words such as "form" or "copy" would, however, give rise to non-functional statements. This would correspond to the production of modified proteins or enzymes which may be either still functional but perhaps impaired, or completely non-functional.

There may thus be scope in the analogy for evolution.

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### Palaeo-electron Microscopy of Mummified Tissue

THE histology of Egyptian mummified tissues was first described by Ruffer in 1911 (refs. 1 and 2). He was able to demonstrate by light microscopy that some tissues

were well preserved, when rehydrated and embedded in paraffin. Because some skin and muscle from a mummified hand of ancient Egyptian origin dating approximately 600 B.C. recently became available, it was of interest to extend this field to the ultrastructural level with electron microscopy.

The mummified material when unwrapped was dry and brittle, and easily disintegrated. Such material was rehydrated according to Ruffer's method. Small pieces of tissue 1–2 mm in length were suspended for 24 h from a platinum loop in small tubes containing Ruffer solution, consisting of a mixture of 30 ml. absolute alcohol, 50 ml. of distilled water and 20 ml. of a 5 per cent sodium carbonate solution. Following hydration, tissues tended to fragment and therefore had to be handled with great care.

After rehydration, small fragments of tissue were suspended in 0.1 molar phosphate buffer solution for 5 min, and then were fixed for 1 h in 1 per cent osmium tetroxide buffered with 0.1 molar phosphate buffer (pH 7.2). After dehydration in a graded series of ethanol solutions, the tissues were embedded in a mixture of 'Epon' and 'Araldite' (modification of Luft's method<sup>3</sup>). Sections were cut with a glass knife on a microtome and picked up on uncoated grids, stained with lead hydroxide according to the method of Karnovsky<sup>4</sup>, and examined in an electron microscope. Micrographs were taken at initial magnification of 1,400–32,000 and were enlarged photographically as required. Thin sections of plastic embedded tissue were stained with toluidine blue according to the method of Trump, Smuckler and Benditt<sup>5</sup>.

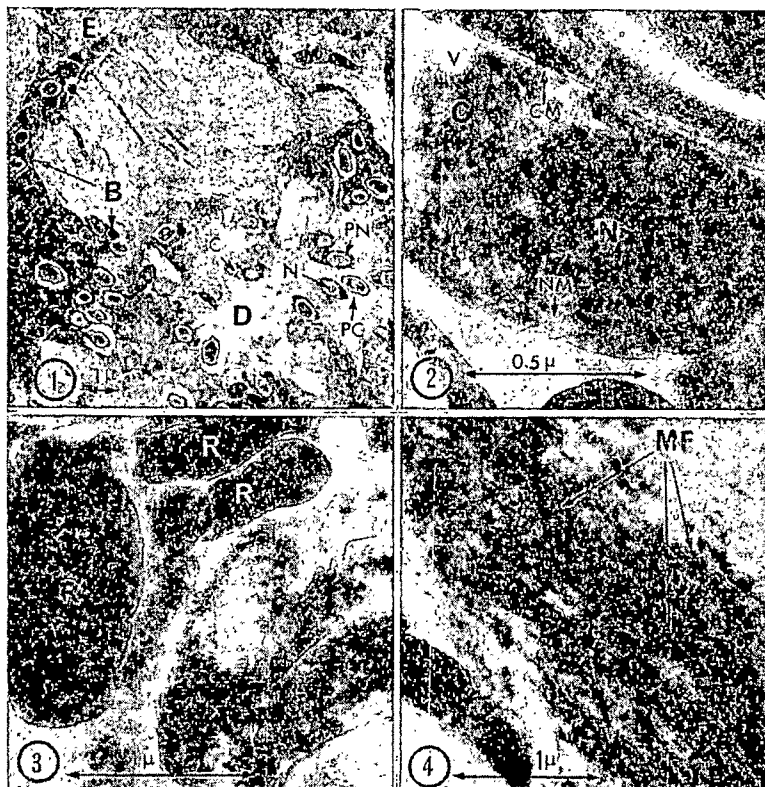


Fig. 1. Low power micrograph of mummified skin. B, Basal epidermal cell layer; D, dermis; E, epidermis; N, cell nucleus; C, cytoplasm; PC, pericellular space; PN, perinuclear space. (Lead hydroxide "stain".)

Fig. 2. Mummified dermal cell. CM, Cell membrane; NM, nuclear membrane; M, cytoplasmic organelle; V, vacuole. (Lead hydroxide "stain".)

Fig. 3. Three non-nucleated cells, possibly erythrocytes (R). (Lead hydroxide "stain".)

Fig. 4. Bundle of muscle fibres. MF, muscle fibres.

The distinct histological features of skin were seen in the embedded sections, stained with toluidine blue. Nucleated cells were most numerous at the epidermal and dermal junctions.

Fig. 1 is a low power micrograph of mummified skin at the epidermal and dermal junctions, with numerous cells mainly in the dermal area. The cells are markedly shrunk with large perinuclear and pericellular spaces. A number of cells with intracytoplasmic vacuoles can also be seen. Fig. 2 is a high power micrograph of a dermal cell with an intact nuclear and cytoplasmic membrane. The nuclear membrane contains some nuclear pores and in an occasional area is double. A membranous organelle, possibly a mitochondrion, is also seen in the cytoplasm, which in addition contains a number of vacuoles of unknown origin. Two of the surrounding cells have pronounced perinuclear spaces. Fig. 3 shows three distinct intact cells which contain homogenous cytoplasm with occasional granules and no nuclei. These cells were surrounded by nucleated cells with perinuclear spaces, and possibly represent red blood cells in a small dermal vessel. Fig. 4 is a section showing a bundle of voluntary muscle fibres from the hand.

It is a credit to the ancient Egyptians that their method of mummification has so well preserved their dead, even at the cellular level. The marked shrinkage of cells observed in the processed tissue may have resulted, in part, from the soaking for 70 days in a brine bath which was commonly used in the mummification process in 600 B.C. (ref. 5). The perinuclear and cytoplasmic spaces are almost certainly artefacts following the crude re-hydration process.

There has recently been an increased interest in the field of palaeopathology<sup>6</sup>, and with the introduction of electron microscopy the examination of mummified material can be extended to the ultrastructural level. The pathology of mummified and even fossilized material may thus be compared with recent pathological specimens, and in the process infectious agents, such as viruses, bacteria and parasites, may be demonstrated.

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### Reflected Light Interference Microscopy of Living and Fixed Biological Surfaces

WHEN malignant and normal mammalian cells were grown directly on metal surfaces, a new technique was developed to photograph living cultures on opaque surfaces<sup>1</sup>.

We considered that modification of an existing technique<sup>2</sup> could be used to photograph biological surfaces other than those of mammalian cells. Linskens and Krner<sup>3</sup> described a method whereby plant surfaces such as

leaves could be examined by means of surface interference microscopy of plastic and gelatine replicas. They state that, because plant surfaces are generally dull and reflect little light, it is difficult to record their surfaces photographically without the use of replicas.

Direct reflected light interference microscopy of the surface of philodendron and sweet potato leaves, by the method described for mammalian cells, did not give satisfactory photographs. A modification of the technique, however, gave superior surface detail both with transparent and semitransparent biological material.

Leaves of various plants were placed loosely between the two coverslips of Rose chambers<sup>4</sup>; a sheet of aluminium foil or a silver mirror surface was inserted behind the specimen so as to permit the light rays to be reflected back by the shiny surface. The Rose chamber was then put together loosely, taking care not to squash the specimen. The leaves were photographed in colour or black-and-white by means of the Nomarski reflected light interference attachment to the Reichert inverted universal camera microscope MeF (refs. 4 and 5). As can be seen in the pictures (Figs. 1, 2 and 3) the contour of the leaf surfaces is readily visible.

Leaf surfaces resting on glass did not photograph satisfactorily. This suggested that the insertion of a mirror or a shiny metal surface behind other biological surfaces might enable us to photograph these directly by reflected light. We chose cell cultures fixed for 1 sec in 10 per cent formalin. Fig. 4 shows KB cells grown on a glass coverslip and photographed by this method.

Investigation of a variety of transparent and semi-transparent biological specimens shows that this technique



Fig. 1. Leaf of *Philodendron hastatus*, lower surface showing guard cell.

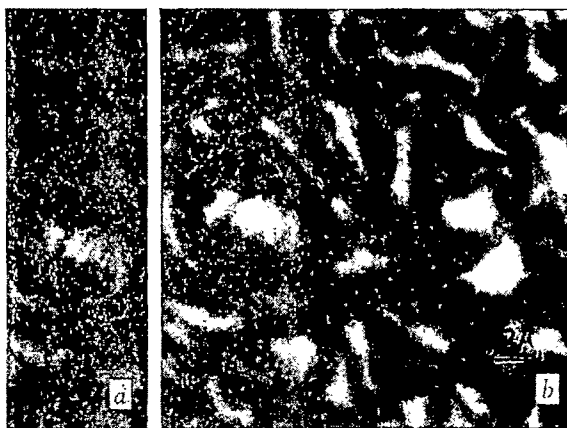


Fig. 2. Leaf of sweet potato (*Ipomoea batatas*), upper surface. a, Details of guard cell.

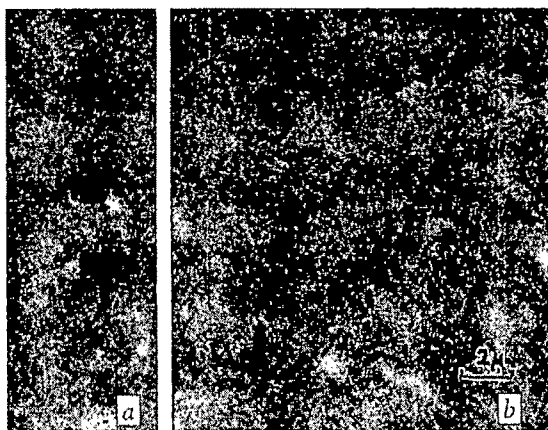


Fig. 3. Leaf of sweet potato (*Ipomea batatas*), showing details of another region of the upper surface photographed in two optical planes.

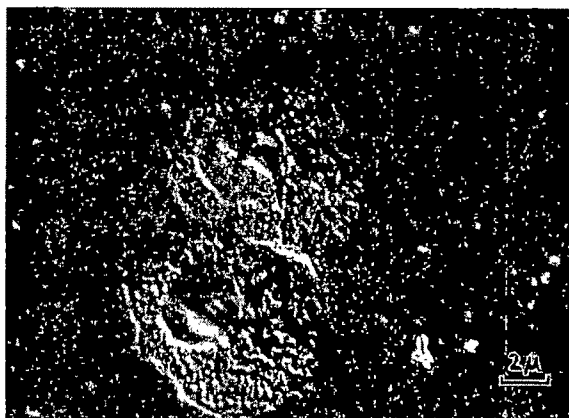


Fig. 4. KB cells growing on a glass surface and photographed by inserting a mirror behind the glass.

may be used for direct photography of many types of plant and animal surfaces. By placing a mirror behind a culture of animal or plant cells growing on a glass surface, this method can be used to record changes in the surface of these cells as they are exposed to changes in their environment.

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### Effect of Water Stress on the Photochemical Activity of Chloroplasts

GROWTH and accumulation of dry matter in plants are dependent on the water status of the photosynthetic tissues<sup>1-3</sup>. Stomatal closure, resulting from the increased water stress in the leaf, is usually considered to be the main cause of reduced photosynthesis<sup>4,5</sup>. Stomatal closure is not, however, a satisfactory explanation for reduced photosynthesis, especially in those cases where it has been

shown that there is no direct relationship between the degree of stomatal opening and carbon dioxide fixation<sup>6,7</sup>. It was suggested that damage by desiccation is caused, at least partly, by structural changes in the cytoplasm<sup>8</sup> and changes in the hydration of chloroplasts<sup>9</sup>. Some evidence showing a good correlation between the degree of hydration of the tissue and its photosynthetic activity in leaves with fully opened stomata has been produced<sup>10</sup>. We have therefore investigated the effect of drought and dehydration on the photochemical activity of chloroplasts.

Leaves of Swiss chard (*Beta vulgaris*—Stiel Mangold—Grüner breitrippiger, Riesen—Mauser Zuerich) were used throughout the experiments. The plants were grown in the greenhouse. Only fully expanded, mature leaves were taken for the experiments. Chloroplasts were isolated in sucrose buffer solution (0.05 molar *tris*, 0.4 molar sucrose, 0.01 molar sodium chloride, pH 7.8) by sedimentation at 1,000 g in the cold. Chlorophyll was estimated according to Arnon<sup>11</sup> and protein according to Lowry *et al.*<sup>12</sup>. Cyclic photophosphorylation mediated by phenazine methosulphate (PMS), and the Hill reaction mediated by ferricyanide or dichlorophenol-indophenol (DPIP), were measured according to Avron<sup>13-15</sup>. Plants were dehydrated by cessation of irrigation until permanent wilting occurred. Sometimes detached leaves were partially dried over calcium chloride.

The photochemical activity of chloroplasts isolated from leaves with different water content was compared. To ensure that the results obtained were caused by photochemical activity of chloroplast and not by the activity of other organelles, such as oxidative phosphorylation of mitochondria, dark controls were run in all the experiments. These dark controls were always negative.

The water content of leaves partially dried in desiccators was measured and chloroplasts were isolated from them. The results for the photochemical activity from some representative experiments are summarized in Table 1. These experiments show clearly that the loss of water from leaves results in a considerable reduction in photophosphorylative and photoreductive activity of the chloroplasts. Severe dehydration (loss of 50 per cent of the water of the leaf) abolishes these activities almost completely.

Table 1. PHOTOCHEMICAL ACTIVITY OF CHLOROPLASTS ISOLATED FROM LEAVES WITH DIFFERENT WATER CONTENT

No. of experiment	Condition of leaves	Percentage water content in leaves	Cyclic photophosphorylation	Photoreduction Ferricyanide	DPIP
1	Turgid	88.6	984	426	119
	Flaccid	71.7	692	290	79.5
	Percentage reduction		29.6	32.0	33.0
2	Turgid	90.2	760	330	138.5
	Flaccid	34.8	158	47	27.5
	Percentage reduction		79.2	85.2	80.6

Results for photoreduction expressed as  $\mu$ moles of ferricyanide or DPIP reduced/mg of chlorophyll/h. Results for photophosphorylation expressed as  $\mu$ moles of ATP formed/mg of chlorophyll/h.

Is this reduction in photochemical activity of chloroplasts, induced by dehydration, a transient effect or is more permanent damage caused? The effect of repeated cycles of drought is of special interest in this respect, and the effect of several such cycles was therefore investigated.

Control plants were irrigated regularly, and wilting never occurred. The treated plants, however, were re-irrigated whenever permanent wilting had occurred. The amount of water given allowed the restoration of full turgidity for 1 day only. The treated plants were exposed to six cycles of drought. Restoration of turgidity in the wilted plants was complete within 12 h of irrigation. The colour of the leaves, however, was not as deep green as that of the control leaves. Chloroplasts were repeatedly isolated from leaves taken from the control plants and from treated plants at the end of the fifth and sixth cycles of drought and again after the wilted plants had been



Table 2. EFFECT OF SEVERAL CYCLES OF DROUGHT ON PHOTOCHEMICAL ACTIVITY OF CHLOROPLASTS

Treatment	Condition of leaves	Percentage water content in leaves	Photo-reduction Ferricyanide	Remarks
Control	Turgid	90.0	387	At the end of fifth cycle of drought
Treated	Flaccid	81.6	242	
	Percentage reduction		37.5	
Control	Turgid	90.0	514	At the end of sixth cycle of drought
Treated	Flaccid	75.8	273	
	Percentage reduction		47.0	
Control	Turgid	90.0	532	After restoration of turgor
Treated	Turgid	90.0	474	
	Percentage reduction		11.0	

Results are expressed as in Table 1.

irrigated and full turgidity was restored. Only photo-reduction was investigated in these experiments. The results are given in Table 2. These results show that the ability of photoreduction in chloroplasts is reduced when the leaves lose their turgor. On restoration of turgor most of the activity is also restored. In other experiments it was shown that photoreduction, on re-irrigation, is restored to a much higher degree than photophosphorylation.

In another experiment the correlation between water content in the soil and in leaf and the effect of water loss on photochemical activity and on the ratio of protein:chlorophyll were investigated. The results are given in Table 3.

Table 3. PHOTOCHEMICAL ACTIVITY OF CHLOROPLASTS AND RATIOS OF PROTEIN:CHLOROPHYLL AS AFFECTED BY PROGRESSIVE LOSS OF WATER FROM THE LEAVES

Treatment	Days after irrigation	Condition of leaves	Percentage water content in soil	Percentage water content in leaf	$\mu$ moles of ferricyanide reduced	Ratio of protein/chlorophyll
Control	0	Turgid	25.4	90.0	339	
Treated (before treatment begun)	0	Turgid	25.4	90.0	335	
Control	0	Turgid	25.4	90.3	320	
Treated	5	Incipient wilting	13.0	80.9	321	
Control	0	Turgid	25.0	90.6	422	2.0
Treated	10	Flaccid	12.4	80.6	264	2.5
Control	0	Turgid	25.4	91.3	466	2.25
Treated	15	Flaccid	10.3	78.4	292	2.23

Control plants were irrigated daily. Treated plants were irrigated at beginning of experiment only.

These results show that large changes in photochemical activity of chloroplasts occur after the soil has been at its wilting point for some time and the plant has been exposed to prolonged water stress (between 5 and 10 days after irrigation). A very slight change in water content of the leaves, at this stage, was accompanied by a large change in photochemical activity. There seems to be a critical point of hydration, below which the photochemical apparatus is damaged.

The more or less equal ratios of protein:chlorophyll in the control and treated plants suggest that it is not the destruction of chlorophyll which causes the damage. A possible cause may be structural changes occurring as a result of the loss of water.

A preliminary experiment was carried out to investigate this possibility. Strips of tissue from turgid and flaccid leaves were fixed in 2 per cent permanganate. They were dehydrated according to the usual procedure, embedded in 'Epon' and prepared for electron microscopy. The control tissue appeared to be completely normal, but the sections from the flaccid leaves showed clear distortion of all the intergranal lamellae.

No precautions were taken to fix the flaccid tissue at the water potential prevailing in the leaf itself. It is possible, therefore, that the distortion of the intergranal lamellae was due to instantaneous rehydration during fixation. It is too early yet to make any assumptions as to the nature of structural changes which take place on

partial dehydration. The different behaviour of the two leaves suggests that such changes do take place.

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### Flavianic Acid as a Hatching Agent for *Heterodera cruciferae* Franklin and Other Cyst Nematodes

MANY efficient artificial hatching agents are known for the beet cyst nematode, *Heterodera schachtii* Schm.<sup>1-7</sup>, some for the potato cyst nematode, *H. rostochiensis* Woll.<sup>7-9</sup>, but few or none for other economically important *Heterodera* species<sup>7</sup>.

Flavianic acid (2,4-dinitro-1-naphthol-7-sulphonic acid) effectively hatched eggs of the cyst nematodes *H. cruciferae*, *H. glycines* Ichinohe, *H. schachtii*, *H. tabacum* Lownsbury & Lownsbury and *H. trifolii* Goffart. It was less effective with *H. goettingiana* Liebs. and failed to hatch eggs of *H. carotae* Jones and *H. rostochiensis*. Of these species, eggs of *H. goettingiana*<sup>10</sup> and *H. glycines*<sup>7,11,12</sup> do not hatch *in vitro* in any root diffusates so far tried. *H. goettingiana* eggs do, however, hatch in soil when pea roots are present<sup>10</sup>; the reason for their different response *in vitro* and in the soil is unknown.

Table 1 shows the hatching activity of flavianic acid and of three other synthetic hatching agents with eight *Heterodera* species. Except for *H. goettingiana* and *H. glycines*, where the hatch in the compound is given as a percentage of that in distilled water and is in italics<sup>7</sup>, activity is expressed as a hatch rating from the formula at the foot of Table 1 (ref. 4).

A 0.6-3 mmolar aqueous solution of flavianic acid hatched more eggs of *H. cruciferae*, *H. schachtii* and *H. trifolii* than the appropriate root diffusates, and as many eggs of *H. tabacum* as hatched in root diffusate. Pea root diffusate was used as the standard for *H. trifolii* because clover root diffusate does not hatch this species *in vitro*<sup>13</sup>. Egg-plant root diffusate was used for *H. tabacum*. Table 1 also shows that flavianic acid hatched many eggs of *H. glycines*.

All four compounds hatched eggs of *H. schachtii*, but other species responded to some and not to others. None was active with *H. carotae*. Although very similar in morphology and host range, *H. tabacum* and *H. rostochiensis* responded differently to the four compounds. Anhydrotetrone acid was the only one that hatched the eggs of both species. *H. rostochiensis* was also hatched by picrolonic acid<sup>9</sup> but not by picric acid or flavianic acid, whereas the reverse held for *H. tabacum*. These two species also responded differently to inorganic ions<sup>7</sup>.

Table 1. HATCHING ACTIVITY OF FOUR COMPOUNDS FOR EIGHT SPECIES OF *Heterodera*, EXPRESSED AS A HATCH RATING

<i>Heterodera</i> species	Flavianic acid		Compound		Picolonic acid		Pleric acid	
	Hatch rating	(mmoles/l.)	Hatch rating	(mmoles/l.)	Hatch rating	(mmoles/l.)	Hatch rating	(mmoles/l.)
<i>H. carotae</i> , carrot cyst nematode	2*	0.6	2	10	1	3	4	10
<i>H. cruciferae</i> , cabbage cyst nematode	158†	0.6	-38§	3	-77	3	-55	2
<i>H. glycines</i> , soybean cyst nematode	1,146†	3			446	3	-93	3
<i>H. goettingiana</i> , pea cyst nematode	331	0.6	204	0.6	116	3	116	3
<i>H. rostochiensis</i> , potato cyst nematode	-3	0.6	90	3	102	0.6	4	2
<i>H. schachtii</i> , beet cyst nematode	110	0.6	68	3	130	3	238	2
<i>H. tabacum</i> , tobacco cyst nematode	90	3	107	3	14	2	70	3
<i>H. trifolii</i> , clover cyst nematode	311	3	9	10	5	3	-36	3

Based on the total number of eggs hatched after 3 weeks from three batches of 100 cysts, except for *H. tabacum* where batches were of 50 cysts.

\* Ratings in roman type: calculated from formula  $\frac{H_r - H_w}{H_d - H_w} \times 100$

where  $H_r$  is the hatch in compound, and  $H_d$  and  $H_w$  are the hatches in root diffusate and water, respectively. A hatch rating of -11 to -100 is inhibitory -10 to 10 inactive (water = 0), 11 to 20 weakly active, 21 to 90 moderately active and > 90 very active (root diffusate = 100)\*.

† Rating in italics: hatch in compound expressed as a percentage of the hatch in water. A hatch rating of -11 to -100 is inhibitory, -10 to 150, inactive (water = 100), 151 to 250 weakly active, 251 to 500 moderately active and > 500 very active.

‡ Bold type: very active stimulation.

§ Negative sign: percentage inhibition compared with hatch in water\*.

Flavianic acid, like the other compounds in Table 1, has an acidic enolic group connected to a conjugated unsaturated system. It also has a sulphonic acid group, but simple sulphonic acids, for example, *p*-toluene sulphonic acid, were inactive or only slightly active with *H. schachtii* and *H. rostochiensis*. With these two species, chain length between terminal polarizable atoms seems an important determinant of hatching activity.

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### Inhibition of the Action of Phytochrome by the Herbicide CIPC

CIPC, (Chlorpropham) isopropyl *N*-(3-chlorophenyl) carbamate, and its analogues are inhibitors of protein synthesis in excised segments of seedling plants<sup>1</sup>. Investigation suggested that this inhibition is actually exercised on the synthesis or release of messenger RNA (ref. 2, and in preparation by Mann, Cota, Robles, Moretti and Yung). Thus it was of interest to determine whether this particular inhibitor could prevent the effect of light in stimulating germination of Grand Rapids lettuce seeds.

This requirement for light is mediated by phytochrome. Considerable evidence indicates that phytochrome must be converted to the  $P_{fr}$  form (with an absorbance maximum at 735 mμ) for germination to occur<sup>3</sup>. We have administered CIPC to seeds imbibing in darkness, irradiated them with red light (forming  $P_{fr}$ ), and removed CIPC by washing while at the same time giving far-red in order to inactivate  $P_{fr}$ . If the first stable effector substance synthesized by  $P_{fr}$  is neither a messenger RNA nor a protein, then CIPC should merely inhibit the expression (that is, germination) of such an effector substance. The ability to remove CIPC (by leaching) and  $P_{fr}$  (by irradiation with far-red) at will permits us to distinguish between an inhibition by CIPC of synthesis of a  $P_{fr}$ -induced effector substance, as opposed to an inhibition of further expression of this hypothetical effector. Our results, however, indicate that CIPC blocks the synthesis of effector substance rather than its expression.

The data are given in Table 1. Dry seeds were first irradiated with far-red light in an effort to minimize the percentage of light-insensitive seeds. They were moistened with a little distilled water or with a 30 p.p.m. solution of CIPC, stored in darkness for 8 h at 25° C, and then given a 15 min exposure to 660 mμ illumination obtained by filtering a fluorescent tube. After an additional 20 h of storage in darkness the seeds were irradiated with far-red illumination, obtained by filtering the output of a 25 W incandescent lamp. In darkness, the seeds were washed into an aluminium tea strainer, rinsed with a large volume of distilled water, and transferred to a Petri dish containing moist filter paper. After an additional 3 days of incubation in complete darkness (obtained by the use of aluminium foil wrappings), the percentage germination was determined. Any radical protrusion was rated as germination. In our experiments obvious differences in the length of roots were apparent only after treatment with *para*-fluorophenylalanine.

In some cases, the addition of CIPC was delayed until after 3 h of imbibition. A considerably increased sensitivity to red light occurs during this period<sup>4</sup>. Delayed addition of CIPC, however, had no effect on our results.

Table 1. EFFECT OF CIPC OR PFPA ON STIMULATION BY RED LIGHT OF GERMINATION OF GRAND RAPIDS LETTUCE SEEDS

Light regime	Water	Percentage germination		
		CIPC at start	CIPC at 3 h	PFPA at start
Darkness*	31	30	—	0
Red at 8 h, far-red at 28 h	96	43	27	71
Red at 8 h, far-red and then red at 28 h	96	87	90	67

Concentration of CIPC was 30 p.p.m. All seeds were washed in complete darkness with distilled water after 28 h of imbibition, irradiated as described, and replated on moist filter paper. Phenylalanine (30 mg/l.) was added to seeds which had been treated with PFPA. One to two hundred seeds were used for each treatment. (Other details in text.)

\* Seeds kept in darkness were not washed free of inhibitors.

CIPC almost completely counteracted the effect of  $P_{fr}$  in stimulating germination, yet it did not affect the germination of the 30 per cent of light-independent seeds which germinated in the absence of red irradiation. CIPC did not cause any permanent injury to the seeds. If, after leaching and irradiation with far-red for 28 h, an additional dose of red light was given, the percentage germination increased to normal levels (Table 1). Similar findings with regard to the effect of anaerobiosis on the "escape phenomenon" have been reported<sup>5</sup>.

Experiments with other nominal inhibitors of protein synthesis (puromycin, azetidine-2-carboxylic acid, and *para*-fluorophenylalanine, PFPA) were carried out. Only PFPA penetrated the intact seed coat, as measured by inhibition of light-independent germination. PFPA did not block germination which was stimulated by  $P_{fr}$  other than with a slight generalized toxicity (Table 1).

We conclude that the first stable product of the action of  $P_{fr}$  is a specific messenger RNA. This explanation can



be generalized to other actions of phytochrome concerned with, for instance, flowering, and pigment synthesis, if it can be assumed that not only a "germination messenger" is under phytochrome control, but other messengers as well, according to the species and tissue in question. A similar hypothesis for the action of phytochrome has been suggested<sup>6</sup>, but it is not clear whether the high energy response in *Sinapis alba* is truly a phenomenon controlled by phytochrome.

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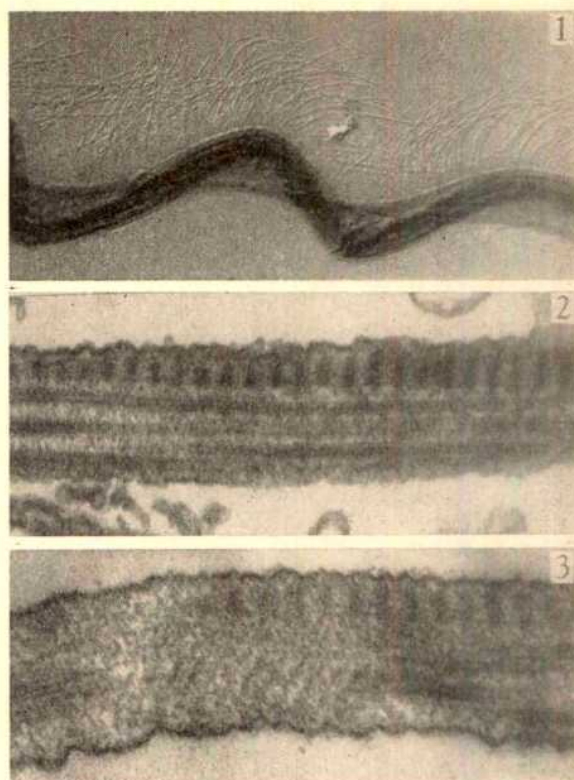
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### Fine Structure of the Dinoflagellate Transverse Flagellum

THE dinoflagellates have for some time been known to possess two flagella which differ from one another in their movements and in the orientation of the beating portions. One, called the posterior or longitudinal flagellum, has a relatively slow movement which has been shown to be in the form of a planar wave<sup>1,2</sup>. Electron microscopy shows that this flagellum is constructed of the normal axoneme plus organized packing material in the proximal two-thirds, all surrounded by a membrane. The transverse flagellum, often situated in a groove around the cell, has a completely different type of motion. With the light microscope it appears ribbon-like<sup>3</sup> and has a rapid undulatory motion which recent investigations have shown to be in the form of a circular or elliptical helical wave<sup>1</sup> (in *Ceratium*). In an early electron microscopical study of *Gyrodinium*<sup>4</sup>, it was found that this flagellum had an expanded sheath much wider than the axoneme, with an array of fine hairs along one side. Our observations have confirmed these findings and have revealed other structures which account for the unusual behaviour of this flagellum.

The transverse flagellum is a true flagellum, since it possesses the normal axoneme of 9+2 fibrils. These emerge from a basal structure identical to that of the longitudinal flagellum and only slightly different from that of other flagellates. When direct mounts are made (Fig. 1) the axoneme always shows a wave form which never completely straightens out, and it bears long fine hairs. Also present within the loose flagellar sheathing membrane is a banded structure—the striated strand (Figs. 2, 4 and 5) and organized packing material (Fig. 3) which occupies space not filled by the axoneme and the strand. The striated strand is composed of fibrous protein with banding showing an overall periodicity of 660 Å. This strand is much shorter than the axoneme and follows an almost straight path.

Because the short striated strand and the long axoneme are held together by packing material and flagellar sheath, the flagellum adopts a helical form rather like that of a drawn-out spiral staircase. A model constructed of tight string and wire in a sheath gives a similar result. Thus the structure of the flagellum dictates the form which its movement must take. The flagellum is held in position around the dinoflagellate cell by a relatively loose attachment of the tip in the region of the flagellar bases. There



Figs. 1-3. Electron micrographs of *Woloszynskia micra*, transverse flagellum.

Fig. 1. Dried and shadowed transverse flagellum showing the wavy profile of the axoneme, the striated strand running in a straight line, the flagellar sheath and the fine hairs attached to this. ( $\times 12,000$ .)

Fig. 2. Median longitudinal section showing the banded nature of the striated strand (top) and filaments of the axoneme. ( $\times 58,800$ .)

Fig. 3. Longitudinal section showing some of the organized packing material in addition to the axoneme and striated strands. ( $\times 58,800$ .)

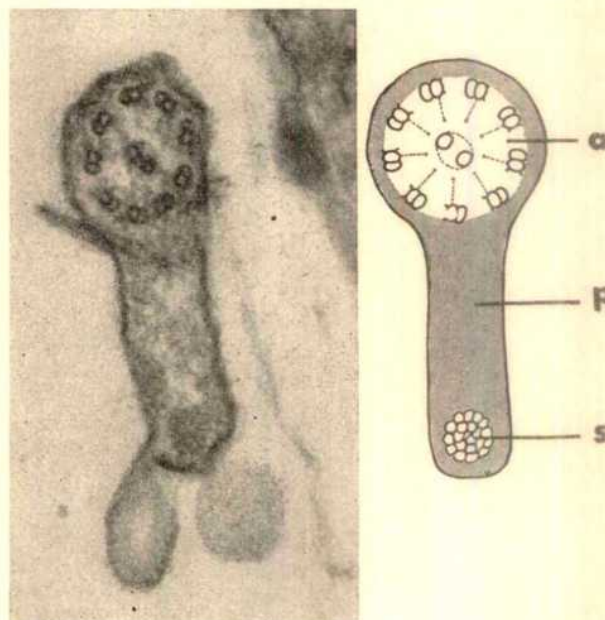


Fig. 4.

Fig. 5.

Fig. 4. Electron micrograph of *Woloszynskia micra*, transverse section of the transverse flagellum showing the axoneme, the striated strand and the space between occupied by packing materials. ( $\times 78,400$ .)

Fig. 5. Drawing to explain Fig. 4. a, Axoneme; p, packing material; s, striated strand.

is no membranous connexion all round the girdle of the type which has often been postulated.

Electron microscopical observations have therefore revealed that the dinoflagellate transverse flagellum is one of the more complex types of flagellum known. In view of its specialized and unusual function this is perhaps not altogether surprising. Further details of the structure of dinoflagellate flagella will be published elsewhere.

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### Production of New Physiologic Races in *Puccinia striiformis* (Yellow Rust) by Heterokaryosis

THERE is no known pycnial stage in *P. striiformis*, and so it is impossible for new physiologic races to arise as a result of sexual recombination, the best known method in *P. graminis*<sup>1</sup>. Nevertheless, there are at least thirty known races on wheat alone in Europe and Asia<sup>2</sup> and two new races, 2B and 8B, have appeared in Britain within the past 15 years<sup>3</sup>, and the epidemic in 1966 on the wheat variety Rothwell Perdix may well be caused by a further new race. Mutation and cytoplasmic change are possible causes of the production of new races, but it seemed to us that heterokaryosis<sup>4</sup> or parasexual phenomena<sup>5</sup> in the uredospore stage (both known in *P. graminis*) could be involved.

Experiments were carried out involving the mixture of uredospores of two physiologic races. A mixture of the uredospores of races 2B and 8B was inoculated on to seedlings of Strubes Dickkopf (a wheat susceptible to both races) and thirty single uredospore cultures were made from the resulting infection. When tested on the eleven standard differential wheat varieties<sup>6</sup> with the addition of Cappelle Desprez (distinguishing race 2B from race 2) and Heines VII (distinguishing race 8B from race 8), twenty-seven isolates resembled one or other parent, but the other three differed significantly from either (Table 1) in their reactions on four of the differential hosts. The reactions are given on the standard scale<sup>6</sup>, 0 being immune and IV highly susceptible. SSC 4 (and SSC 27, which closely resembled it) are less virulent than the parent races, but SSC 21 is more virulent, and could be of considerable importance if it became established in the field. Both races appear to be different from any previously described<sup>2</sup>.

Table 1. REACTIONS OF NEW PHYSIOLOGIC RACES AND THEIR PARENTS

Race	Vilmorin 23	Rouge prolifique barbu	Heines VII	Cappelle Desprez
2B	IV	II-IV	0	IV
8B	0-I	0-II	0-IV	0
SSC 4	I-II	0	0	IV
SSC 21	IV	II-IV	0-IV	IV

The production of these races can be explained on the basis of heterokaryosis: the parasexual cycle may be involved, but it is not necessary to invoke it to explain the results obtained, and the simplest explanation, that of simple reassortment of heterokaryotic nuclei, is preferred. Each uredospore possesses two nuclei, and if these were, after hyphal fusion, to change partners, two but not more than two new races would be expected if the parent races were heterokaryotic. The formation of hyphal fusions, and the passage of cell contents from one germ tube to another, have been observed by us, and will be described fully elsewhere.

The production of new physiologic races by hybridization in the uredospore stage provides a method for determining, in the absence of any sexual stage, the genetical factors responsible for virulence in the rust. The results reported here are insufficient to make more than a very preliminary determination, but they are in accordance with the postulate that three factors for virulence in the rust genome are involved. The action of the factor postulated as conferring virulence to Heines VII is very distinct. The factor conferring virulence to Vilmorin 23 is distinct from the one conferring virulence to Cappelle, since SSC 4 gives a type IV reaction on Cappelle, but only a type I-II reaction on Vilmorin 23, in contrast to the type IV reaction given by race 2B and SSC 21, the other two isolates attacking Cappelle. The difference between these two reaction types is accepted as being highly significant<sup>7</sup>. If the absence of parasexual phenomena is assumed, the factor conferring virulence to Cappelle must be inherited as a dominant character; the evidence is insufficient to determine whether the other two factors are recessive or dominant.

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### Juvenile Sterility in Male Ticks of *Ornithodoros tholozani*

DURING an investigation of the physiology of reproduction in ticks, we observed that young male ticks of *Ornithodoros tholozani* are temporarily sterile, although they may copulate.

Young unfed males of *O. tholozani*, *O. lahorensis* and *O. tartakovskyi* generally copulate at 4-6 days old<sup>1</sup>, but they sometimes do so as soon as a few hours after ecdysis. To ascertain whether fertilization had taken place, the female was dissected after copulation and the uterus was inspected for the presence of a spermatophore. A spermatophore in the uterus was taken as an indication that fertilization had taken place. This conclusion should be modified, at least in the case of *O. tholozani*.

Our observations on the fertility of *O. tholozani* also showed that young unfed males are able to copulate. In our experiments some males began to copulate on the day after ecdysis and continued to do so at short intervals. Other males began to copulate later in their life. The presence of a spermatophore in the female's uterus does not, however, suffice to prove the ability of the male to fertilize a female.

Only microscopic examination of a spermatophore which has been dissected out of the uterus reveals the true contents of the spermatophore. The first spermatophores produced by a male generally do not contain sperm. They do, however, contain the sperm symbiotes *Adlerocystis ornithodori* which develop in one pair of the complex of accessory lobes of the male genital system which function as a mycetome<sup>2</sup> (Figs. 1 and 2).

Sterility is generally observed in young males of *O. tholozani* for 2 weeks after ecdysis. During this time a male may copulate up to five times, but all the spermatophores produced will contain no sperm. The entire process of copulation, as well as the spermatophore itself, is apparently normal, apart from the absence of sperm.



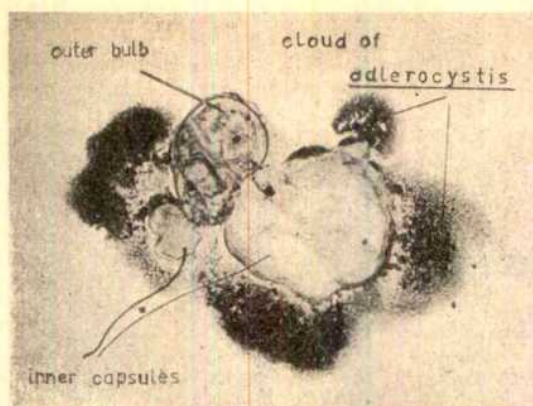


Fig. 1. A whole spermatophore of *Ornithodoros tholozani* before its introduction into the female ( $\times 20$ ). On light pressure on the cover glass the inner capsules, with all their contents, oozed out of the outer bulb. Note the large number of *Adlerocystis* forming "clouds" and the total absence of sperm cells. It was the first copulation of a male 24–48 h old.



Fig. 2. "Spermal fluid" of a spermatophore containing no sperm cells, but *Adlerocystis ornithodori* ( $\times 160$ ). The spermatophore was dissected out of the uterus 5 days after copulation. At copulation the male was 8 days old, and it was its second copulation.

One third (eight out of twenty-four) of the males in our experiments did not copulate before they became fertile.

The starved males usually start to produce sperm and become fertile at age 9–13 days. One male, however, produced sperm on the day after ecdysis, while another produced a spermatophore, which did not contain sperm at its first copulation, when it was 80 days old; the same male produced sperm at its second copulation, 13 days later. If the young male accepts a feed soon after ecdysis, sperm are found in its spermatophore about 5 days after the feed.

The presence of the spermatophore in the uterus is therefore no evidence of the ability of a male to fertilize, but only to copulate. So far as we know, this is the first observation of regular and normal sterility in young male individuals, who nevertheless copulate in an apparently normal way. We propose to call this phenomenon "juvenile sterility".

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## Attraction of *Apis mellifera* Drones by the Odours of the Queens of Two Other Species of Honeybees

ETHANOL extracts of the queens of three species of honeybees, *Apis mellifera*, *A. cerana* sub-species *indica* and *A. florea*, contain substances that inhibit both queen rearing by workers of *A. mellifera* and development of the ovaries of workers of this species<sup>1,2</sup>. Rearing of queens and development of workers' ovaries in *A. mellifera* colonies are inhibited by the pheromone, 9-oxodec-2-enoic acid, which is produced in the queen's mandibular glands<sup>3</sup> and which also serves as the olfactory sex attractant in this species<sup>4,5</sup>. It has been suggested that it would be interesting to find out whether the odours of the queens of the other three species of honeybees, *Apis cerana*, *A. dorsata* and *A. florea*, attract drones of *A. mellifera* and, if so, whether such attraction is also caused by 9-oxodecenoic acid<sup>2</sup>. Nubile virgin queens of these species were not available, and so tests were made with mated queens of *A. cerana* sub-species *indica* and *A. florea* which had been preserved in ethanol for several years.

The ethanol in which the *A. cerana* and *A. florea* queens had been stored was evaporated to a small volume on a water bath, poured over the respective queens and allowed to dry at room temperature. The two queens and an *A. mellifera* queen were hidden in separate wire gauze cages, each 1 cm  $\times$  2 cm  $\times$  8 cm, and exposed, one at a time on a warm afternoon when drones were flying freely, at the top of a tripod 6 m high. A dead *A. mellifera* worker in another wire gauze cage was used as a control. Using the technique devised by Butler and Faurey<sup>6</sup>, each cage was exposed in turn for 2 min and the drones which approached a dead worker bee suspended by a nylon thread about 1.2 m directly downwind of the cage were counted. Each cage was exposed ten times; results are shown in Table 1.

Table 1. ATTRACTION OF ODOURS OF MATED QUEENS OF *Apis cerana* SUB-SPECIES *indica*, *A. florea* AND *A. mellifera* TO FLYING DRONES OF *A. mellifera*

	<i>A. cerana</i> queen	<i>A. florea</i> queen	<i>A. mellifera</i> queen	<i>A. mellifera</i> worker
Mean No. of drone visits in 2 min	25.3	25.9	24.5	0.2
S.E. of mean	4.41	5.19	5.89	0.13

The mean number of drones attracted by the odours of the three species of *Apis* queens did not differ significantly, but did differ significantly from the number attracted by the *A. mellifera* worker used as a control ( $P < 0.001$ ), indicating that queens of *A. cerana* sub-species *indica* and of *A. florea* produce one or more volatile substances that attract *A. mellifera* drones.

When the attraction of the queens to *A. mellifera* drones had been established each was stored in ethanol and, later, examined chemically. The head of each queen was removed and extracted with ether. This extract was combined with the ethanol in which the queen had been kept, the whole evaporated to dryness and the residue was methylated<sup>6</sup>. The resulting pentane extracts containing methyl esters were evaporated and the residues dissolved in acetone. Care was taken to avoid any possibility of contamination during preparation and analysis of these extracts. Portions (usually 0.1 per cent) were analysed by gas chromatography using a Perkin-Elmer F11 chromatograph with dual 6 ft. columns of silicone gum rubber E-301 on 'Chromosorb W' at 152°C. A major peak was observed, in the chromatogram of material from each queen, which had the same retention time as methyl 9-oxo-dec-2-enoate and was not separated from this on co-chromatography. It is therefore inferred that the queens of both *A. cerana* and *A. florea* contained 9-oxodecenoic acid and this attracted the *A. mellifera* drones.

Because of the previous history of these queens, their original content of 9-oxodecenoic acid could not be accurately estimated, but the amounts detected were of



the order of 10 µg/queen, which can be taken as a minimum value.

Whether, given the right conditions, 9-oxodecenoic acid attracts drones of *A. cerana* and *A. florea*, and whether queens of the fourth species of honeybee, *A. dorsata*, also produce 9-oxodecenoic acid has still to be determined, but both seem probable. If it is found that 9-oxodecenoic acid forms the whole, or part, of the olfactory sex attractant of several species of *Apis*, the question arises how interspecific attraction is prevented in regions where two or more species co-exist. Interspecific matings are unlikely because of disparity in size and anatomical differences between the drones.

Most olfactory sex attractants may be species specific, but enough exceptions are known to show that such specificity is not essential to prevent indiscriminate cross-mating even between closely related species, for example, gypsy moth, *Porthetria dispar*, and nun moth, *Porthetria monacha*<sup>7</sup>, tobacco moth, *Ephestia elutella*, and Mediterranean flour moth, *Ephestia kuehniella*<sup>8</sup>.

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### Larval Moulting Cycle and DNA Synthesis in *Drosophila hydei* Salivary Glands

IN *Drosophila*, cell division stops from the time the salivary gland primordium is formed in the embryo, and no mitoses have been observed in the glands after this stage. The number of the cells present remains the same throughout the entire larval development and the increase in size of the glands is merely due to cell growth<sup>1</sup>.

Histophotometric measurements of the DNA content per nucleus show that during the larval development DNA undergoes progressive duplication. On the basis of data by Swift and Rasch (see Alfert<sup>2</sup>), it seems that this process leads to an increase of total DNA content per cell up to 1,024 times the normal haploid value.

According to Plaut<sup>3</sup>, the number of duplications of original non-polytene DNA should be eight, and "... since it takes about 1 week under normal culturing conditions for eggs to develop into late third instar larvae, the average time for each of the eight replication cycles is about 20 hours". Plaut suggests also that the cells with polytene chromosomes of *Drosophila* salivary glands have a pattern of DNA synthesis similar to that of the other non-polytene cells; each synthetic activity should lead to a precise doubling of the pre-existing DNA and should be separated from the next period of synthesis by a pause in which there is no DNA synthesis.

Some authors<sup>4,5</sup> are in favour of the view that larval development, with the profound metabolic changes taking place at each moult, is a discontinuous process. Certain hormones, such as ecdysone, seem to play an important part in this connexion. There is some evidence suggesting that ecdysone may interfere with nucleic acid metabolism (see review by Wigglesworth<sup>6</sup> and the experiments on puffing patterns induced by injection of ecdysone<sup>6</sup>).

The experiments reported here have been carried out in order to ascertain whether the replication cycles in the salivary glands occur independently of the moulting cycle or are somehow related to it. Our observations have been confined to larvae from the late second to the late

third instar. This period has been chosen because it is more suitable for our experimental approach.

The entire larval development in our culturing conditions lasts 196 h from the time of oviposition to the early pupa. Based on the presence of shed cuticles on the culture medium, the moult takes place at 116–118 h. The period from 100 to 196 h was divided into eleven periods each of 8 h; the tenth period, however, lasted 16 h.

From synchronous cultures, groups of ten larvae were collected from each period of the development. Tritiated thymidine was administered in the food so that the uptake conditions were as near physiological as possible and the treatment uniform for all larvae<sup>7</sup>. Each group of larvae remained in the radioactive food for 8 h, in standard culture conditions. After incubation, the incorporation of the isotope was stopped by the addition of unlabelled thymidine, and the larvae were transferred to normal food for growth until the early pre-pupa.

In autoradiographs of squashed preparations of the salivary glands, the percentages of labelled nuclei have been calculated for each of the eleven periods (Fig. 1). After an initial period of intensive synthesis in the whole gland, the percentage of labelled nuclei rapidly decreases, reaching a minimum (less than 20 per cent) in correspondence with the moult. A new period of synthesis takes place soon after (70–80 per cent of cells are labelled from 132 to 156 h), followed by a continuous decrease until the puparium is formed.

Fig. 2 represents the distribution of the numbers of silver grains per labelled nucleus; on the whole, this graph is similar to that in Fig. 1. Moreover, it shows that the fairly high percentage of labelled nuclei from 156 to 172 h results entirely from a large number of cells with low levels of uptake (only the 2.3–5.5 per cent of nuclei show

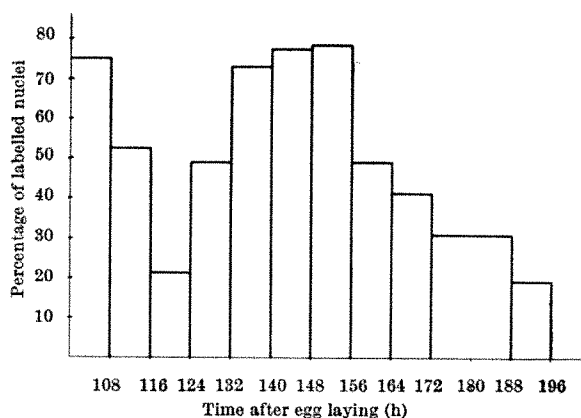


Fig. 1. Percentages of labelled nuclei in the different 8 h periods. As a rule, nuclei with at least twenty silver grains were considered. The counts were made on six slides for each period of the development.

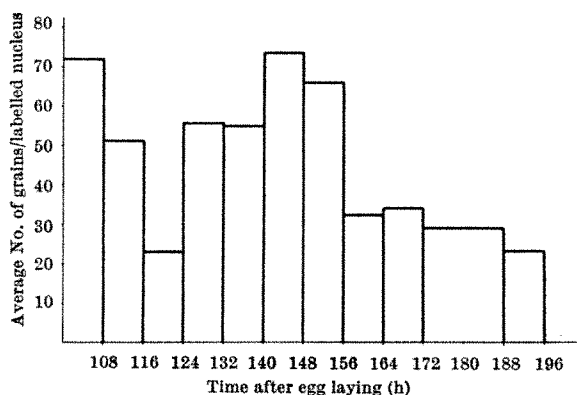


Fig. 2. Average number of silver grains in a labelled nucleus, in the different periods. The grains were counted on fifty nuclei, at random, for each slide.

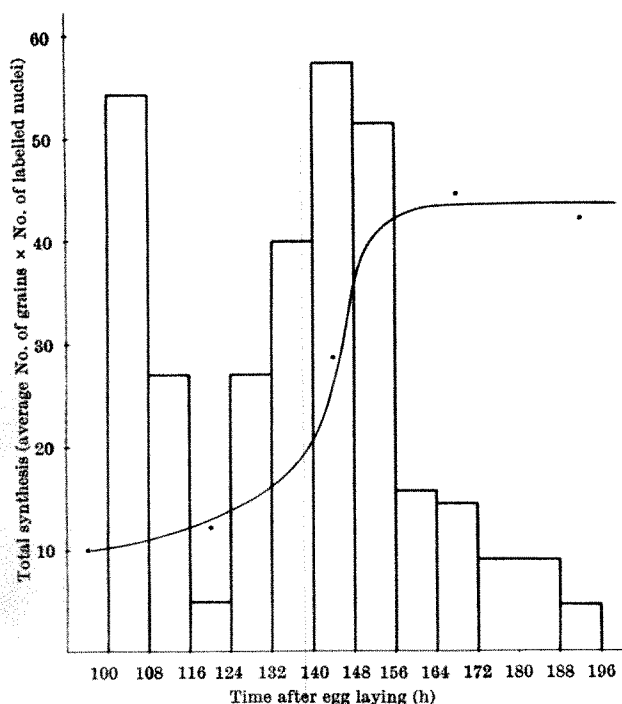


Fig. 3. Total synthesis in the gland for each 8 h period, represented as the average number of grains/labelled nucleus  $\times$  percentage of labelled nuclei. The continuous curve describes the relative increase of the average DNA content per gland (initial amount = 10).

a number of grains large enough to suggest active synthesis). This situation may arise from the presence of nuclei which are completing their replication cycle or, rather, of nuclei in which, after a complete DNA replication, some DNA synthesis may continue in several active "replicones".

The total synthesis in the gland is represented in the histogram in Fig. 3 as the average number of grains per nucleus  $\times$  the number of labelled nuclei in each sample (300 cells). The shape of the graph shows that a wave of DNA synthesis occurs during the first half of the third instar.

In order to determine whether during this wave of synthesis there are one or more doublings of DNA, a biochemical approach to the problem was attempted. At various stages of the larval development, nucleic acids were extracted according to Schneider's technique and the DNA content determined by Burton's modified DPA method. The extractions were made on 100 pairs of glands for each period of the development, excised from synchronized larvae. The details of the procedure are discussed elsewhere<sup>9</sup>.

The contents of DNA are also reported in Fig. 3. The continuous curve describes the relative increase of the average content of DNA per gland. The curve shows that, in the period of massive synthesis revealed by autoradiography, there is an increase in the amount of the initial DNA of about 4.5 times.

The comparison between the autoradiographic and the biochemical data indicates that in a restricted period of the third instar there is an extensive synthetic activity which leads to an increase of the average content of DNA per gland, corresponding to at least two doublings. Because the synthesis stops at the second moult, resumes, and stops again before the beginning of the metamorphosis, it seems reasonable to relate the perturbation of the cell cycle with the physiological events involved in the moulting cycle.

Results similar to ours have been obtained by Krishnakumaran *et al.*<sup>10</sup> in *Samia cynthia ricini* (Lepidoptera). They observed that "... in the day of the moult to the

fourth instar, synthesis of DNA and RNA was at its lowest during the moult cycle; their synthesis increased during the rest of the instar and reached another low point on the day of the moult to the fifth instar". They concluded that "the stimulation of DNA synthesis is a primary effect of ecdysone".

Becker<sup>11</sup> has shown that in *Drosophila* the release of the ring gland hormone occurs a few hours before the formation of a puparium. It has also been demonstrated that the effect on the metabolism of nucleic acids is almost immediate, as suggested by the appearance of puff patterns within less than an hour of the hormone being injected<sup>6</sup>.

Similarly, other authors think (see Wigglesworth's review<sup>5</sup>) that a high concentration of hormone in insect larvae is reached only shortly before ecdysis.

With our results it is difficult to ascribe the increased synthesis of DNA to the influence of the hormone. In fact, a rapid decrease in the synthetic activity occurs between 108 and 124 h, just when the concentration of the hormone in the haemolymph is quite high; if the effect of ecdysone on the metabolism of nucleic acids is primary<sup>10</sup> and immediate<sup>6</sup>, an activation of the synthetic activity would be expected rather than the over-all arrest which is actually observed in our experiments.

The questions of whether the partial synchronization of the cells synthesizing DNA results from the action of ecdysone, and of whether the ecdysone acts as an activator or an inhibitor, are now being investigated.

We thank Prof. B. Battaglia and Prof. G. Marin for their helpful suggestions in this work.

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### Effect of Packaging on the Major Microbial Flora of Irradiated Haddock

A NUMBER of investigations has been made to determine the alterations in the microflora of fish caused by irradiation, and in some cases, the subsequent growth patterns of the survivors during refrigerated storage<sup>1-4</sup>. These investigations have indicated that radiation drastically reduces the Gram-negative micro-organisms normally associated with fish or with fish spoilage and leaves larger numbers of the more radioresistant Gram-positive bacteria and fungi. The nature of the microbial spectrum surviving irradiation depended on the dosage and the initial level of contamination. Subsequent growth of the radiation survivors was then found to be a function of the storage temperature. One variable which has been relatively neglected is the effect of packaging and the accessibility of air on the growth patterns of irradiated seafoods, although certain investigations have indicated that this could be important for ground beef<sup>5</sup> and for poultry packed and canned under nitrogen<sup>6</sup>. In order to evaluate the relationships between radiation, refrigerated storage, and the presence of air, haddock fillets were

packaged in cans without hermetic sealing but covered with aluminium foil to allow free exchange of air (FP), sealed with a limited air headspace (LP), or vacuum packed (VP). The samples were then exposed to 150 krad and stored at 3° C for 3 weeks. For taxonomic identification of the various organisms the determinative scheme of Shewan<sup>7</sup> was used for the Gram-negative organisms. Gram-positive organisms were determined by standard morphological and biochemical criteria. Three per cent hydrogen peroxide was used for detection of catalase, and for cytochromes the benzidine test was used<sup>8</sup>. The results indicated that, in addition to a shift in the major microflora caused by radiation, the microbial spectrum is further altered by the presence or absence of air during refrigerated storage.

The total counts (Fig. 1) of the unirradiated controls (all of which were found to be spoiled after 3 weeks) were much higher for packs FP than for packs LP and VP. After 3 weeks in storage all the irradiated samples were judged by odour and appearance to be in an acceptable condition. The microflora for all samples remained essentially psychrotrophic, as shown by the greatly reduced plate counts at 37° C.

Table 1. THE SELECTION OF *Pseudomonas* BY IRRADIATION AND THE PRESENCE OF AIR DURING STORAGE

<i>Pseudomonas</i> Group	Percentage of microflora					
	Unsealed		Air-sealed		Vacuum	
	U	I	U	I	U	I
I	28	0	0	0	0	0
II	28	4	0	0	9	0
III and IV	4	0	40	0	44	0
Total	60	4	40	0	53	0

U, Unirradiated; I, irradiated.

In the irradiated samples, *Pseudomonas*, the dominant flora in the unirradiated controls, were replaced by the *Achromobacter-Alcaligenes* group which had been present in reduced numbers in the controls. This was especially true for the FP samples (Fig. 2). The three predominant groups present on the irradiated samples were *Achromobacter-Alcaligenes*, *Lactobacteriaceae*, and catalase-positive cocci. The *Flavobacter-Cytophaga* group was only found in a high percentage in the FP samples; the percentage of *Lactobacteriaceae* was highest in the VP and of intermediate value in the LP fillets. Further taxonomic division of the *Pseudomonas* was made and, in agreement with a

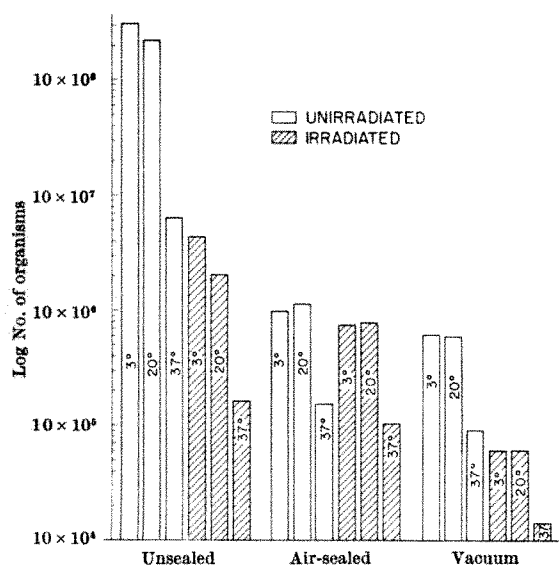


Fig. 1. Total aerobic plate counts of samples after 3 weeks storage at 3° C. A 50 g portion of haddock was blended for 3 min with 450 ml. of trypticase diluent. Total aerobic pour plate counts were made in trypticase soy agar supplemented with 0.5 per cent yeast extract in artificial sea water. For each sample, three sets of plates were prepared and incubated at 3°, 20°, and 37° C, respectively.

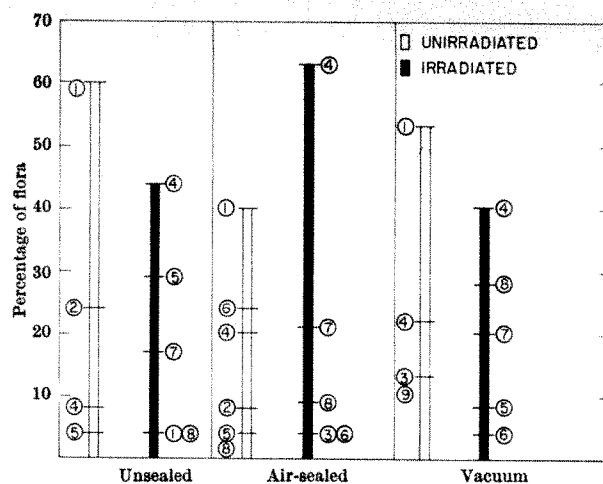


Fig. 2. Microbial flora of the samples after 3 weeks storage at 3° C as determined taxonomically. (1) *Pseudomonas*; (2) *Vibrio*; (3) *Aeromonas*; (4) *Achromobacter-Alcaligenes*; (5) *Flavobacterium-Cytophaga*; (6) *Paracolon*; (7) Gram-positive cocci, catalase (+); (8) Gram-positive cocci and rods, catalase (-); (9) others (not identified).

recent report<sup>9</sup>, it was found that groups I and II, which were 56 per cent of the microflora, become the dominant flora associated with the unirradiated FP pack. Limiting the available air in the packs, however, caused a shift to the less oxidative groups III and IV, which were 40 per cent for the air sealed (LP) packs and 44 per cent for the vacuum packed (VP) samples. Groups III and IV of the *Pseudomonas* had been found by others<sup>10</sup> to be the major spoilers of unirradiated sole fillets in samples prepared in a similar manner to the LP and VP cases. The spoilage potential of the *Pseudomonas* sub-groups therefore requires further elucidation. One should also consider that in the LP samples carbon dioxide produced by microorganisms during growth may have led to an inhibition of the more oxidative organisms<sup>11</sup>.

It was noted that a large portion (67 per cent) of *Pseudomonas* groups III and IV required sea salts for growth on the various media and also had the highest incidence of proteolytic activity on skim milk agar. Relatively few of the *Achromobacter-Alcaligenes* group were proteolytic whereas a high percentage of the *Vibrio*, *Aeromonas*, and *Flavobacter-Cytophaga* groups were proteolytic. These were usually a small portion of the microbial flora, however, and only a small number of them appeared to require sea salts for growth.

Although higher doses of irradiation might have altered the survival pattern and favoured the survival of micrococci, yeasts, and *Lactobacteriaceae*, only certain of these groups are capable of growth at 3° C.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, January 30

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "Ion Implantation" opened by Dr. J. Dearnley and Mr. L. Large.

UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 5.30 p.m.—Prof. S. J. Pirt: "The Cultivation of Microbes and Cells" (Inaugural Lecture).\*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Gordon Sutherland, F.R.S.: "Science" (third of four Cantor Lectures on "Some Aspects of the U.S.A. Today").

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Meeting on "Timber & Reinforced Plastics for Boat-Building".

## Tuesday, January 31

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.20 p.m.—Prof. G. Belyavin: "Influenza—The Mysterious Scourge".\*

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers and the IEE/RAES London Joint Group, at Savoy Place, London, W.C.2), at 2.30 p.m.—Colloquium on "Adaptive Control for Aircraft".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. J. R. H. Otter, Mr. A. C. Cassell and Mr. R. E. Hobbs: "Dynamic Relaxation".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "Some Recent Developments in Servo-Operated Recorders" opened by Mr. L. A. C. Dopping-Hepenstal and Mr. T. F. Prowse.

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Mr. J. P. von Wartburg (Bern): "Alcohol—Its Effects and Metabolism".\*

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. P. Alexander: "Repair of DNA in Mammalian and Microbial Cells". (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

SOCIETY OF INSTRUMENT TECHNOLOGY, MEASUREMENT TECHNOLOGY COMMITTEE (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Prof. D. de Jong: "Fine Mechanics—The Process from Craft to Science".

## Wednesday, February 1

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the National College of Rubber Technology, Holloway, London, N.7), at 2.30 p.m.—Panel Discussion on "Opportunities in Industry".

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Prof. J. D. H. Widdess: "The Royal College of Physicians of Ireland and some of its Famous Personalities".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. R. A. Mostyn and Mr. A. F. Cunningham: "Some Applications of Atomic Absorption Spectroscopy to the Analysis of Fuels and Lubricants".

UNIVERSITY OF LONDON (at the Institute of Laryngology and Otology, Royal National Throat, Nose and Ear Hospital, Gray's Inn Road, London, W.C.1), at 5.30 p.m.—Dr. P. D. Byers: "Pathology of Lesions of Facial Skeleton".\*

SOCIETY FOR ANALYTICAL CHEMISTRY (in the Chemistry Department, Imperial College, London, S.W.7), at 7 p.m.—Mr. C. Whalley and Mr. R. Goulden: "Detection and Determination of Small Quantities of Material".

## Thursday, February 2

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. E. J. Denton, F.R.S.: "On the Organization of Reflecting Surfaces in Some Marine Animals".

ROYAL SOCIETY OF MEDICINE, NEUROLOGY SECTION (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Dr. J. V. T. Gostling, Prof. M. R. Grist and Dr. H. E. Webb: "Viral Diseases of the Nervous System".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. S. Brenner: "Control and Mechanism of Protein Synthesis". (Seventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. E. F. Ball: "A Simple Transient-Flow Method of Measuring Thermal Conductivity and Diffusivity".

## Friday, February 3

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Dr. P. Suppan: "Reactivity of Excited States".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. David Attenborough: "BBC-2".

## Saturday, February 4

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at the Westminster Hospital Medical School, Horseferry Road, Westminster, London, S.W.1)—Annual General Meeting, followed by a meeting on "Vitamins in Clinical Biochemistry".

## Monday, February 6

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Gottmann (Paris): "The Growing City as a Social and Political Process. I. City Form and Function in a Changing Society".\*

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 6 p.m.—Dr. H. D. Anthony: "The Study of Scenery—2".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Emil Kekich: "Commerce" (last of four Cantor Lectures on "Some Aspects of the U.S.A. To-day").

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 6 p.m.—Prof. A. Williams: "British Ordovician Shelly Faunas. I. Palaeogeographic Setting and the Principal Faunas".\*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. B. J. Hardy: "Some Problems in the Design and Use of Power Cables".\*

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION AND THE INDUSTRIAL MARKETING RESEARCH ASSOCIATION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. J. A. Nicoll: "A Technique of Long Term Forecasting"; Mr. B. N. P. Hutchesson: "Market Research—Forecasting for the Chemical Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Alwyn A. Ruddock: "The Earliest English Voyagers Across the North Atlantic".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH BIOCHEMIST/BIOLOGIST (suitably qualified graduate) to work on immunological and biological aspects of human tumours—The Secretary, The London Hospital Medical College (University of London), Turner Street, London, E.1 (February 2).

LECTURER (qualified biochemist) IN THE DEPARTMENT OF CHILD LIFE AND HEALTH—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, Scotland (February 4).

SENIOR RESEARCH ASSISTANT in the BIOMECHANICS RESEARCH UNIT for research in the DEPARTMENT OF MECHANICAL ENGINEERING on problems associated with blood flow through valves—The Registrar, The University, Liverpool 3, quoting Ref. RV/353 (February 4).

CHAIR OF TELECOMMUNICATION SYSTEMS within the DEPARTMENT OF ELECTRICAL ENGINEERING SCIENCE—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (February 7).

PHYSICIST (with wide experience of the physical aspects of radiotherapy and radioisotope techniques) to fill a Senior Grade (N.H.S.) vacancy—The Secretary, Mount Vernon Hospital, Northwood, Middlesex (February 10).

LECTURER (educational psychologist) IN EDUCATION—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (February 11).

LECTURER IN THE DEPARTMENT OF PHYSICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (February 14).

LECTURER or ASSISTANT LECTURER (well qualified in mycology and general microbiology and preferably with interests in plant pathology, soil microbiology, or microbial genetics) in the DEPARTMENT OF BOTANY—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (February 15).

LECTURER or ASSISTANT LECTURER (with research experience in acoustics) IN PHYSICS—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (February 17).

UNIVERSITY RADIATION PROTECTION OFFICER (suitably qualified graduate) to implement codes of practice throughout the University and to be concerned with radiation work in the area generally—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR, 88C (February 18).

CHAIR OF BIOCHEMISTRY at the Royal Free Hospital School of Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 20).

LECTURER/ASSISTANT LECTURER (with at least a good honours degree or equivalent and capable of undertaking teaching and research in one or more of the following fields: biogeography, hydrology, climatology, oceanography and cartography) in the DEPARTMENT OF GEOGRAPHY, University of Malaysia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, February 21).

LECTURER or ASSISTANT LECTURER in THEORETICAL NUCLEAR PHYSICS—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11 (February 23).

ASSOCIATE PROFESSOR, SENIOR LECTURER or LECTURER GRADE I (preferably with experience in medical teaching hospitals) in the DIVISION OF MICROBIOLOGY, DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY, Medical School, University of Lagos—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 24).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT OF GEOLOGY—The Registrar, The University, Keele, Staffs (February 24).

LECTURER (preferably with suitable qualifications and experience in some area of theoretical or laboratory astrophysics) in ATOMIC PHYSICS—The Registrar, The University, Newcastle upon Tyne 2 (February 25).

LECTURERS or ASSISTANT LECTURERS (2) (preferably with special interests in plant metabolism or algology or mycology) in BOTANY—The Registrar, The University, Leicester (February 25).

SENIOR LECTURER (science or medical graduate) in the DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY at the Western Infirmary—Secretary of the University Court, The University, Glasgow (February 27).

LECTURER or ASSISTANT LECTURER (with a degree in biochemistry, physiology, zoology, or veterinary science, and prepared to undertake research in the field of avian physiology) in PHYSIOLOGY—The Secretary, Wye College (University of London), near Ashford, Kent (February 28).

LECTURER and an ASSISTANT LECTURER (qualified in the fields of palaeontology or structural geology) in GEOLOGY—The Registrar, The University, Leicester (March 1).

LECTURER in GENERAL PHYSIOLOGY in the School of Biology—The Registrar, The University, Leicester (March 1).

SENIOR LECTURER/LECTURER (with a post-graduate degree and preferably some experience of teaching biochemistry at university level, and a special knowledge of the agricultural aspects of biochemistry) in BIOCHEMISTRY at Lincoln College, University of Canterbury, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, March 4).

LECTURER or SENIOR LECTURER in BIOLOGY in the field of entomology or applied entomology at the University of Malawi—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 7).

**HEAD OF THE PROTEIN BIOCHEMISTRY DEPARTMENT** (Senior Principal Scientific Officer grade)—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen, Scotland (March 15).

**RESEARCH FELLOWS/SENIOR RESEARCH FELLOWS** (2) (preferably whose principal interests lie either within the field of social, moral and political philosophy or the field of logic and methodology) IN PHILOSOPHY, Research School of Social Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 15).

**UNIVERSITY NUCLEAR PHYSICS RESEARCH FELLOWS** (of Ph.D. standard with a special aptitude for research into high or low energy nuclear physics)—The Registrar, The University, Liverpool, 3, quoting Ref. RV/355 (April 1).

**CHAIR OF ENGINEERING DYNAMICS** at Monash University, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Academic Registrar, Monash University, Melbourne, Australia (Australia, April 17).

**ASSISTANT or ASSOCIATE PROFESSOR** (with a Ph.D. in monogastric nutrition and prepared to teach and conduct research in poultry nutrition) of POULTRY NUTRITION—Head, Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada.

**BIOCHEMIST or BIOLOGIST** (with a Ph.D.) to work in research in the DEPARTMENT OF MEDICINE (possibility of an academic appointment)—Dr. R. A. Camerini, Department of Medicine, New York Medical College, 106 St. and Fifth Avenue, New York, N.Y. 10029, U.S.A.

**EDITOR** (honours degree scientist, preferably physicist with some editorial experience) of the *Annals of the I.Q.S.Y.*, to edit a series of volumes on the results of the International Years of the Quiet Sun sponsored by the International Council of Scientific Unions—I.Q.S.Y., 6 Cornwall Terrace, London, N.W.1.

**FELLOW** (post-doctoral) or **RESEARCH ASSISTANT** in the DEPARTMENT OF CHEMISTRY to study viscosity, molecular relaxation processes and structure of liquids using ultrasonics—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex.

**LECTURER or ASSISTANT LECTURER** in the DEPARTMENT OF SOCIAL SCIENCE and HUMANITIES to teach in the Department's new Social Science Degree course and to contribute to the teaching of sociology to students of engineering and applied science—The Academic Registrar, The City University, St. John Street, London, E.C.1, quoting Ref. 14/SS.

**LECTURER** (with an honours B.Sc. in biochemistry and preferably a Ph.D., with relevant postgraduate experience) in PHARMACOLOGICAL BIOCHEMISTRY in the PHARMACOLOGY GROUP of the School of Pharmacy—The Registrar (S), The University, Bath, Somerset, quoting Ref. 67/3.

**PRINCIPAL LECTURER** IN COMPUTING to be responsible to the Head of the DEPARTMENT OF MATHEMATICS for the organization and administration of the Polytechnic's computer activities—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

**RESEARCH ASSISTANT** (preferably with experience in modern techniques in gas and blood/gas analysis) to organize a laboratory of the DEPARTMENT OF ANAESTHESIA, and to supervise junior staff mainly for research into subjects associated with anaesthetics and respiratory physiology—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. ASC/841, Project No. 802.

**RESEARCH ASSISTANTS** (2) (postdoctoral chemical engineers, physical chemists or physicists) for (1) highly novel work on detonation threshold phenomena (supervised by Dr. G. Munday); and (2) reaction kinetics in shock-heated homogeneous and heterogeneous systems (supervised by Dr. D. H. Napier)—Prof. A. G. Gaydon, F.R.S., Chemical Engineering and Chemical Technology Department, Imperial College, London, S.W.7.

**RESEARCH ASSISTANTS** (2) (with a good honours degree in mechanical engineering, metallurgy or physics) in ENGINEERING METALLURGY, to work in the DEPARTMENT OF MECHANICAL ENGINEERING on exciting new developments in the prediction of the fatigue life of metals—The Secretary, University College London, Gower Street, London, W.C.1.

**RESEARCH TECHNICAL ASSISTANT** (preferably B.Sc. in microbiology) to assist and participate in a research project concerned with the chemotherapy of synchronous bacterial cultures—The Director, Bland-Sutton Institute of Pathology, Middlesex Hospital, London, W.1.

**SENIOR FELLOW IN HUMAN GENETICS** at the John Curtin School of Medical Research, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

**TECHNICAL OFFICER** (preferably with a degree or diploma in agriculture) in the HERBAGE SECTION of the SEED PRODUCTION BRANCH—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

**UNIVERSITY DEMONSTRATOR** IN ORGANIC CHEMISTRY—Dr. P. Maitland, University Chemical Laboratory, Lensfield Road, Cambridge.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Society for the Promotion of Nature Reserves. Handbook 1966, Forty-ninth Annual Report, year ending 31st December, 1965. Pp. 124. (London: The Society for the Promotion of Nature Reserves, British Museum (Natural History), 1966.) 15s. [1111]

Sponsorship of Music: The Role of Local Authorities. (A PEP Report.) Pp. 77. (London: Political and Economic Planning, 1966.) 10s. [1111]

General Register Office. Studies on Medical and Population Subjects, No. 20: Accuracy of Certification of Cause of Death—a Report on a Survey conducted in 1959 in 75 Hospitals of the National Health Service to Obtain Information on the Extent of Agreement between Clinical and Post-Mortem Diagnoses. By M. A. Heasman and L. Lipworth. Pp. vii+133. (London: H.M. Stationery Office, 1966.) 21s. net. [1111]

Ministry of Agriculture, Fisheries and Food. Bulletin No. 38: Sex-Linkage in Poultry Breeding. Sixth edition. Pp. iv+38+4 plates. (London: H.M. Stationery Office, 1966.) 8s. 6d. net. [1411]

The National Central Library. 50th Annual Report of the Executive Committee for the year ending 31 March, 1966. Pp. 28+3 plates. A Description of the New Building in Store Street opened by Her Majesty the Queen, March 18th, 1966. Pp. 17. (London: The National Central Library, 1966.) [1411]

Cement and Concrete Association. Report for the year 1965. Pp. 72. (London: Cement and Concrete Association, 1966.) [1411]

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The Zoological Record, Vol. 101. Section 7 (1964): Brachiopoda. Compiled by B. F. Owen. Pp. 31. (London: The Zoological Society of London, 1966.) 12s. 6d.; \$1.80. [1511]

Institution of Gas Engineers. Communication 720: 27th Report of the Chairman's Technical Committee—1965-66. Pp. 14. Communication 721: 43rd Report of the Gas Education Committee—1965-66. Pp. 19. Communication 722: Looking Backward at French Natural Gas. By Georges H. Robert. Pp. 20. Communication 723: Automatic Control of Combustion Characteristics. By R. W. J. Andrews, W. H. French and T. A. Lucas. Pp. 12. Communication 724: Institution Gas Research Fellowship Report—1961-63. The Decomposition of Ethane Over Lime Under Continuous-Flow Conditions. By Dr. V. Moran. Pp. 10. Communication 725: The Application of Electronic Digital Recorders to Domestic Load Research. By J. H. Collins. Pp. 16. Communication 726: High-Pressure Measurement. By John W. Harriger. Pp. 6. Communication 727: The Houldsworth School of Applied Science—The Fuel Department After 60 Years. By Prof. A. L. Roberts. Pp. 11. Communication 728: Propane, as Enrichment to Increase Calorific Value and Meet Peak Demands. By L. Langfield, J. L. Wilson, G. A. Woodward and C. E. Sanderson. Pp. 13. Communication 729: Some Fundamental Viewpoints Concerning Gas Quality, Conversion of Appliances, and the Design of Multi-Gas Appliances. By A. van der Linden. Pp. 12. Communication 730: Conversion at Canvey. By R. P. Rhodes. Pp. 14. Communication 731: Trends in the Scandinavian Gas Industry Since 1945. By Norman V. Steenstrup. Pp. 19. Communication 732: The Resistance of Ductile Iron to Corrosion by Sulfur. By D. R. Whitchurch and H. H. Collins. Pp. 9. (32nd Autumn Research Meeting, London, 15th and 16th November, 1966.) (London: The Institution of Gas Engineers, 1966.) [1511]

### Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1214-E: Element Distribution in Some Shelf and Eugeosynclinal Black Shales. By James D. Vine. Pp. iv+31. \$0.20. Bulletin 1222-B: Geology of the Northwest Quarter of the Anaconda Quadrangle, Deer Lodge County, Montana. By Alexander A. Wanek and C. S. Venable Barclay. Pp. iii+28+plates 1 and 2. Bulletin 1222-C: Geology and Mineral Deposits of the Powell River Area, Claiborne and Union Counties, Tennessee. By Arnold L. Brokaw, John Rodgers, Deane F. Kent, Robert A. Laurence and Charles H. Behre, Jr. Pp. iii+56+plates 1-4. (Washington, D.C.: Government Printing Office, 1966.) [711]

Organization for Economic Co-operation and Development. Economic Growth 1960-1970—a Mid-decade Review of Prospects. (Economic Studies.) Pp. 113. (Paris: Organization for Economic Co-operation and Development; London: H.M. Stationery Office, 1966.) 10 francs; 15s.; \$2.50. [711]

Stanford Ichthyological Bulletin. Vol. 8, No. 3: Megalomycteridae, a Previously Unrecognized Family of Deep-sea Cetomimiform Fishes Based on Two New Genera from the North Atlantic. By George S. Myers and Warren C. Freihofer. New Distributional Records of the Butterfly Fish *Chaetodon falciifer*. By Warren C. Freihofer. Description and Osteology of *Lepidarchus adonis*, a Remarkable New Characid Fish from West Africa. By Tyson R. Roberts. Pp. 193-227. (Stanford, California: Division of Systematic Biology, Stanford University, 1966.) [711]

Australia: Prime Minister's Department. The Commonwealth Government in Education. Pp. 56. The Design of Science Rooms. Prepared by The Commonwealth Advisory Committee on Standards for Science Facilities in Independent Secondary Schools. Pp. 56. (Canberra, A.C.T.: Prime Minister's Department, 1966.) [711]

United States Department of the Interior: Geological Survey. Professional Paper 542-D: The Alaska Earthquake, March 27, 1964—Effects on Communities in the Homer Area, Alaska, by Roger M. Waller. With a Section on Beach Changes on Homer Spit. By Kirk W. Stanley. Pp. vi+28+plate 1. \$0.65. Professional Paper 543-C: The Alaska Earthquake, March 27, 1964—Regional Effects. Gravity Survey and Regional Geology of the Prince William Sound Epicentral Region, Alaska. By J. E. Case, D. F. Barnes, George Pfafker and S. L. Robbins. Pp. v+12. \$0.20. (Washington, D.C.: Government Printing Office, 1966.) [711]

Deutsches Hydrographisches Institut, Hamburg. Jahresbericht Nr. 20 für das Jahr 1965. Pp. 134. (Hamburg: Deutsches Hydrographisches Institut, 1966.) [811]

Indian Forest Records (New Series). Statistical, Vol. 1, No. 2: Standard Volume Tables for Bori (Hoshangabad) Teak. By Sabita Das. Pp. 51-60. (Delhi: Manager of Publications, 1966.) Rs. 1.05. [811]

The Carlsberg Foundation's Oceanographical Expedition Round the World 1928-30 and Previous "Dana" Expeditions. "Dana" Report No. 70: Ocular Anatomy of some Deep-Sea Teleosts. By Ole Munk. Pp. 62+16 plates. (Copenhagen: Andr. Fred. Høst and Son, 1966.) 25 Danish kr. [811]

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United States Department of the Interior: Geological Survey. Professional Paper 527-G: Evaporation Study in a Humid Region, Lake Michie, North Carolina. By J. F. Turner, Jr. Pp. iv+137-150. \$0.20. Professional Paper 486-C: Hydrologic Regimen of Salton Sea, California. By Allen G. Hely, G. H. Hughes and Burdige Ireland. Pp. iv+32. \$0.80. Professional Paper 523-C: Late Pleistocene Marine Paleogeology and Zoogeography in Central California. By W. O. Addicott. Pp. iii+21+plates 1-4. \$0.35. Professional Paper 523-E: *Tinsleya*, a New Genus of Seed-bearing Callitrid Plants from the Permian of North-Central Texas. By Sergius H. Mamay. Pp. iii+15+plates 1-3. \$0.30. (Washington, D.C.: Government Printing Office, 1966.) [911]

United States Atomic Energy Commission. 1966 Financial Report. Pp. vi+50. (Washington, D.C.: Government Printing Office, 1966.) \$0.50. [1011]

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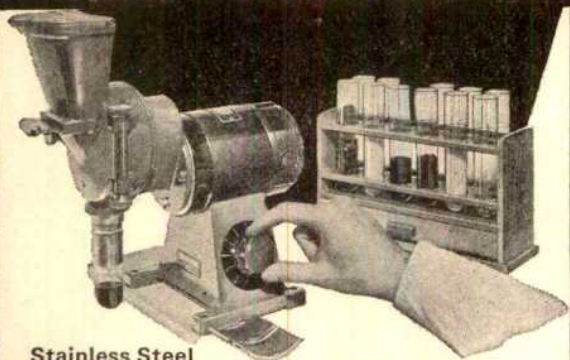
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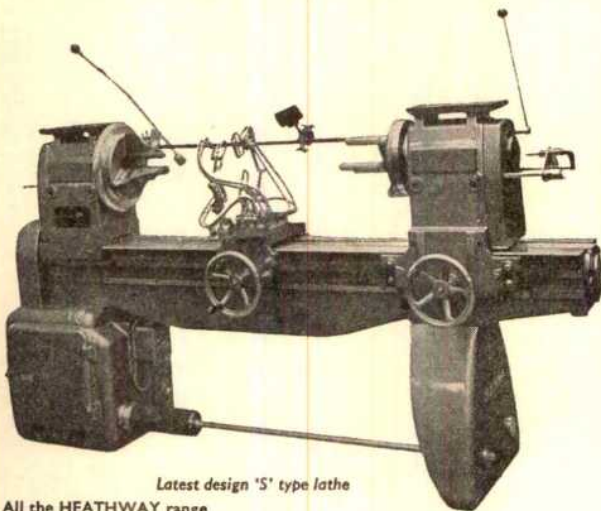
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Further particulars and forms of application (quoting A62/66), can be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.1, with whom applications should be lodged by February 18, 1967. (397)

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The appointee will be required to develop a research programme related to an important problem of the livestock industry and to lead the present biochemical work of the laboratory, which is concerned with certain bacteriological diseases of sheep and with studies on host-parasite relationships, particularly with regard to helminth infections of sheep. At present, five professional workers are engaged in biochemical research and there is a vacancy for another biochemist which could be filled in accordance with the interests of the appointee. Adequate laboratory space, technical assistance and experimental animals are available. The laboratory is well-equipped with modern instruments, including gas-chromatography, spectrometry and those required for radio-isotope studies.

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Mr. R. F. Turnbull, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2.

to whom applications (quoting Appointment No.: 202/293) should be addressed by the **14th April, 1967**. (428)

## **MARINE BIOLOGISTS NATIONAL MUSEUM OF CANADA OTTAWA, CANADA**

A Canadian Oceanographic Identification Centre, under the sponsorship of the International Biological Programme, has been established at the National Museum to provide identification services in zooplankton, benthos, fishes and phytoplankton for qualified research institutions.

Two positions will be available on April 1, 1967, one to be filled by an experienced biologist who will act as head of the Centre and the second by a more recent graduate.

Applicants should have a Ph.D. plus related experience and publications in planktonology, oceanography and marine biology. Salary will be commensurate with training and experience and will be in the range \$9,500–\$15,000.

**Résumés should be submitted to the Civil Service Commission, Ottawa 4, Canada, quoting file reference 67-100KM. For further information, write to the Director, Natural History Branch, National Museum, Ottawa, Canada. (352)**



## WHAT MR. WEBB SHOULD DO

It is sad that the three American astronauts should have been killed at Cape Kennedy a week ago. They were brave men. Like everybody else, they knew that there must be a fatal accident sooner or later. Indeed, it is a wonder that it has not happened before. Even the irony that the accident now should have happened at a rehearsal, not the real thing, is not entirely unexpected. In the circumstances, of course, it is quite inevitable that Mr. James Webb, the administrator of the National Aeronautics and Space Administration, should have announced that the Apollo programme will continue. Now that so much effort has been spent, it would be folly to turn back.

But what are the chances of success or failure? Mr. Webb would be the last to conceal the uncertainties which remain. Indeed, for several years he has left Congress in no doubt of how the Apollo programme has been trimmed down to its bare essentials, chiefly so as to keep the budget within acceptable bounds. It is no surprise that some of the tasks which remain to be performed before a man can travel to the Moon are almost hair-raising in their difficulty and novelty. The Saturn V rocket still has to leave the ground as an integrated assembly; so far, only the booster has flown on its own. It is unlikely that the landing of Surveyor rockets on the Moon has already provided a good understanding of the difficulties of using rockets as a means of settling gently on a distant surface—though in this respect the intervention of men could be a help and not a hindrance. The problems of arranging for bits and pieces of rockets to reunite in orbits about celestial objects are unfamiliar, to make the lightest of them. Yet hazards such as these have been obvious for some time. The accident a week ago, by contrast, has not previously been given much public attention. Inevitably, the way ahead must now seem even more hazardous than at the end of 1966. If Mr. Webb were now to go to Congress for more money, the chances are that he would have a sympathetic hearing. His difficulty is that he is short of time as well as money. Making the Apollo programme more deliberate would probably imply that the old target of "before the end of the decade" would have to be forgotten.

But would this be a tragedy? Would it matter if the first American to reach the Moon did not arrive until 1970? Or 1971? Or even 1981? The truth is that the objectives which the Apollo programme has set itself are largely arbitrary. They are numbers out of a hat. There may have been something in the argument frequently advanced by NASA at the beginning of the Apollo programme that a tighter programme would be better co-ordinated and even cheaper, but that was always founded on the assumption that the plan proposed was feasible and safe. Now what force it may have had is substantially diminished.

The promise of incidental technological benefits for industry in the United States has not been fulfilled. The side effects there have been do not match in any way the cost of the Apollo programme, and most of those that have some substance are attributable to the whole programme of rocket and satellite development and not just to the part of it concerned with sending men away from the Earth. It is also clear that international prestige is neither dependent on nor won by success in launching men with rockets. The real world is more subtle and more interesting than that, and it has changed a lot in five years. But now even the old Everest argument that Americans must travel to the Moon "because it is there" will seem a little less like courageous daring. Its essential irrationality will be more apparent. In the circumstances, if Mr. Webb has to go back to Congress for a substantial revision of the Apollo programme, he will find more outright opposition as well as more sympathy.

What then should happen? Given the existing commitment to the Apollo programme, it is only sensible to continue. At the same time, however, any means of making the programme more deliberate should be seized on. The extra cost and time involved in mounting more test launchings of the critical pieces of equipment would not be outrageous. If the target of a landing on the Moon sometime this decade should become unattainable, nobody should be made to feel ashamed. But such lessons as there are to be learned from the accident at Cape Kennedy will bear on future programmes, not Apollo. They will serve to reinforce the tendency already apparent in the United States to shy away from spectacular programmes. The budget for NASA for the financial year beginning in July (see page 431) shows that the Administration has taken a sober view of what should lie ahead. There are no funds for further spectacular adventures. Instead, NASA is to be encouraged to make the fullest use it can of the rockets that will have been developed by the end of Apollo. This is sensible, and in line not merely with the recommendations published a year ago by a committee of the National Academy of Sciences in Washington, but also with the growing disenchantment with space travel among people and politicians. In the years since Apollo was begun there has grown up in the United States a healthy preoccupation with real problems on the surface of the Earth. Inevitably, the surface of the Moon has come to seem less exciting and less important. That is entirely as it should be.

## UNIVERSITY GRANTS

THE latest report of the University Grants Committee (see page 434) has done very little to clarify the relation-

ship between the British Government and British universities. In many ways it is a curiously complacent document. Although it recognizes that stresses are accumulating within the system, the committee makes no outward sign to show that it recognizes how far-reaching some of the consequences may be. Instead, the report leaves the impression that the committee is convinced that outstanding problems can be dealt with by comparatively minor changes—an extra deputy chairman here, an extra sub-committee there, and a general exhortation to the universities to look ahead as well as to the past. The trouble, of course, is that the University Grants Committee may be overtaken by events. The demand by the Public Accounts Committee that the books of the universities should be opened to public inspection is a sign of how the wind is blowing, if not a serious threat to academic freedom in its own right. At the same time it is now plain that the universities—and, for that matter, the whole system of higher education—are now so huge and so complicated that a much more professional management is required than circumstances now provide. What is to be done?

The first need is to decide what the University Grants Committee is for. It is a long time since it could serve as a kind of insulator between the public purse and the universities. When the university system was smaller, when the cost of operating it was less, and when university education appeared to be a kind of academic luxury and not a central pivot of a nation's economic strength, it was comparatively easy for the British Government to agree every five years to spend a certain sum of money and then to allow a distinguished and impeccable band of academics to share this out among themselves and their colleagues.

But all this has changed. The government quite properly expects to see that the increasing sums of money spent on the universities are properly spent, and the University Grants Committee has in any case been forced to keep a closer watch on how money is spent in the universities in order that it can make a proper case on their behalf. One obvious danger in these circumstances is that of being turned into a glorified book-keeper—a kind of Auditor-General working from within. Another is that the University Grants Committee, lacking all but the most negative influence on the universities but saddled with responsibility for presenting their case to the outside world, will not be able to urge the universities towards desirable innovation and experiment. Indeed, pushed this way and that as it has been by circumstances, the committee has too often been required to foster uniformity and dullness. There is a case for saying that if it is to continue to exist at all, it should be made stronger and more independent—and more responsible. Indeed, with existing institutions, it is even possible that it will only be possible for individual universities to be given a real sense of their public responsibilities if the University Grants Committee is less committed to the defence of what is called academic freedom, and more

able to thump the table from time to time. The best analogy is that of the nationalized industries and similar public corporations in Britain and elsewhere. The objective is to transfer day-to-day responsibility for some particular task from the government to some other body of men without compromising in any way the right of the government to have its say on broad matters of policy and finance. The corporation is given the responsibility and—just as important—the resources to see that things are well administered. If they are not, presumably the chairman can be sacked. Among the advantages of such an arrangement in the management of the British universities would be that the U.G.C. could then hope to carry out the continuing review of higher education and the demand for it which is essential if orderly progress is to replace the process of transformation by occasional upheaval which served well enough when the system was smaller. In such an arrangement, it would also be possible for the University Grants Committee to carry out the kind of studies on which the qualitative improvement of the university system must in the long run be based. From audio-visual teaching aids and novel kinds of examinations to better means of forward planning, there is a host of innovations to be attempted. As things are, there is a danger that some innovations are overlooked altogether, and that others are tried out on too small a scale in widely scattered universities. A stronger Grants Committee could help enormously to give the system greater cohesion.

But would not a stronger University Grants Committee be as great a threat to the independence of the universities as inspection by the Auditor-General or even direct control by the Department of Education and Science? This is what many academics will instinctively say. The simple answer is that this danger is much less serious than many others. For one thing, there is no reason to fear that an independent corporation asked to find the best match between higher education and the public need would fail to appreciate not merely the importance of real independence in the universities but also the folly of attempting anything else. But in any case, whether the real power lies with departments of government or with the University Grants Committee, there is an urgent need that the universities should be equipped with the machinery of what is sometimes called, in other connexions, collective bargaining. This, with luck, is what the Committee of Vice-Chancellors and Principals will become. In the past few months, the committee has undertaken several tasks which show that its heart is in the right place, though there is still some way to go before it will be in a position to resist all pressure. The most urgent need in the months ahead is to find some way of channelling the energies of the universities through the committee. In the process, it is inevitable that individual universities will have to bury some of their often wayward and idiosyncratic tendencies, but it is in any case time for that to happen.

## NEWS AND VIEWS

### Retreat on Drugs

THE British Government is about to abandon its traditional position on the treatment of drug addiction in the United Kingdom, and to go part of the way to meeting the recommendations of the Brain Committee on Drug Addiction which reported in 1965. In particular, the government will arrange for addicts to be treated at regional centres and not, as at present, supplied with drugs by general practitioners. There is, however, no intention at this stage of making treatment in regional centres compulsory as the Brain committee recommended.

The statement of the British Government's intentions was made in the House of Commons on January 30 when Miss Alice Bacon, Minister of State at the Home Office, agreed with critics of the present position that new legislation would be necessary. Earlier the Minister of Health, Mr. Kenneth Robinson, said that there were now apparently 1,000 people addicted to drugs such as heroin and morphine, an increase of more than a factor of two since 1959 (when there were 454 addicts known to the Home Office). Although the number of addicts in Britain is still small compared with that in the United States and Canada, the rate of increase in recent years has worried the authorities as well as their critics. Although the effects of other drugs such as hallucinogens may be less damaging to an individual than those of heroin and morphine, there is at least reason to suspect that increasing use of these drugs among young people may eventually lead to increasing numbers of adult addicts to the drugs officially classified as dangerous.

To study these questions, the Minister of Health is proposing to establish a research unit at the Institute of Psychiatry of the Maudsley Hospital. As yet there seems to be no approved pattern for the centres at which treatment for drug addiction will be provided. In the debate in the House of Commons the government was criticized for failing to recognize how much financial support would be necessary if the problem is to be treated energetically. More should be known on these matters when legislation is introduced in the early summer.

### Budget for Science

THE balance of public support for science and technology in the United States will not be markedly affected by the budget for the fiscal year 1967-68 published in Washington on January 26. The predicted reduction of the cost of space research and development during the fiscal year from \$5,600 million to \$5,300 million is cancelled out by the increase of defence research and development expenditure, which is expected to increase from \$6,700 million to \$7,200 million. Support for the National Science Foundation and the National Institutes of Health will be modestly increased, as will be the research spending of the United States Department of Agriculture. The most striking feature of the budget, however, is the way in which the United States Government is now embarked

on schemes for controlling the environment, natural and artificial. Although much of this work cannot at present be labelled either as research or development, its growth cannot fail to have important consequences for science.

During the fiscal year to 1968, total expenditure on research and development will amount to \$16,147 million, which is divided almost equally between civil and defence research and development. In the three years 1965-68 expenditures under these heads, together with the expenditure of research and development (as such) by the big spending agencies, are given in the budget as follows:

U.S. RESEARCH AND DEVELOPMENT (MILLIONS OF DOLLARS)

	1966	1967 (est.)	1968 (est.)
NASA	5,350	5,310	5,126
NIH	536	680	662
NSF	176	196	226
Total civil	6,049	7,175	7,180
Total military	7,890	8,381	8,967
Total, civil and military	14,839	15,555	16,147

These figures do not include the \$151 million which the National Science Foundation will spend in 1967-68 on the development of new curricula for teaching science in schools and on other educational activities such as the retraining of teachers. With the growth of other activities, and particularly of the processing and handling of information, the total expenditure of the National Science Foundation will amount to \$455 million (compared with \$395 million in 1966-67).

The increase in the estimated expenditure of the Department of Defense on research and development is largely accounted for by the work now being undertaken to develop a more effective ballistic missile to be launched from a submarine. This same project accounts for some of the expected increase in the budget of the Atomic Energy Commission, although that is partly offset by a reduction in the cost of nuclear space rockets and other devices. The cost of space research and development is being reduced now that the principal expenditure on the lunar landing programme lies in the past, but the National Aeronautics and Space Administration is being given \$82 million to embark on further studies of more distant objectives.

The steady increase in the expenditure of the United States Government on environmental studies and the management of natural resources goes back for at least a decade. In 1968 the Administration is proposing to spend \$3,538 million on projects ranging from the desalination of water (\$28 million) and the control of water pollution (\$229 million) to the management of fish and wildlife resources (\$139 million) and the conservation of soil (\$104 million). Expenditure on new transport techniques and the modern planning of cities is also increasing; for example, some \$250 million will be spent in 1968 on "special projects in model cities". It is expected that some seventy cities will be involved in this programme during the financial year for which plans are now being made.

### Transatlantic Comparisons

THE danger that some European scientists may be affected by "an unjustifiable sense of inferiority" in respect of the United States was one of the themes of an address to the Royal Society of Arts in London on January 30 by Sir Gordon Sutherland, Master of Emmanuel College, Cambridge, and previously pro-



fessor of physics at the University of Michigan and Director of the National Physical Laboratory. According to Sir Gordon, European inferiority is the contemporary equivalent of a sense of inferiority among scientists in North America thirty years ago, and which has now "entirely disappeared". But because the United States, by virtue of its greater population and productivity, is bound to play a much more important part in the development of science and technology, it does not follow that countries such as Britain "should throw up the sponge"; rather, Sir Gordon said, scientists in Europe should reflect how it is possible to make small resources accomplish great things, from radio-astronomy to protein structure.

The reasons for the growth of confidence among American scientists are to be found, Sir Gordon believes, at least in part in the structure of the institutions responsible for higher education and for the management of public expenditure on science and technology. Among the sources of strength in American universities he cited the quality of postgraduate teaching, the flexibility stemming from the absence of a hierarchical structure in university departments and, in particular, the presence of several professors in many departments, the freedom of American universities to compete financially for good staff, the attachment to universities of research centres financed from outside sources, and the fact that university staffs in the United States "have never been ashamed" that research work can sometimes be usefully directed towards practical problems. In his comparison of the arrangements in Britain and the United States for providing public support for scientific research, Sir Gordon was somewhat less envious of arrangements in the United States, although he made it plain that he considers the existence of several government agencies able to spend money on science and technology an assurance that good ideas do not go begging for lack of money. At the same time, he admires the way in which the organization of the budget in the United States makes it possible for "a highly professional group of men" without departmental axes to grind to subject the whole pattern of spending to "central scrutiny and co-ordination". Sir Gordon Sutherland went on to hope that the Zuckerman Committee would emerge as the British equivalent of the Office of Science and Technology in the United States. Speaking of the brain drain, which may be considered a tangible expression of feelings of inferiority or otherwise, he said that "it should not be too difficult to devise self-denying ordinances in the form of quotas for permanent immigration"—a statement that will not easily win agreement.

## What Next for Apollo?

WHETHER the United States Apollo programme will be delayed by the accident which killed the crew of the first spacecraft on January 27 is not yet known. The device, which was due to be launched on February 21 by a Saturn IB rocket, is presumably beyond repair, and statements from Cape Kennedy have suggested that at least three months would go by before another can be brought to the launching site and tested. But the most serious potential delay is that the enquiry now being conducted may make it necessary to re-design some parts of the Apollo system.

The past few months have been plagued by a number of technical troubles. The device in which the fire took place a week ago consisted of two of the three components of the spacecraft intended for the Moon. This has been at Cape Kennedy since October 1966. Early in the programme of testing, the environmental control system failed and a water heater had to be replaced. There has also been difficulty about the fuel cells, which had at various times threatened to produce less power than the project needs. The latest setback will no doubt take even longer to put right.

Even so, it is by no means impossible that the National Aeronautics and Space Administration will be able to land a man on the Moon before 1970—the original target. Indeed, towards the end of last year, some optimists were talking of 1968 as the year in which the first American might land on the Moon. The next step in the programme, which need not be delayed by the accident a week ago, is to launch the Saturn V booster rocket (with 7.5 million lb. of thrust). This test will provide valuable information about the rocket system and will also help to test the efficiency of the heat shields on the Apollo system. Later in 1967 it had been intended to carry out a complicated operation in which the lunar landing capsule would be put into an orbit about the Earth and then occupied, 24 h later, by one of three astronauts launched separately. This experiment will no doubt now be postponed.

## Canadian Appointments

OPERATIONAL Research at Canadian Forces Headquarters in Ottawa has a new Director-General, Dr. George R. Lindsey. He succeeds Dr. R. J. Sutherland, who died recently. Dr. Lindsey graduated from the University of Toronto in 1942, and served with the Royal Canadian Artillery and in operational research with the British Army during the War. In 1946 he worked at the Cavendish Laboratory in Cambridge, and carried out post-graduate work in high energy physics, obtaining his doctorate in 1950. He has worked for the Canadian Defence Research Board, the Canadian Air Force, and the Department of National Defence, and in 1961 was for a year chief of the operations research groups at the NATO station at La Spezia. His work in recent years has been concerned with air defence and ballistic missile defence, and naval matters.

In the background is the question of what Dr. O. M. Solandt will do when his term as Vice-President in charge of Research and Development at Hawker Siddeley of Canada ends shortly. Dr. Solandt, who was a lecturer in physiology at Cambridge in 1939, has also been concerned with operational research. It has been suggested that he might become President of the National Research Council, or return to his old job as Chairman of the Defence Research Board.

## New Radio-telescope

THE Science Research Council has awarded a grant of £45,000 to the University of Manchester for a design study for a new radio-telescope. At the Jodrell Bank Observatory, which will naturally be in the thick of the design process, it is hoped that the new instrument may be a telescope 400 ft. or so in diameter, and capable of high resolution. The cost could easily amount to £4 million—a sum which exceeds by a factor of more

than four the cost of the 250 ft. telescope with which Jodrell Bank pioneered the construction of very large steerable telescopes a decade ago. Evidently the experience of the past few years has persuaded the designers that it will be possible to construct a telescope of this size without presenting the Science Research Council with an impossibly large bill.

As yet, there has been no final decision about the location of the new instrument. It has been arranged that the Atomic Energy Authority should act as an agent for the Science Research Council, supervising the process of design and collecting whatever information will eventually be necessary for making a decision. With luck, design will be complete by the end of this year, so that a prompt decision by the Science Research Council would make it possible to have the new instrument in service by 1972.

## Initial teaching alphabet

In 1960, the University of London Institute of Education and the National Foundation for Educational Research in England and Wales decided jointly to support an experimental study of a simplified and more regular alphabet to be used to teach children to read. The Reading Research Unit, which was formed to undertake the study, has now released its findings (*The i.t.a. Symposium, Research Report on the British Experiment with i.t.a.*, by John Downing, 25s. National Foundation for Educational Research). They justify the enthusiasm of the early pioneers of simplified reading. The alphabet used, Sir James Pitman's forty-four character initial teaching alphabet (i.t.a.), was used in forty-one schools to teach 873 children to read. The same number of pupils were taught by the conventional method. The unit took considerable care that the tests should be as objective as possible, given the notorious difficulties of controlled tests on human subjects.

The children taught with the i.t.a. learned faster than those taught by the normal method. At the age of eight (after three years at school) they were six months in advance of their normally taught contemporaries. Their ability in comprehension tests was greater, and their essays were as much as 50 per cent longer. Curiously enough, the i.t.a. children were even able to spell better after four years than were children taught by the traditional method. The report concludes that "the traditional orthography of English is an important cause of difficulty in learning to read". The children taught with the i.t.a. cannot of course entirely escape the effects of this problem—there is a setback when they finally go over to using the traditional letters, but it is not as great as might have been thought.

The report emphasizes that i.t.a. is not necessarily the best simplified alphabet that can be devised. There is obviously further scope for research into reading and methods of teaching it. Such research might well improve considerably the "productivity" of infant schools, and even pave the way to a simplified alphabet for adults. It is depressing then to find that the report may well have been the last fling of the Reading Research Unit; Mr. Lionel Elvin of the Institute of Education thinks it may be forced to close in May unless further funds are made available. The work of the unit so far has opened up a host of new questions

about teaching methods and it deserves a chance to investigate them more fully.

## Collaboration on Abstracts

THE United Kingdom Atomic Energy Authority has come to an arrangement with *Nuclear Science Abstracts* by which the British literature cited in the abstracting journal will be abstracted in Britain and then sent to the United States for publication. Work on the scheme has already begun and the Atomic Energy Research Establishment at Harwell is responsible for co-ordinating the British effort. The agreement on collaboration, which has the blessing and partial financial support of the Office of Scientific and Technical Information, gives the Atomic Energy Authority responsibility for selecting which items in the British literature shall be abstracted. Later on it is planned that the authority shall also supply *Nuclear Science Abstracts* with indexing terms.

One of the arguments in favour of collaboration is that the libraries of the Atomic Energy Authority are already engaged on similar work to that of *Nuclear Science Abstracts*, so that duplication of effort will be avoided. Similar considerations have led to the decentralization of work on the Canadian and Japanese literature, and no doubt other agreements with *Nuclear Science Abstracts* will follow. To the journal itself, which is at present abstracting nearly 50,000 items a year, it will also be a great advantage that the cost of the operation will be shared with other countries; recently the journal has been under pressure from Washington to ensure that its size and cost do not grow exponentially but, rather, remain constant. At the same time, the efficiency with which the journal has operated since its foundation in 1947 has raised in some quarters the fear that devolution of responsibility may mean less speedy service. From this point of view, further extensions of the international agreement will be scrutinized with care. It also remains to be decided how *Nuclear Science Abstracts* will develop in the years ahead. Changing over to publication on magnetic tape may be tempting but is probably at present unacceptable. Forms of collaboration still have to be worked out with international organizations such as the International Atomic Energy Agency and the EURATOM organization in Europe, both of which are known to be interested in information processing. Yet another possibility, however remote, is that *Nuclear Science Abstracts* will wither away as the subject matter with which it is primarily concerned is transformed either into commercial engineering or into physics, chemistry and other more familiar disciplines.

## Decimal Pound

THE British Government seems to be determined not to change its mind on the decimalization of the British currency. In the House of Lords on January 30, Lord Winterbottom, Parliamentary Secretary at the Ministry of Public Building and Works, said that the government did not believe the difficulties of making a transition to the system based on a pound rather than a unit half as big would be anything like as great as critics made out. He suggested that the critics were trying to frighten ordinary people and that the decimal currency board would ultimately launch a campaign

of public education to put people's fears at rest. Lord Halsbury, the chairman of the committee which recommended that the pound rather than ten shillings should be used as the basic unit, backed up the government with the argument that the choice of the pound would ensure that people were not cluttered up with "unwanted coins". And Lord Shackleton, opening a debate on the subject for the government, said that, even though he welcomed the opportunity for discussion, it should be clear that "the government had made up its mind".

## University Money

THE University Grants Committee this year combines its annual report with the review of university development in Britain in the first four years of the present quinquennium from 1962-64 (H.M.S.O., Cmnd. 3192, 4s.). The committee, now twenty-one strong and on a part-time basis, rejects suggestions that it should be radically reduced in size and made professional. Its present size is "probably about right". The U.G.C. now has more sub-committees from which it can seek advice; new ones, covering Arts, Audio-Visual Aids, Biological Sciences, Education, Mathematics, Physical Sciences and Social Studies, have been set up.

The new quinquennium—the five-year period for which the U.G.C. establishes grants—begins on August 1, 1967. Grants for the whole period will not be announced for several months, but a provisional announcement has already been made of the minimum level of the recurrent grant in the first year of the quinquennium. On December 20, 1965, the Secretary of State for Education and Science said that the recurrent grant (which excludes capital investment in building and equipment) would be £147.5 million. This figure excludes the cost to the U.G.C. of taking over certain research projects from the research councils and also a contribution of roughly £6 million which the central government will eventually make towards local authority rates (taxes). The comparable figure for 1966-67 is £133.2 million, which implies an increase of 10.7 per cent. The U.G.C. has now allocated this sum to the individual universities so that they can plan their recruitment of staff for 1967-68 and also indulge in more elaborate forward planning if they are so inclined. The U.G.C. is plainly hoping to encourage the universities to make fuller use of modern management techniques. Organization and method can hardly be said to be spreading like wildfire through the British universities, though, and the U.G.C. has enlisted the Committee of Vice-Chancellors and Principals to fan the flames. Organization and method exercises have been mounted in north-east England and in Scotland; several other universities, the report hints, are about to take the plunge.

The report also announces a new type of grant which should be available in 1968-69, if any university is thinking ahead that far. This will be an equipment grant, designed to enable universities with old departments to re-equip them occasionally. At present new equipment is supplied with new buildings, so that university departments tend to be either new from top to bottom, or antique both in fabric and equipment. Most, of course, have been buying equipment piecemeal from their recurrent grants, but the new proposals would make special grants available for this purpose.

After the publication of the report, Sir John Wolfenden, chairman of the U.G.C., suggested that an investigation of the way university laboratories are used might well be profitable. Once again the U.G.C. is nominating the Committee of Vice-Chancellors and Principals for the job, in collaboration with the Confederation of British Industries. (The investigation would be on the same lines as that conducted by Sir Gordon Sutherland and his committee into government laboratories, which will shortly be published.) Sir John Wolfenden also said that universities which are deterred from undertaking research contracts from industry because these are short-term operations should get in touch with the U.G.C., which will occasionally be prepared to take over in the same way as it has been doing for contracts with the research councils.

## Nottingham Agriculture

THE University of Nottingham has decided to amalgamate the departments of agriculture and horticulture into one "Faculty of Agricultural Science". This decision will no doubt bring cheer to the University Grants Committee, which has recently been attempting to encourage the rationalization of agricultural teaching at British universities. The registrar at Nottingham, Mr. A. Plumb, explained earlier this week that the university had suggested a "re-alignment" in view of the imminent departure of the professor of agriculture, Dr. J. P. Hudson, for Bristol, and had not had to wait for prompting from the U.G.C. A statement by the university says that the present department of agricultural sciences will be divided into two—a department of physiology and environmental studies and a department of applied biochemistry and nutrition. This will emphasize the increasingly scientific character of agricultural education. The school of agriculture at Nottingham is in fact the second largest in Britain with an entry of 93 undergraduates in 1965-66 and with five professors, two readers and 26 lecturers on the staff. The department of agricultural economics will be unaffected by the proposed change.

## Living on Air

A boost in British hovercraft development may result from a decision announced by the Minister of Technology, Mr. Anthony Wedgwood Benn, on January 24. The Technical Group of Hovercraft Development Ltd., a subsidiary of the National Research Development Corporation, is on April 1 to become the responsibility of the National Physical Laboratory. Almost all the activities of Hovercraft Development Ltd. were concentrated in the Technical Group at Hythe, so that NRDC is left with little but the power to issue licences for hovercraft development. The move seems sensible if only to make use of the considerable experience of the Ship and Aerodynamics Divisions at the NPL; the Ship Division has been working on air cushion vehicles for some time, both in collaboration with HDL and on its own account. The performance of free flight models in waves has been studied, in experiments designed to be of direct value to hovercraft engineers. A constant force towing dynamometer using a slipping clutch has been designed, allowing the model to surge 30 ft. downstream quite freely, and minimizing the risk that it will overturn.

More rapid progress would be welcome, since developments elsewhere in the world are beginning to make British efforts look leaden footed. In France, the Aerotrain test vehicle, propelled by a temporary rocket attachment, reached a speed of 188 m.p.h. along its short test track recently. In Britain, the corresponding project is still at the model building stage, and seems to have raised no enthusiasm in British Rail. On the naval side, there is interest in vehicles in which the sidewalls are immersed so that the air cushion is trapped; these are particularly suitable for comparatively low speeds. Military strategists on both sides of the Atlantic should by now have firm ideas about how useful hovercraft are likely to be in a military role, since both the United Kingdom and the United States have used them in operations in the Far East.

At the moment, too many hovercraft projects in Britain seem to be hanging fire, which makes it easy for American companies to coax British engineers to emigrate. NRDC, whose source of funds is now the Ministry of Technology, has spent more on hovercraft than on any other project, but the total since 1958 is only £2.5 m. This is derisory in comparison with the share in the Anglo-French variable geometry project—at least £100 m—which Britain has cheerfully taken on. Perhaps Mr. Benn's enthusiasm will divert similar largesse towards the NPL when it starts work; until then the British effort, like the hovercraft themselves, appears to have no visible means of support.

## X-Ray Camera

THE resolution of X-ray diffraction patterns is increased if the X-ray beam is well collimated. While good resolution is always important in crystallography, it is particularly important when partially ordered materials are being studied, since even in the ideal case the patterns are likely to be diffuse. If very fine apertures are used to provide a collimated beam, the exposure time is greatly increased, and may run into weeks. The Franks camera, commonly used for work on partially ordered materials at low Bragg angles, focuses and collimates the beam by reflexion from two mirrors at grazing incidence, but suffers from the disadvantage of a very low angular aperture. Reflexion at grazing incidence has now been used by Dr. A. Elliott of the Department of Biophysics at King's College, London, for a new camera which he has developed for work at higher Bragg angles. (For spacings  $< 50 \text{ \AA}$ .)

In Dr. Elliott's camera the X-ray beam is focused as it passes along a capillary tube the internal profile of which is barrel-shaped. The tube, about 3 mm wide at its widest point and several cm long, is coated internally with gold, which has the highest known reflexion for X-rays. Even so, for copper  $K\alpha$  radiation the glancing angle is only about  $\frac{1}{2}^\circ$ ; a similar design built in the United States by Henke and DuMond (*J. Appl. Phys.*, **26**, 903; 1955) was not suitable for X-rays of wavelength shorter than aluminium K radiation, for which the glancing angle is three times greater. Dr. Elliott has succeeded in producing a mirror small enough for use with copper  $K\alpha$  radiation by a casting technique with epoxy resins.

The camera is suitable for high angle work; the difficulty at low Bragg angles is that scattering of the main beam interferes with the pattern. At high

angles, however, the camera gives about a ten-fold increase in intensity and very good definition, as well as acting as a monochromator. The high angle diffraction pattern of muscle has been studied in greater detail than before, and liquid crystals have also been studied (Parry and Elliott, *Nature*, **206**, 616; 1965). The design can also be used in powder cameras, and is being used in X-ray fluorescent analysis by Mr. R. W. Fearnhead at the London Hospital Medical College. The method has not yet been applied at King's for the study of active muscle, despite the impression given by a previous item in *Nature* (**212**, 969; 1966), but it is hoped that this will be done in the future. The advantages of the camera should become more generally available soon, when it is marketed commercially.

## Geophysics in India

THE annual meeting of the Indian Geophysical Union was held on January 5 this year. In his presidential address Dr. M. S. Krishnan spoke about the structure of south-east Asia with particular reference to the Mesozoic and Tertiary island arcs. The chief guest at the meeting was Dr. Atma Ram, Director-General of the Indian Council of Scientific and Industrial Research, who emphasized the importance of geophysics in exploring the natural resources of the country at a time when India desperately needed natural resources. The Krishnan medal for the year 1966 was awarded to Dr. H. M. Iyer for his outstanding contribution in the field of seismology. The medal for 1965 was presented to Dr. D. Lal at the meeting. Honorary Fellowships of the Indian Geophysical Union were conferred on the following: Dr. K. R. Ramanathan, Dr. Maurice Ewing, Dr. Tuzo Wilson, Professor V. V. Belousov, Sir Edward Bullard, Dr. T. Nagata and Madam Kosminskaya.

## East African Academy

MAN in his East African environment was the principal theme of the fourth symposium of the East African Academy, which was held in Kampala in September. In the opening address the vice-president of Uganda, Mr. J. K. Babuha, challenged the academy not to "look at East Africa and its problems from the dizzy heights of [an] academic ivory tower", but to use its knowledge to help the less fortunate to a better way of life. In five plenary sessions, evolutionary and cultural aspects of life in East Africa were discussed. The role of nationalism, urbanism and tribalism in contemporary Africa was examined, and in another session the changing pattern of disease and the effects of environmental change on this were traced. In a special plenary session the president of the academy, Dr. W. K. Chagula, discussed the role of the educated East Africans in developing their countries, and Dr. Chidzero of the United Nations, from Nairobi, in the distinguished lecture—a new feature of this symposium—examined the reasons for international aid programmes and the need to expand them. These programmes must be synchronized with local efforts if living standards are to be much improved. There is a danger, he warned, of using economic assistance either to provide jobs for international civil servants or as a camouflage to sabotage the political independence of recipient countries.

Four of the physics papers, given in the disciplinary section, were geophysical in content, which reflects the fact that perhaps 80 per cent of physics research in the three colleges of the University of East Africa is concerned with investigation of the environment. An Institute of Tropical Geophysics was proposed, as a centre of expertise and a stimulus for the progress of physics in tropical universities. Half of the biological papers given were parasitological, most of them from the East African Trypanosomiasis Research Organization; much research into sleeping sickness, which has long been a barrier to human settlement and farming activities, is in progress. The possibility of using antigenic classification for trypanosomes was discussed in relation to the biological control of the disease in cattle. A survey of trypanosomiasis in the fly-belt region revealed that the area of tsetse fly invasion is increasing, mainly because of the encroachment of game into some stock areas. Another zoological paper described how the breeding seasons of equatorial free-tailed bats coincide with the rainfall peaks of Uganda. Such research is valuable, for at least fourteen different viruses have been isolated from bats at the East African Institute of Virus Research.

### Nature by Regions

DURING 1966, 3,387 scientific communications of all kinds were published in *Nature* and the following list shows that these originated in a total of 59 countries. As in previous years, the United Kingdom and the United States each contributed roughly a third of them. The countries of the British Commonwealth and Western Europe were the principal sources of other communications although contributions from Japan and the Soviet Union are a large and growing feature of the journal. It is now some years since there have been communications to *Nature* from the Chinese People's Republic.

Argentina	13	Korea	1
Australia	184	Lebanon	1
Austria	12	Malaysia	6
Belgium	27	Mexico	1
Brazil	5	Monaco	1
Bulgaria	2	New Zealand	31
Canada	112	Nigeria	8
Ceylon	2	Norway	26
Chile	8	Pakistan	1
China (Taiwan)	4	Philippines (The)	1
Czechoslovakia	34	Poland	15
Denmark	13	Portugal	3
Egypt	6	Rhodesia	2
Finland	23	Romania	9
France	41	South Africa	38
Gambia, The	1	Spain	12
Germany	53	Sweden	55
Ghana	5	Switzerland	22
Greece	4	Sudan	1
Holland	64	Tanzania	4
Honduras	1	Thailand	2
Hungary	19	Turkey	3
India	69	Uganda	4
Iran	1	United Kingdom	1,140
Ireland (South)	16	United States	1,045
Israel	26	Uruguay	1
Italy	52	U.S.S.R.	55
Jamaica	2	Venezuela	4
Japan	76	Yugoslavia	11
Kenya	9		

### Parliament in Britain

IN reply to questions in the House of Lords on January 23, Baroness Phillips said that outbreaks of disease affecting salmon, sea trout and brown trout had occurred during the past two months in the rivers Conder, Crake, Gilpin and Kent in Lancashire, the River Eden in Cumberland, and the rivers Dee, Annan and Nith in south-west Scotland. The position was sometimes obscured by the natural mortality among spawned fish at this time of year. In a written answer in the House of Commons on January 24, Mr. J. Hoy, joint parliamentary secretary, Ministry of Agriculture, Fisheries and Food, stated that extensive tests at the Waterville fish farm and on fodder fish elsewhere had disclosed no grounds for believing that the farm is responsible for the disease. The cause had not yet been established, but a leading authority in the United States had stated that he had not found among infected salmon in Ireland the particular organism responsible for columnaris disease in open waters and in some fish hatcheries in America: neither had that organism been isolated from stocks of rainbow trout or from fodder fish used in the Waterville fish farm.

IN reply to questions in the House of Commons on January 24, the Minister of Technology, Mr. A. Wedgwood Benn, said that the terms of reference of the Advisory Board on Relations with the Universities, set up with Dr. S. C. Curran as chairman, were "to consider and report on proposals for the formation and development of Institutes of Advanced Technology and to keep under review collaboration between the Ministry of Technology and the universities and other higher educational establishments on projects designed to promote technological progress in industry".

IN written answers on the same day, Mr. Benn stated that in the financial years 1965-66, £365,000 of Government money was committed to the computer industry under the Advanced Computer Techniques Project while the National Research Development Corporation had made a further £1.6 million available. Development since the National Electronics Research Council was set up in July 1964 had profoundly affected the role of the Council which would now become the National Electronics Council under the Ministry of Technology with a more limited interest in research but responsibility for the impact of major developments in electronics on society. It would consider and advise the Government on the application of electronics to national life and, if it saw fit, would take steps to promote research or other actions. The membership of the governing body of the Council would be widened and the Secretariat would be provided by the Ministry of Technology.

IN reply to questions in the House of Commons on January 24, Dr. J. Bray, parliamentary secretary to the Ministry of Technology, said that construction of the Steam Generating Heavy Water Reactor at Winfrith Heath was on schedule and it should be on power this autumn. Government expenditure of £85,000 on whisker technology last year was shared by the Ministry of Aviation, the Atomic Energy Authority and the Science Research Council. The future of the programmes in this expanding field was currently under review and there was room for an increase in the £5,000 at present sponsored with the universities.



## University News:

## Edinburgh

PROFESSOR W. L. M. PERRY, professor of pharmacology, and Professor D. Talbot Rice, professor of fine art, have been appointed vice-principals of the university as from January 23.

## London

DR. L. HOUGH, reader in the University of Bristol, has been appointed to the chair of chemistry, tenable at Queen Elizabeth College. The title of professor has been conferred on the following: Dr. P. Alexander (radio-biology, in respect of his post at the Institute of Cancer Research: Royal Cancer Hospital); Dr. D. F. Cheeseman (biochemistry, in respect of his post at Bedford College); Dr. D. R. Hughes (mathematics, in respect of his post at Westfield College); Dr. J. F. McGhie (organic chemistry, in respect of his post at the Chelsea College of Science and Technology).

## Manchester

DR. F. LIONS, of the University of Sydney, has been appointed to a nine months visiting readership at the university. Dr. Lions, whose appointment has been financed by a Science Research Council grant, is a leading authority on the synthesis of heterocyclic compounds.

## Appointments

SIR HAROLD HIMSWORTH, Secretary of the Medical Research Council, has been appointed Deputy Chairman and a member of the Council for the duration of his tenure of office as secretary. Lord Platt becomes a member of the Council.

## Announcements

At the end of the annual Winter Meeting of the American Institute of Physics this week in New York, Dr. Charles H. Townes, professor of physics at the Massachusetts Institute of Technology, assumed office as president from Dr. John Bardeen of the University of Illinois. The president-elect is Professor Luis Alvarez of the Lawrence Radiation Laboratory of the University of California at Berkeley, who works in high energy physics. Dr. William Havens of Columbia University has been appointed executive secretary of the institute, and will assume most of the duties of Dr. Karl Darrow, who has been secretary for 26 years. Other appointments to the council are Dr. Shirley Quimby (Columbia University), who will be treasurer, Dr. Walter Gordy (Duke University) and Dr. Robert R. Wilson (Cornell University). Dr. Samuel Goudsmit, hitherto managing editor of the professional publications of the American Institute of Physics, is now to be known as editor-in-chief. He remains the senior of the two editors of *Physical Review Letters*.

THE Linnean Society medals and awards have been awarded to the following: *The Linnean Gold Medal* to Dr. C. E. Hubbard, formerly of the Royal Botanic Gardens, Kew, and C. S. Elton, director of the Bureau of Animal Population, Oxford; *The H. H. Bloomer Award* to Mr. A. G. Long, of Berwickshire High School, Duns, Berwickshire; *The Trail-Crisp Award* to Professor J. Heslop-Harrison, of the University of Birmingham.

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Mathematics*, Prof. M. Deuring (Göttingen); *Chemistry*, Prof. H. Kölbl (Berlin); *Physical Chemistry*, Prof. I. N. Stranski (Berlin); *General Biology*, Prof. M. Hašek (Prague); *Anatomy*, Prof. D. A. Shdanov (Moscow); *Physiology*, Prof. P. G. Kostjuk (Kiev); *Neurology*, Prof. B. Horányi (Budapest); *Dermatology*, Prof. F. Földvari (Budapest) and Prof. W. Ocklitz (Berlin); *Stomatology*, Prof. I. Reichborn-kjennerud (Oslo);

*Hygiene*, Prof. D. Blaškovič (Bratislava), Prof. G. Piekarski (Bonn) and Prof. E. Witebsky (Buffalo).

SIR EDWARD BULLARD is to give the annual Bakerian Lecture of the Royal Society of London.

THE Horticultural Trades Association is offering a prize of £20 for a description, in 90 words or less, of the "F.1 hybrid seed process". The intention is that the "ordinary man in the street" should be able to understand it.

THE London International Engineering and Marine Exhibition and the International Welding Exhibition are to be held in London during April 25–May 4.

THE Lighting Exhibition will be held at Earls Court, London, during April 25–May 4. Further information can be obtained from F. W. Bridges and Sons, Ltd., Commonwealth House, 1–19 New Oxford Street, London, W.C.1.

THE third European Congress of Neurosurgery, organized by the Belgian, Dutch and Portuguese–Spanish Societies of Neurosurgery, will be held in Madrid during April 23–26. Further information can be obtained from Dr. S. Obrador, Eduardo Dato, 23, Madrid 10.

AN international symposium on "Physical Separation Methods in Chemical Analysis", organized by the Analytical Chemistry Section of the Koninklijke Nederlandse Chemische Vereniging, is to be held in Amsterdam during April 10–14. Further information can be obtained from Dr. C. L. de Ligny, Analytical Chemistry Laboratory, The Rijksuniversiteit, Utrecht.

THE twenty-first annual symposium on fundamental cancer research entitled "The Proliferation and Spread of Neoplastic Cells", sponsored by the University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, will be held in Houston during February 27–March 1. Further information can be obtained from The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

A CONFERENCE on "Spark Discharges", organized by the Atomic and Molecular Physics Sub-Committee of The Institute of Physics and The Physical Society, is to be held in the University of Liverpool during April 5–7. The topics will be restricted to spark discharges in gases. Further information can be obtained from the Meetings Officer, The Institute of Physics and The Physical Society, 47 Belgrave Square, London, S.W.1.

THE second international congress for stereology, organized by the International Society for Stereology, will be held in Chicago during April 8–13, under the auspices of the National Science Foundation, the Chicago Medical School and the Chicago Academy of Sciences. Further information can be obtained from the International Society for Stereology, 2020 W. Ogden Avenue, Chicago, Illinois.

A SYMPOSIUM on "Lubrication and Wear in Living and Artificial Human Joints", organized by the Lubrication and Wear Group of the Institution of Mechanical Engineers in association with the British Orthopaedic Association, is to be held at the Institution of Mechanical Engineers on April 7. Further information can be obtained from E. P. Davies, Head of Groups Dept., The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

ERRATUM. In the communication "Depression of Muscle Spindle Function with Vincristine" (*Nature*, 212, 90; 1966) the dose of vincristine given in the twenty-second line should be 2.0 mg/m<sup>2</sup>.

ERRATUM. In the article "Dielectric Absorption of Microwaves in Human Tissues", by J. R. Mallard and D. G. Lawn, which appeared in *Nature*, 213, 28; 1967, the second formula on line 1 of page 29 should read

$$B_j = \begin{bmatrix} e^{i\varphi_j} & 0 \\ 0 & e^{-i\varphi_j} \end{bmatrix} \begin{bmatrix} 1 & R_{j+1} \\ R_{j+1} & 1 \end{bmatrix}$$

# Money for Universities

by our Special Correspondent

Should university finance be subject to inspection by the Comptroller and Auditor-General? The Committee of Public Accounts of the House of Commons has recently considered the question, and has recommended that inspection should begin in 1967-8. The minutes of evidence taken by the committee show that many people doubt the wisdom of this decision.

SIR JOHN WOLFENDEN, chairman of the University Grants Committee, told the committee how the U.G.C. finances the universities. The five year block grant, he said, was seen as imposing on the universities a very serious responsibility. "A university knows for five years in advance, at any rate at the beginning of the quinquennium, what its income will be, and within that income it must stay." The academic in-fighting in the senate, Sir John said, means that if a university spends its money on one thing it cannot spend it on another; this is a built-in inducement to economy. By nature, he added, universities are bound to try to be economical, though he doubted if that was quite the same thing as knowing in detail how to. Asked if the U.G.C. consults the Department of Education and Science about the more expensive building projects, Sir John said that the criterion of cost was not discussed. The arrangement seems to be that the U.G.C. submits a list of building starts to the department, and if the total falls beneath the ceiling established for that year's building starts, the list is approved. Any building start of value more than £30,000, however, is examined individually by the department. Being more specific about the procedure, Sir John described it in terms of a three phase process; first, the universities tell the U.G.C. what they want to do. Then the U.G.C. finds out from the D.E.S. how much money is going to be available, and knowing this, reconsiders the bids submitted by the universities and decides which are the best to back within the ceiling set. D.E.S. approval is then likely to be a formality.

## Cost Control

Moving to cost control, Sir John said that his experience had convinced him that the universities would not object to an exercise in cost control; the difficulties that the U.G.C. had found in trying to work out the costs of an individual student had been caused by the crude and unsophisticated way that it had set about it. Sir John went on to add that there was a danger in this sort of accounting—"I would really rather earnestly plead that this is not simply an arithmetical matter . . . if examination by the Comptroller and Auditor-General resulted simply in pressure for an arithmetical formula as the controlling thing, then I would be very sorry". Sir John also spoke of "centres of excellence", a phrase for which he expressed some distaste. The concept, however, he seems to accept, with the reservation that "it is not really an either/or situation, that you have got to have concentration and specialization, or you are landed with a uniform spread which can only result in mediocrity. . . . I think we have got to combine the best elements of both".

In its memorandum to the Public Accounts Committee, the Committee of Vice Chancellors and Principals expressed its fears about the involvement of the Comptroller and Auditor-General in university finance. The principle that the D.E.S. should not interfere with

university freedom was not at issue, the memorandum says; this is agreed by everybody. What was at issue was whether the investigations by the Comptroller and Auditor-General would involve the D.E.S. in parliamentary controversy which would in turn force the department to take a much more detailed interest in university expenditure. The D.E.S. would no longer remain at one remove from the universities, with the U.G.C. acting as a buffer, and this would be damaging to the universities, whose efficiency is "a function of their academic freedom and initiative". This theme is the basis of the memorandum, and it was expanded when the committee gave evidence. Asked by the chairman of the Public Accounts Committee, Mr. Boyd-Carpenter, whether the committee would find control by the U.G.C. more or less objectionable than control by the department, Sir Charles Wilson said that he thought it would be less objectionable, but that it would certainly be followed, in his opinion, by an increase of control by the department. He agreed that intervention by the U.G.C. in "housekeeping details" of university expenditure had increased in recent years, and suggested cautiously that this might have reduced academic freedom. The area of discretion left to the universities was, he thought, vital.

Asked to give a concrete example of the way in which he felt examination by the Comptroller and Auditor-General would be an intrusion into university freedom, Sir Charles quoted the example of his own university, the University of Glasgow. In the medical school, a change had been made in which the fourth year of the five or six year course had been turned into an integrated year, in which everybody was taught together. The cost of this experiment had turned out to be considerable. How could one say whether the experiment was good value for money? The Comptroller and Auditor-General, Sir Bruce Fraser, replying, said that if he were auditing the books and found an increase of this type, he would ask why. If he were told that the increase had come about as a deliberate decision of academic policy, that is all he would wish to know. "I would say no more about it. It would be ridiculous for me to try to substitute my judgment for the academic judgment of the people who know about it. This is not the way the Comptroller and Auditor-General ever, ever works." Sir Charles Wilson said that if his fears had been based on a misunderstanding of the memorandum prepared for the Public Accounts Committee by the previous Comptroller and Auditor-General, he would be glad to have misunderstood it. The original memorandum had, he thought, been more wide-ranging than Sir Bruce Fraser had suggested.

## Hospitals

The discussion then turned to the hospitals, which are subject to the inspection of the Comptroller and Auditor-General. Sir Robert Aitken said that he thought that adverse consequences had followed from this inspec-

tion; the controls were closer, and the machine operated much more slowly. Sir Bruce Fraser, drawing on his previous experience as Permanent Secretary of the Ministry of Health, said that it was not the audit but the fact that the ministry had never provided enough money which had caused this situation.

Mr. Sheldon of the Public Accounts Committee suggested that it was unreasonable to object to an audit by the Comptroller if the universities were prepared to accept increasing control by the U.G.C. "It seems very much like the question of just accepting the devil one knows even if his horns are growing rapidly", he said. Sir Charles Wilson replied that U.G.C. control might be acceptable alone, but increased departmental control in addition would be too much. "It is to pile Pelion upon Ossa", he said.

The Association of University Teachers, which met the Committee of Public Accounts two days later, was treated far less tenderly, probably because it criticized the effect the committee's decisions about Cranfield Aeronautical College had had on the morale of the staff and, by implication, on the quality of the research carried out there. Dr. T. G. Halsall of the University of Oxford said that Cranfield was the single example available from which inferences could be drawn, and from the information he had received from colleagues there, the recommendations of the Public Accounts Committee were incompatible with the freedom to conduct the pattern of research which they would wish to conduct. This stung the chairman of the committee into a brisk exchange. "Are you aware that as a result of this committee's investigations in Cranfield, very considerable economies were achieved?" Dr. Halsall replied, "I understand, sir, at the expense of very considerable developments and research". On academic freedom, Dr. K. Urwin, the general secretary of the association, suggested that there was greater emphasis on supplying places at universities for science and technology students than for arts students. This made it very much easier for a mediocre student to get in to read scientific subjects than to read arts subjects. If this trend were to continue, it might be interpreted as an intolerable infringement of academic freedom, although this was not yet the case. The association did not think that inspection by the Comptroller and Auditor-General would make this situation worse.

### Cost Effectiveness

The difficulties of deciding when money is well spent were well illustrated by one exchange. Professor Griffiths asked if this meant universities should get tenders before they bought things. "That is one element of it," replied Sir Douglas Glover of the Committee of Public Accounts. "We do that now," said Professor Griffiths. "The point is, if we took the lowest tender, a Japanese estimate, which caused us to spend valuable money outside the country, would that be well spent?" The association repeated an argument used by the U.G.C. that the real safeguard for public money in university research was the fact that there was never enough of it, so that the internal struggles made sure that it was not wasted. Mr. M. Hookham of the University of Leicester said that the tenacity with which university lecturers fought proposals which they believed to be a waste of money was really quite remarkable. "Even the university chancellors do not know with what intensity this sort of thing is watched."

The committee was more deferential to its next witness, Lord Butler of Saffron Walden, among other things a former Minister of Education. Lord Butler said that there were dangers in enlarging the access of the Comptroller and Auditor-General to the detailed books and papers of the U.G.C. He said that it really was possible to criticize the shortness of money and expenditure rather than the laxity of the amount of money spent. His case rested on the psychological effect which the proposals would have, rather than any specific intrusion into university freedom.

His claim that "Dictatorship always starts by detailed criticism of the universities" did not, he said, imply any criticism of the present government. Lord Franks, Provost of Worcester College, Oxford, said that if the Comptroller and Auditor-General was to investigate universities, his net might well be spread even wider. "It is not obvious to me why, if this is the proper scope of the Comptroller and Auditor-General, he should not, on the same reasoning, have access to the records to the books and records of many other bodies besides the universities—for example, the nationalized industries, the BBC, local government authorities, and indeed, private companies doing work under contract to government, especially where a great part of their work is of this kind". He added that he had had the impression that the activities of the Comptroller and Auditor-General resulted in a steady pressure towards uniformity.

### Auditing

Lord Murray of Newhaven, Chancellor of the University of Southampton, feared that auditing would necessarily involve the Department of Education and Science more closely with the universities. "I think that the accounting officer would have to protect himself to meet his obligations as accounting officer for the universities. . . . I think it would be difficult for him to stop at asking why as well as what expenditure is incurred". Lord Heyworth, a former member of the U.G.C. and a former chairman of Unilever, Ltd., said that the position of the academic members of the U.G.C. was delicate: "Only by the fact that nobody knows who contributed to what can they still retain the respect of their colleagues, and the U.G.C. can retain its authority". He said that central control would necessarily need formulae, and he did not believe that formulae were a reasonable means of reaching this kind of decision. "The interrelation between teaching and research elevates the element of judgment to such a degree that it really changes the whole operation".

Lord Robbins, Chairman of the Committee on Higher Education, held to the line established by his committee. "I regard the U.G.C. with its present functions as one of the most felicitous constitutional inventions of this century". Lord Robbins was unconvinced by the explanation that the Comptroller would not be concerned with academic matters; "I personally find it extremely difficult to believe that, with the best will in the world, the Comptroller and Auditor-General could avoid becoming involved in judgments of value and questions of policy if he were to undertake this particular function". This difficulty was returned to when Sir John Wolfenden gave further evidence, with several members of the U.G.C. "I find it difficult, as many of your other witnesses have found it difficult, to see how, if one investigates methodology, one can refrain from, perhaps, calling into question some of the decisions based on that methodology".

The last to give evidence was Sir Herbert Andrew, Permanent Under-Secretary of State at the D.E.S. He, too, thought it would be difficult for the Comptroller to avoid treading on academic toes. "It does seem to me that sooner or later questions would arise that would throw doubt on or put into question the allocations which the U.G.C. had made." Sir Herbert felt that any change might affect the standing of the U.G.C., and that the universities would become concerned if the position of the U.G.C. as a buffer were seen to move. Sir Herbert thought that the relation between the U.G.C. and the D.E.S. would have to be worked out again, which would be difficult, although perhaps not impossible. An audit would, he said, "make it a little bit easier for any government that was minded to extend control over the universities to do it". On the other hand, he thought that refusal to let the Comptroller and Auditor-General look at the books would hardly constitute a serious obstacle to any government that was so minded.

# Melting at High Pressures

by

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There has been a flurry of speculation about the variation of melting pressure with temperature in the past few years. There are obvious limits to the extrapolation of familiar equations, particularly at high pressures. Model calculations have thrown light on some parts of the problem.

THE shape of the melting curve—that is, of the melting pressure as a function of temperature—for a metal or a molecular crystal has long been the subject of speculation. Gas-liquid curves end in critical points at quite moderate pressures, usually below 100 bar, but there is still some doubt about the fate of the solid-fluid curve. It has been followed experimentally up to pressures of the order of 50 kbar for many metals.

The discussion of these curves is hampered by the absence of an adequate theory of phase transitions. There has, however, recently been a spate of new proposals, and discussion of old proposals, for the representation of such curves. Some of these are related to the hypotheses of Lindemann and Grüneisen, and some are based on computer calculations of melting in model assemblies of hard spheres.

Three old approximations, all of uncertain validity, are widely used in the discussion of the properties of solids. Briefly, they are: (1) Grüneisen's hypothesis that the frequency spectrum of a solid (and in particular the temperature  $\Theta$  used to characterize it) is a function only of the volume. Grüneisen's constant  $\gamma$  is defined by the equation

$$\frac{d \ln \Theta}{d \ln V} = -\gamma \quad (1)$$

(2) Lindemann's proposal that a crystal melts when the molecular vibrations reach a certain amplitude. This has led to a relation between the melting temperature  $T_m$  at an arbitrary pressure, the relative molar mass  $M$ , and the molar volume  $V$ :

$$T_m = CM \Theta^2 V^{(2/3)} \quad (2)$$

The constant  $C$  should be the same for all substances.

(3) Simon's equation for the effect of the pressure on  $T_m$  is

$$\frac{p_m}{a} = \left( \frac{T_m}{T_0} \right)^c - 1 \quad (3)$$

where  $T_0$  is the melting temperature at  $p_m = 0$ , and  $a$  and  $c$  are parameters peculiar to each substance. The equation is useful only for those substances—the great majority—for which  $T_m$  rises monotonically with  $p_m$ .

Salter<sup>1</sup> showed that (1) and (2), with few additional assumptions, led to (3), and that the parameter  $c$  of Simon's equation is related to Grüneisen's constant by

$$c = \left( \gamma + \frac{1}{6} \right) \left( \gamma - \frac{1}{3} \right)^{-1} \quad (4)$$

Kraut and Kennedy<sup>2</sup> recently proposed as an alternative to (3) the equation

$$T_m = T_0 \left( 1 + k \frac{|\Delta V|}{V_0} \right) \quad (5)$$

where  $|\Delta V|$  is the decrease in volume on isothermal compression at  $T = T_0$  to the pressure  $p_m$  that corresponds

to  $T_m$ . At a meeting of the American Geophysical Union in April 1966, Kennedy<sup>3</sup> claimed that (5) held for fifty elements and compounds. Among these are alkali metals for which  $|\Delta V|/V_0$  is about 0.5 at 50 kbar.

This proposal has led to a "flurry of re-examinations"<sup>4</sup>, in which it has been shown independently by Gilvarry<sup>5</sup> and by Vaidya and Gopal<sup>6</sup> that (5) is also closely related to (1) and (2). Equation (5) is obtained by differentiating (2) with respect to volume, using (1) to obtain the volume dependence of  $\Theta$ , and then making a binomial expansion. Hence  $k$  of (5) should also be related to  $\gamma$ , and so to  $c$ , by the equation

$$k = 2 \left( \gamma - \frac{1}{3} \right) = (c - 1)^{-1} \quad (6)$$

In view of the known faults of (1) and (2), it is not surprising that experiment does not confirm the consistency checks (4) and (6). Moreover, these were derived for small values of  $\Delta V$  and  $\Delta T = T_m - T_0$ , while (3) and (5) are used over wide ranges of those variables. Sodium is a metal for which both (3) and (5) represent the melting curve tolerably well, but for which  $c$  is not related to  $\gamma$  or to  $k$  by (6). In contrast, argon is a typical molecular crystal whose melting curve is well represented by (3), and for which the derived value of  $c$  is reasonably close to that found from (4). Even so, (5) cannot be used for argon because of its great initial compressibility. The apparent value of  $k$  for small values of  $\Delta V$  and  $\Delta T$  is about 5 (which disagrees with that obtained from  $\gamma$ ) and this coefficient rises rapidly with increasing pressure. Babb<sup>4</sup> has shown that nitrogen also does not conform to (5).

These comparisons are set out in Table 1, in which much of the uncertainty comes from the failure of Grüneisen's hypothesis and so from uncertainty in the values obtained for  $\gamma$  from the mechanical and thermal properties of the crystal.

Table 1

Substance	$\gamma(\text{obs})^*$	$c(\text{obs})$	$c(\text{from } \gamma)$	$k(\text{obs})$	$k(\text{from } \gamma)$
Sodium	1.18-1.25	3.5†	1.6	1.65†	1.7-1.8
Argon	1.8-2.2	1.5-1.6‡	1.3	(5)§	3.0-3.8

\* From properties of crystal, see ref. 6 for sodium and ref. 7 for argon.

† See ref. 2.

‡ See refs. 8 and 9.

§ See text.

It is hard to say which equation—(3) or (5)—is more likely to be correct in predicting the course of a melting line at very high pressures. Neither has a proper theoretical basis, and the checks provided by (4) and (6) are unreliable guides to their parameters. Moreover, the difference between them can be great when long extrapolations are made. Kennedy<sup>3</sup> has said that at 3 Mbar, the probable pressure at the junction of the inner and outer cores of the Earth, equation (3) leads to a melting temperature of  $\gamma$ -iron of 7,500° C while (5) suggests that it is only 3,700° C.

In the past, some bad extrapolations have been made by using Simon's equation, chiefly because any one set of

results can often be fitted with different pairs of the parameters  $a$  and  $c$ . Nevertheless, the equation has no obvious faults of form and clearly allows both  $p_m$  and  $T_m$  to be unbounded.

The extrapolation of (5) is, at first sight, more attractive since there is only one parameter to be chosen. As Ross and Alder<sup>10</sup> observe, however, the equation must ultimately fail because  $T_m$  is bounded,  $T_m < (1+k)T_0$ . For sodium, which melts at 98° C at zero pressure, this limit is 710° C, which seems most improbable. It is true that some metals—for example, caesium—have maximum temperatures in their melting curves, but it is believed that in all such cases there is a phase transition to a more dense solid at higher pressures, and that this solid then melts along a curve for which  $T_m$  rises monotonically with  $p_m$ .

We can say more about the ultimate behaviour of melting curves by appealing to computer calculations on model assemblies of hard spheres<sup>11</sup>. These show that there is a phase change to a solid structure at values of  $p_m$  and  $T_m$  related by

$$p_m b / RT_m = 18 \pm 1 \quad (7)$$

where the co-volume  $b$  is four times the volume of the spheres. The changes in volume and entropy on melting are independent of  $T_m$ , and therefore  $p_m$  and  $T_m$  are proportional to each other. If real molecules have "hard cores", then this result implies that there cannot be a solid-fluid critical point.

The results for this model can be extended to more realistic models in two ways. Longuet-Higgins and Widom<sup>12</sup> considered an array of hard spheres moving in a uniform negative potential chosen to represent the mean intermolecular configurational energy. This potential raises the melting temperature at  $p_m \sim 0$  from absolute zero, in (7), to a triple-point temperature,  $T_0$ , where

$$RT_0 = -(0.068)U_s(V_s/V_*) \quad (8)$$

where  $U_s$  and  $V_s$  are the molar energy and volume of the solid at  $T_0$ , and  $V_*$  is the molar volume of a close-packed array. If one considers the melting curve in the form of  $\ln p_m$  as a function of  $\ln T_m$ , then this model predicts a line that rises steeply from the triple point to a pressure of the order of  $(-U_s/V_s)$  or 2.8 kbar, where it slowly bends over and then rises more gently, becoming asymptotic to (7) with a slope of unity. The pressure  $(-U_s/V_s)$  plays the same part here as the internal pressure  $\alpha$  in Simon's equation, which is about 2.3 kbar for argon<sup>7,9</sup>. This shape of melting curve, with two different logarithmic slopes above and below the internal pressure respectively, is a good representation of the melting curves of the inert gases<sup>13</sup> and the turning point marks the change from the

region where the crystal is held together by its attractive forces (at low  $p_m$ ) to that (at high  $p_m$ ) where its stability is due primarily to the higher packing density of an ordered array, that is, to the effects of the repulsive part of the intermolecular potential.

At very high pressures the representation of molecules by hard spheres over-estimates the volume they occupy. If, more realistically, the pair potential  $u(r)$  is taken to be proportional to  $r^{-n}$  then  $p_m$  is proportional to a power of

$$T_m \text{ of } \left(1 + \frac{3}{n}\right). \text{ This follows directly}^{13} \text{ from the fact that}$$

the intermolecular virial function of such an assembly is a direct multiple of its intermolecular energy. Hence the limiting form of  $c$  of Simon's equation becomes

$$c = 1 + \frac{3}{n} \quad (9)$$

This equation was obtained for a more restricted model by Domb<sup>14</sup> some years ago.

This extension of the hard sphere results is not restricted to the potential proportional to  $r^{-n}$ , but may be made although with less simple results for any arbitrary but steep potential<sup>13</sup> by means of a Taylor expansion in a parameter that is the reciprocal of a measure of the steepness—for example  $n^{-1}$ . This leads again for all sufficiently steep potentials to a limiting behaviour similar to that of (7) but with a logarithmic slope greater than unity, to a volume change on melting that is non-zero at all finite temperatures, and so to the absence of a solid-fluid critical point or of a change to a transition of higher order.

What is still uncertain is the degree of steepness of the potential required for these extensions of the hard-sphere model to be valid. Since the partition function depends on an exponential function of the configurational energy, then a necessary condition is presumably that all derivatives of  $\exp[-u(r)/kT]$  vanish at  $r=0$ . But this is probably too weak to be a sufficient condition.

<sup>1</sup> Salter, L., *Phil. Mag.*, **45**, 389 (1954).

<sup>2</sup> Kraut, E. A., and Kennedy, G. C., *Phys. Rev. Lett.*, **16**, 608 (1966).

<sup>3</sup> Kennedy, G. C., *Trans. Amer. Geophys. Union*, **47**, 173 (1966) (abstract only).

<sup>4</sup> Babb, S. E., *Phys. Rev. Lett.*, **17**, 1250 (1966).

<sup>5</sup> Gilvarry, J. J., *Phys. Rev. Lett.*, **16**, 1089 (1966).

<sup>6</sup> Valdy, S. N., and Gopal, E. S. R., *Phys. Rev. Lett.*, **17**, 635 (1966).

<sup>7</sup> Pollack, G. L., *Rev. Mod. Phys.*, **36**, 748 (1964).

<sup>8</sup> Babb, S. E., *Rev. Mod. Phys.*, **35**, 400 (1963).

<sup>9</sup> Lahr, P. H., and Eversole, W. G., *J. Chem. Eng. Data*, **7**, 42 (1962).

<sup>10</sup> Ross, M., and Alder, B. J., *Phys. Rev. Lett.*, **16**, 1077 (1966).

<sup>11</sup> Alder, B. J., and Wainwright, T. E., *J. Chem. Phys.*, **33**, 1439 (1960).

<sup>12</sup> Longuet-Higgins, H. C., and Widom, B., *Mol. Phys.*, **8**, 549 (1964).

<sup>13</sup> Rowlinson, J. S., *Mol. Phys.*, **8**, 107 (1964); Jonah, D. A., and Rowlinson, J. S., *Trans. Faraday Soc.*, **62**, 1067 (1966).

<sup>14</sup> Domb, C., *Phil. Mag.*, **42**, 1316 (1951); see also *Nuovo Cim.*, **9**, suppl. 1, 9 (1958), and *Disc. Faraday Soc.*, **40**, 54 (1965).

## Intelligence among University Scientists

by

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Studies on the deviation IQ of scientists at the University of Cambridge show that there is a considerable variation among scientists. However, IQ seems not to be related to success as a scientist, provided that it is greater than a certain threshold which varies according to the particular scientific discipline.

IN an investigation concerned with fertility and social mobility, the test known as the Wechsler Adult Intelligence Scale (W.A.I.S.)<sup>1</sup> was given to one hundred and forty-eight scientists in the University of Cambridge. The scientists were sampled from the thirty-three "science" departments in the University. The Department of

Geography, the Faculty of Economics and Politics and the Farm Economics Branch of the Faculty of Agriculture were included in the sample although they would not be considered as science departments in some universities. The chief sciences represented in the sample are indicated in Table 1.



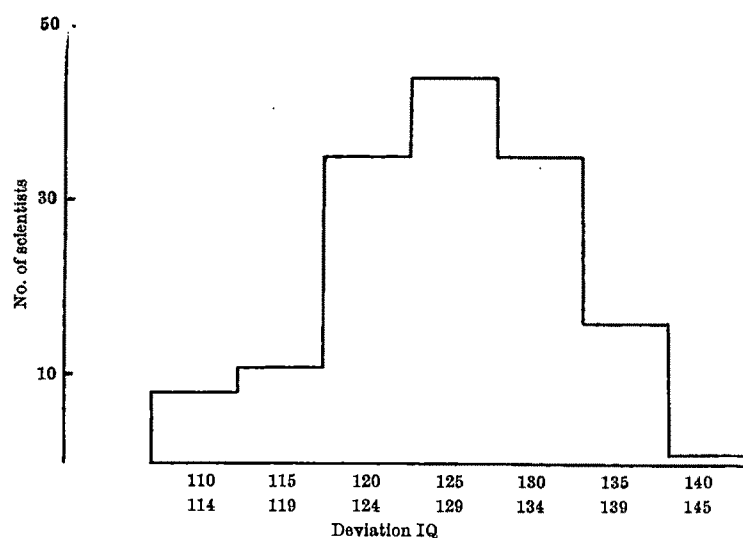


Fig. 1.

The initial sample comprised all the male teaching officers and holders of posts in science departments at June 1, 1965, who were born on or after January 1, 1931. The age range was 25 to 34 years. The total number of scientists meeting these requirements was one hundred and eighty-five—of these, fifteen did not agree to co-operate and thirteen were either abroad or could not be located during the investigation. A further nine members of the sample were already familiar with the W.A.I.S. and were not tested, although in some cases their scores were known; such scores are not included in the data.

### Sample

The age range of this sample, which consisted of 80 per cent of the possible sample, is completely encompassed by one of the standardized W.A.I.S. age ranges. No correction for age was therefore necessary to obtain the deviation IQ scores shown in Fig. 1.

Table 1

Subject of bachelor degree	n	Mean deviation IQs	Range of scores	Variance	Standard deviation of the mean
Agricultural sciences	17	121.6	110-135	41.18	1.55
Biochemistry	10	130.0	122-141	41.33	2.034
Biological sciences	20	126.1	113-135	27.05	1.15
Chemistry	12	129.6	121-138	30.45	1.59
Engineering sciences	16	125.0	111-138	57.46	1.9
Mathematics	16	130.4	124-136	16.0	1.0
Medical sciences	10	127.0	116-134	46.22	2.15
Physics	20	127.7	112-136	39.05	1.4
Social sciences	10	121.8	112-132	41.55	2.04

The scores range from 110 to 141 with a mean of 126.5 and a standard deviation of 6.3 IQ points. All the scores fall within three standard deviations of the mean. The distribution of the scores in terms of Wechsler's classification<sup>1</sup> shows that all the scientists obtained scores above the seventieth percentile rank for the general population—35.2 per cent are classified as "very superior", 51.3 per

cent as "superior", and 13.5 per cent as "bright normal". Approximate percentages of these three groups in a general population sample are 2.2, 6.7 and 16.1 respectively. For any age group in the general population the distribution of W.A.I.S. deviation IQ has a mean of 100 and a standard deviation of 15, the middle 50 per cent of each age group having IQs between 90 and 110. Thus, for this sample of scientists, the range of IQs are similar to the range of scores expected of the higher 25 per cent of a representative general population sample.

The scientists were classified into groups based on the principal subject of the bachelor degree. Fifteen scientists, including those who read geography, geology and metallurgy, were not represented by sufficient numbers to be considered separately and are not included in any of the subject groups. The nine subject groups which could be compared are listed in Table 1. These groupings do not correspond to university departments.

Scientists who had specialized in mathematics for the bachelor degree obtained the highest mean deviation IQ in the sample. The variance for the mathematicians' scores was significantly lower than the variances for the scores obtained by engineering scientists, medical scientists and physicists ( $0.05 > p > 0.01$ ). The scores of the engineering scientists had the highest variance of IQ scores.

### Significant

The significance levels for each of the thirty-six comparisons of mean IQ scores which can be made between the nine groups are given in Table 2. Medical scientists did not differ significantly from any of the groups in mean IQ scores. The mathematicians had significantly higher mean scores than four of the groups, the social scientists had significantly lower mean scores than four of the groups and the agricultural scientists had lower scores than five of the groups.

The deviation IQ scores of 146 scientists could be related to the class of the bachelor degree (Table 3). The regression coefficient of IQ on degree class was small ( $r = +0.15$ ) and non-significant ( $p > 0.3$ ). In the present sample

Table 2. COMPARISON OF THE MEAN IQ SCORES OF THE NINE SUBJECT GROUPS

Subject of bachelor degree	Mean IQ	2 Bioch.	3 Chem.	4 Phys.	5 Med.	6 Biol.	7 Eng.	8 Sociol.	9 Agric.
1 Mathematics	130.4	—	—	—	—	+	+	++	++
2 Biochemistry	130.0	—	—	—	—	—	—	++	++
3 Chemistry	129.6	—	—	—	—	—	—	++	++
4 Physics	127.7	—	—	—	—	—	—	++	++
5 Medical sciences	127.0	—	—	—	—	—	—	—	—
6 Biological sciences	126.1	—	—	—	—	—	—	—	++
7 Engineering sciences	125.0	—	—	—	—	—	—	—	—
8 Social sciences	121.8	—	—	—	—	—	—	—	—
9 Agricultural sciences	121.6	—	—	—	—	—	—	—	—

Probabilities. \* < 0.001; + < 0.01; ++ < 0.05; --- > 0.05.

Table 3a

Class of bachelor degree	n	Mean IQ score	Variance
First class	77	127.5	40.85
Upper second	36	126.8	35.36
Lower second	17	124.4	40.25
* Others	16	122.2	29.0

\* Includes third class and pass degrees.

Table 3b

Table 3b. PROBABILITY LEVELS OF "t" IN COMPARISONS OF MEAN IQ SCORES FOR DEGREE CLASS

Degree classes	2:1	2:2	Others
1	>0.5	<0.05	<0.001
2:1		>0.1	<0.01
2:2			>0.3

scientists who had gained first and upper second class degrees had significantly higher mean IQ scores than those with lower degrees ( $p < 0.01$ ) although the variances were not significantly different. It is interesting that both the highest and lowest individual IQ scores were found among scientists with first class degrees. This relationship between the class of the bachelor degree and IQ is in part reflected in the differences in mean IQ scores between the subject groups described here. The proportions of the different degree classes were not similar in all the nine subject groups (Table 4).

Table 4. PROPORTIONS OF VARIOUS DEGREE CLASSES IN SUBJECT GROUPS

Subject of bachelor degree	Degree class			Others
	1	2:1	2:2	
Agricultural sciences	4	3	2	8
Biochemistry	6	3	1	—
Biological sciences	10	8	2	—
Chemistry	7	4	—	1
Engineering sciences	8	3	2	3
Mathematics	13	2	—	1
Medical sciences	5	1	2	1
Physics	9	8	3	—
Social sciences	5	2	2	1

The present IQ data can be compared with Mackinnon's findings<sup>2</sup>, using Terman's Concept Mastery intelligence test on samples of distinguished American scientists, and with Hudson's findings<sup>3</sup> using the A.H.5 intelligence test on English sixth formers. Each of these studies indicates that above a certain threshold level (in the region of IQ 110) a conventional intelligence test measures qualities unrelated to the success of an individual in a scientific career. The present data support the suggestion that there may be a threshold level of IQ below which an individual is unlikely to become a scientist. Whatever qualities are measured in the W.A.I.S. test, however, they are not equally represented in each of the nine subject groups in the present sample and thus the level of the IQ scores may be relevant in some scientific subjects. The biochemists, chemists and mathematicians all had IQs above 120, while some scientists in the other groups had IQs below 120.

The significant finding in the present study is the large proportion of scientists in all subjects with IQ scores below 130 (Fig. 1). Whether those with higher scores enjoy more success in a particular scientific subject is not known and to generalize from the present data would be as misleading as the statement often made that all scientists have high IQs.

We thank all the scientists in the sample (and others) who generously helped in this work. One of us (J. G.) acknowledges the financial support of the Eugenics Society (London) and the Hopkins Donation Funds (U.S.A.).

<sup>1</sup> Wechsler, D., *Manual for the Wechsler Adult Intelligence Scale* (The Psychological Corporation, New York, 1955).

<sup>2</sup> Mackinnon, D. W., *Amer. Psychol.*, 17, 484 (1962).

<sup>3</sup> Hudson, L., *Contrary Imaginations* (Methuen, London, 1966).

## BOOK REVIEWS

### OLD MASTER

#### Collected Papers of G. H. Hardy

Including joint papers with J. E. Littlewood and others. Vol. 1. Edited by a committee appointed by the London Mathematical Society. Pp. 700. (Oxford: Clarendon Press; London: Oxford University Press, 1966.) 105s. net.

G. H. HARDY was the author or part author of more than three hundred original papers and several influential books. His work had a profound influence throughout the whole of analysis and, more than any other mathematician, he was responsible for the renewal of interest in "real" mathematics in Britain. The London Mathematical Society intends to publish all Hardy's papers, including those written in his very fruitful collaboration with Littlewood and Ramanujan, and this handsomely produced volume is the first of seven. The papers are arranged in groups according to subjects; this volume contains those on Diophantine approximation and additive number theory.

The work of Hardy and Littlewood on Diophantine approximation arose primarily out of their interest in problems on uniform distribution modulo 1. Let  $f(n)$  be a function defined on the positive integers and write  $(f(n))$  for the positive fractional part of  $f(n)$ —that is, for the "decimal part" of  $f(n)$ . Then the problem of uniform distribution is that of finding conditions on  $f$  so that the frequency with which the values of  $(f(n))$  fall in any sub-interval of the interval (0,1) is proportional to the length of the sub-interval. This problem is closely related to that of estimating the sums

$$\sum_{n=1}^N e^{2\pi i f(n)} \quad \text{and} \quad \sum_{n=1}^N [(f(n)) - \frac{1}{2}]$$

In its turn, the latter sum is related to the problem of finding the number of points with integral co-ordinates in a given right-angled triangle.

Hardy and Littlewood developed a method, now known as "the Hardy-Littlewood method", which provides a general line of attack on all problems concerned with finding the number of ways in which a given number can be expressed as a sum of numbers of certain special types. There is, for example, the problem of representing a number,  $n$ , as the sum of  $s$  squares. If  $r_s(n)$  denotes this number, then  $r_s(n)$  is the coefficient of  $q^n$  in the expansion in powers of  $q$  of  $(1 + 2q + 2q^4 + 2q^9 + \dots)^s$ . One takes  $q = e^{2\pi i \tau}$  and then the problem of picking out  $r_s(n)$  becomes a problem of the behaviour of the resulting function of  $\tau$ . Similar arguments can be used to investigate the problem of representing  $n$  as a sum of  $k$ th powers (Waring's problem) and the problem of finding the number of ways in which  $n$  can be expressed as a sum of smaller numbers (the problem of partitions). Another variant of the method can be used to attack the celebrated conjecture of Goldbach, which asserts that every even number from 6 onwards is the sum of two primes other than 2. If this is true—it is still unproved—then every number greater than 5 is the sum of three primes and, using their analytical method, Hardy and Littlewood were able to prove that this result is true for all sufficiently large numbers. To do this they had to make an assumption about the zeros of a certain function—an assumption which is still unproved. (On the other hand, Vinogradov

has proved the three primes theorem without this assumption.)

Each group of problems in the *Collected Papers* has a lucid introduction and commentaries on subsequent developments and unsolved problems. The papers themselves make absorbing reading—I am ashamed to admit that I was reading many of them for the first time—and reflect the extremes of creativity and technical facility which were characteristic of the authors. One is impressed, too, by the elegance of the style. It is not inappropriate to compare it with accounts given in *Wisden* of the cricket of the day; both present us with glimpses of a more generous age.

J. V. ARMITAGE

## THE THYMUS

### The Thymus

Experimental and Clinical Studies. Edited by G. E. W. Wolstenholme and Ruth Porter. (Ciba Foundation Symposium.) Pp. xiii+538. (London: J. and A. Churchill, Ltd., 1966.) 80s.

THE true nature of thymic function has always been one of the most intriguing mysteries in biology. Suggested roles have ranged from the filling of the thorax during contraction of the lung to the elaboration of a fluid to attenuate the blood, and apart from one or two notable contributions to our knowledge during the first fifty years of this century, there has been very little evidence on which to base better theories. The deficiencies are now being made good and a wealth of information has been collected, particularly in the past five years.

In this volume are some twenty contributions covering a fairly wide range of processes in which the thymus appears to play a leading part. The papers were read at the meeting arranged in Melbourne in August 1965, in honour of Sir Macfarlane Burnet on his retirement as director of the Walter and Eliza Hall Institute of Medical Research. Fittingly, all the contributions are of a high standard, and from them and the discussions which follow a very comprehensive survey emerges of what is known about the gland and of the present trends of thought preoccupying those who are actively engaged in research.

During the past few years, following the work of J. F. A. P. Miller, attention has been focused mainly on the role of the thymus in immunity and on the associated—indeed inseparable—problem of the origin, function and fate of the lymphocyte. Miller's own contribution to the symposium emphasizes the central part which the thymus plays in the development of immunological capacity and suggests that the chief functions may be the provision of lymphocytes or precursor cells and the elaboration of a factor which induces competence. Supporting evidence for thymic hormone production is provided by the electron microscope studies of S. L. Clark, but D. Metcalf provides convincing evidence against any massive migration of thymic lymphocytes to peripheral lymphoid tissues as was previously thought to occur. Elegant experiments by J. L. Gowans and his co-workers demonstrate the close association between antibody production by lymphocytes and the mode of presentation of the antigen. The macrophage may be an essential intermediary in the inductive process. An important theme which emerges from this and from other work, including that of Auerbach on embryogenesis of thymus and lymphocytes, is the possibility of the existence of two functionally and developmentally separate systems of cells, one responsible for antibody formation and the other for the mediation of transplantation reactions. As yet, the only direct experimental evidence for the existence of two such systems derives from work on the bursa of Fabricius of chickens. There have been reports, however, post-dating the Melbourne meeting, of the ability of neonatally thymectomized animals to produce antibody

to certain antigens, and in this volume Professor Good directs attention to the same kind of dissociation found in man.

Other contributions deal with the relationship between the thymus and tolerance, the competence of cloned cell populations and with the thymus in ontogeny and phylogeny. Sir Macfarlane Burnet describes some of his own observations on mast cell metaplasia in the thymus, and some of the work done on the relationship of the gland to the auto-immune haemolytic disease of the NZB strain of mice is also presented.

With the increasing awareness of the importance of the thymus in immunobiology, there has been a re-awakening of interest in the pathology of the human thymus and in the association between it and immunological deficiency syndromes and auto-immune disease. These topics are dealt with in a number of interesting papers. The lecture given by Dameshek is particularly stimulating.

In any symposium, as in any single paper, only a few aspects of a topic can be touched on. It is out of the question, therefore, to lament the absence of reports dealing with the other myriad facets of thymic function. In any event, the recorded discussions between members of the symposium cover such a wide field that almost all the related problems are included. Indeed, the sections devoted to discussions form the most valuable part of the whole volume. It remains true, however, that although better theories of thymic function have now been formulated, they still do not fit all the known facts. It is to be hoped that the next few years will see further dramatic advances in our knowledge, and perhaps the answer to the problems of human auto-immune disease.

R. MAWDSLEY

## THE FUNGUS SPORE

### The Fungus Spore

Edited by M. F. Madelin. (Proceedings of the 18th Symposium of the Colston Research Society, held in the University of Bristol, March 28–April 1, 1966.) Pp. xvi+338. (London: Butterworth and Co. (Publishers), Ltd., 1966.) 120s.

THE spore represents in some respects the most significant part of the life cycle of a fungus, and as such is of interest to all mycologists. This volume, therefore, in bringing together up-to-date information on many facets of spore behaviour, is a welcome addition to the mycological literature.

It comprises a series of authoritative papers from a symposium held at the University of Bristol, and the organizers are to be congratulated on the balance that has been attained between the diverse aspects of the main subject. The initial stages of the meeting were concerned with the process of spore production as observed in the main systematic groups. This topic, together with the associated one of spore structure, was enlivened by the prominence given to studies pursued with the aid of the electron microscope. A stimulating account of spore liberation by Professor C. T. Ingold introduced that part of the symposium devoted to spore dispersal and germination, and the latter aspect in particular received excellent coverage. Professor L. E. Hawker describes the "morphological and anatomical changes" that can be observed during germination, while Professors V. W. Cochrane and D. Gottlieb elucidate so far as is possible the associated biochemical metabolism. Some of the factors influencing spore germination were also discussed at some length, although perhaps a more ecological bias to this interesting subject would have been more rewarding. The concluding papers considered the part played by spores in the epidemiology of plant and animal diseases.

The compilation of these papers has, therefore, the essential ingredients of a first class reference book—

authority and a wide range of discussion around the main theme. The material is well presented and adequately illustrated, and the various authors have interpolated sufficient pertinent speculation both about future lines of investigation and the interpretation of present results to sustain interest through some of the more involved passages. It is guaranteed a wide readership and, although an expensive publication, is a worthy accession to any library.

R. K. ROBINSON

## RUSSIAN FORESTS

### The Forests of the U.S.S.R.

By V. P. Tseplyaev. Translated from the Russian by A. Gourevitch. Pp. v+521. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 138s.

A GENERAL introductory chapter covers the classification and general features of the forests of the Soviet Union, and is followed by a short survey of forest distribution, by area, in the several republics of the Soviet Union. An account of forest zonation is given in the third chapter and this is followed by the principal parts of the book—two chapters descriptive of the different types of forests in the Soviet Union. One chapter covers coniferous and the other broad-leaved forests. Forests are dealt with according to the predominant type of tree—pine, fir, larch, oak, for example—so that mixed forests may be found under more than one heading. The more important or extensive types of forest are further subdivided geographically. For each of them there is a short history and account of forest husbandry and exploitation, a brief descriptive account of forest physiognomy, flora and fauna, an indication of quality of the stands, a survey of regeneration, and a statistical survey of forest area, age characteristics, reserves, usage and increment. Much of the information throughout the book is presented in tabular form. There are also short chapters surveying forest utilization in the Soviet Union and the forest resources of the world (based on the FAO survey of 1953).

The comparative vastness of the forest resources of the Soviet Union is clearly apparent from this book, but it is also clear that this vastness has not blinded Russian foresters to the need to conserve, maintain and properly exploit their forests. It is also evident that there are large areas of the Soviet Union, especially in the drier parts of the Middle Asian republics, where forest resources are negligible and conditions for silviculture difficult. Other areas in the south, such as Dagestan, have clearly suffered and still suffer from the bad effects of over-exploitation, over-grazing and neglect. Russian foresters are also fully aware of the amenity value of forests, particularly in the vicinity of large centres of population. An account of the forests of the Moscow "green belt" is of interest to all who are concerned with the wellbeing of city surroundings.

The English translation reads well and is remarkably free from errors of usage and spelling. It should, however, be noted that "cedar" is used to cover certain *Pinus* species, not *Cedrus*, while "aspen" includes more than the one species *Populus tremula*. A few explanatory notes have been added where necessary, but they do not intrude on the original text. In dealing with official Russian names of organizations and institutes, a happy balance has been struck between translation and transliteration.

Throughout the book there are useful references to forest husbandry and recommended practice in the various forest types and regions of the Soviet Union, but the book is in no way a forestry handbook but rather a source-book or compendium of the forest resources of the Soviet Union in the period 1957–59. For a book of this kind, it is remarkably readable—a reflexion of the love of

forests of the original author as well as of the skill of the translator.

C. JEFFREY

## NOBEL SYMPOSIUM

### Muscular Afferents and Motor Control

Edited by Ragnar Granit. (Proceedings of the First Nobel Symposium held in June 1965 at Södergarn, Lidingö, County of Stockholm.) Pp. 466. (New York and London: John Wiley and Sons; Stockholm: Almqvist and Wiksell, 1966.) 150s.

THIS record of the first Nobel symposium contains some 450 pages of scientific papers in 39 separate contributions by 61 authors, most of whom were present at the discussions. We are told in the preface that "it was decided not to publish the lively discussions that took place, unless the discussants themselves wanted it and were prepared to deliver signed contributions". Regrettably for those who set a high value on this form of thinking aloud, very few of the participants delivered their signed contributions, so that only six pages of the volume are given over to discussion. It would, of course, have greatly lengthened the book had discussion been included and perhaps the task of collation and publication would have been exceedingly difficult. The reader is bound to be curious, however, as to what was actually said in discussion of many of the arguable and open statements in these papers.

The inaugural paper by Lord Adrian stresses the importance of this Nobel Foundation experiment in a new technique. He says "Scientists, therefore, are learning how to profit by the classical method, the symposium of experts, few enough to sit round a room, with a definite subject on their agenda and a chairman . . . like Socrates . . . to keep them to the point", and later "... we hope to go home with clearer ideas about the nervous apparatus which regulates muscular contraction so that movements are smooth and posture is adjusted to suit them. The subject is ripe for discussion". But the general reader of this important volume has been prevented from sharing those clearer ideas about nervous action contributed to the discussion. By the publication of the Proceedings of this first Nobel symposium in the present form he is scarcely better off than if the papers had been published without the contributors ever having visited Stockholm.

It is scarcely possible to summarize or even to review the contents of the volume, dealing as it does with nearly forty topics within the very wide scope of its title, from the structural aspects of mammalian muscle spindles through the analysis of their responses to the peripheral and central mechanisms of control of motor systems in man and the cat. It is obvious, however, that this collection of papers will form a valuable source of reference on the sensory apparatus of muscle and the control of motor function. It will particularly serve the needs of those bio-cyberneticians interested in developing theoretical concepts about central nervous activity and the theory of action of the neuro-musculature. In it they will find many suggestions and ideas about possible mechanisms for regulation and control in the central and peripheral nervous and motor systems.

One of the difficulties in writing meaningful accounts of experimental work in neuro-physiology is the vast amount of detail that must be put on record in any statement of results, if the statement is to have any value at all either in its own right or in comparison with other work. The problem is made more difficult by differences over nomenclature. It is especially to be regretted that the final day's discussion on this topic has not been reported.

The difficulty of complexity can be overcome in two ways—by well formulated theory and more research

aimed at deciding critical issues raised by the theory. An urgent need, as judged by the absence of mathematical papers in this symposium, is for the formulation of theories of nervous action based on relatively simple models which can be tested by experiment and by computer.

W. F. FLOYD

## MOISTURE MEASUREMENT

### Humidity and Moisture

Measurement and Control in Science and Industry. Editor-in-Chief: Arnold Wexler. Vol. 1: Principles and Methods of Measuring Humidity in Gases. Edited by Robert E. Ruskin. Pp. xv + 687. \$30. Vol. 2: Measurement and Control in Science and Industry. Pp. xv + 634. \$27.50. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1965.)

EACH of these volumes is a collection of papers presented, under the sponsorship of the National Bureau of Standards and other United States interested organizations, in May 1963 at an International Symposium on Humidity and Moisture in Washington. Their appearance reflects the extraordinary change which has taken place in recent years in what had previously been one of the most neglected fields of physics. The subject of hygrometry rarely merits more than a paragraph or two in text-books of physics, and most science students meet it as a single unpleasant and unrewarding experiment in elementary physics. This state of affairs is all the more inexplicable when the industrial importance of humidity and its control is borne in mind. The change has been brought about (and this is obvious from the subject matter of these volumes) principally by two factors.

The first is the increase in knowledge of the principles of automation, which has made it attractive to develop humidity sensors that can be suitably matched into servo-systems. The huge domestic market for air conditioning appliances in the U.S.A. promises adequate reward for successful solutions. The second is the need voiced by the armed services and by space agencies for successful methods of humidity measurement and control.

It is no surprise, therefore, that of the sixty-eight papers in Volume 1, no fewer than fifty-one originate in the United States or Canada; in Volume 2 the proportion is sixty-nine out of a total of seventy-four.

Volume 1 begins with an excellent review of the underlying theory, and the tables and charts and other computational aids used with that commonest of all hygrometers, the wet- and dry-bulb instrument. The remaining papers deal with various forms of this instrument, with dew-point, electric, spectroscopic and coulometric hygrometers, and finally with a number of miscellaneous methods which cannot be classified under the foregoing heads. The dew-point hygrometer has obviously become a much more attractive instrument following on the invention of Peltier electric cooling elements. It is interesting to find that the oldest and simplest hygrometer of all, the hair hygrometer, still has its uses.

The second volume is concerned with the applications of humidity measurement and control in various fields of interest. These cover biology and medicine, agriculture, controlled humidity chambers, air conditioning systems, process control, meteorology, radio propagation and atmospheric refraction. The range of the volume indicates the general importance of the subject.

In a publication of this type and especially in the second volume, involving so many separate contributions, it cannot be expected that all are of the same standard. The general level is high, however, and the quality of presentation is of an excellence one would expect of a publication sponsored by the National Bureau of Standards. The index to Volume 1 is adequate, but that to Volume 2 is so terse as to be hardly of use. These two

volumes, comprising a collection of papers representative of the present state of the art, can be recommended to all interested in hygrometry, with the usual regret that few individuals outside North America will be able to afford them.

W. H. J. CHILDS

## COMMITTEE BOOK

### History of Mankind

Cultural and Scientific Development. By Caroline F. Ware, K. M. Panikkar and J. M. Romein. Vol. 6: The Twentieth Century. Part 1: Introduction: The Development and Application of Scientific Knowledge. Pp. xlvii + 1-646 + plates 1-20. Parts 2-4: The Transformations of Societies; The Self-Image and Aspirations of the Peoples of the World; Expression. Pp. xiii + 647-1387 + plates 21-64. (London: George Allen and Unwin, Ltd., 1966. Published for the International Commission for a History of the Scientific and Cultural Development of Mankind.) 168s. net.

THIS instalment in Unesco's six volume *History of Mankind* is a vivid proof that committees cannot write books. The project is organized "under the mandate of the International Commission for a History of the Scientific and Cultural Development of Mankind", and the volume on the twentieth century (published in two volumes) has been written by a team of named authors working under the supervision of an author-editor and two co-author-editors. A part of the exercise, which began in 1954, has required that authors should win the agreement or at least invite the dissent of a number of distinguished reviewers. The result, in many chapters, is that the footnotes which say who disagrees with whom are frequently more interesting than the necessarily pallid chapters which they accompany.

Dr. J. Bronowski, who contributed the chapter on "The New Scientific Thought", seems to have had a particularly rough ride. Dutifully, and with commendable brevity, he gives a straightforward Darwinian explanation of the principle of natural selection, only to earn for his pains the rebuke from Academician Ivan Malek that "the principle of natural selection is explained incorrectly, in a Malthusian fashion. . . . Darwin was mistaken in making use of Malthusian ideas and referring to them, but this does not detract from the essential correctness of Darwin's teaching on natural selection". Academician Malek goes on to explain precisely what he means, and the author-editors rally to the defence of their contributor by calling "attention to the fact that the explanation presented in the text is that given by Darwin". But this is only a beginning. Poor Bronowski is taken to task for what he says about Marx and Engels, for allegedly failing to distinguish between "mechanistic determinism and determinism as such", for his espousal of the positivist interpretation of quantum mechanics, and for many other misdemeanours as well. It is all good knockabout stuff, and no doubt faithfully representative of many Unesco meetings. But is it the history of mankind?

JOHN MADDOX

### Catalogue of Meteorites

With special reference to those represented in the collection of the British Museum (Natural History). By Max H. Hey. Third, revised and enlarged, edition. (Publication No. 464.) Pp. lxviii + 637. (London: British Museum (Natural History), 1966.) 120s.

STUDENTS of meteorites in Britain are exceptionally lucky to have the British Museum's large and comprehensive collection of meteorites at their disposal, and to have a tradition of meteorite study behind them which



includes such classic works as Prior's classification of meteorites. Prior also put together the first edition of the *Catalogue of Meteorites* which has now gone into a third edition, enlarged and improved by Dr. M. H. Hey. Apart from the catalogue itself the book has a useful summary of the classes of meteorites and an interesting chapter on the history of the British Museum collection. Also included are catalogues of meteorite craters and of tektites in the British Museum collection. A particularly welcome addition to the volume is the summary of minor element determinations. Clearly a summary of major element determinations as well would involve an enormous amount of research, but perhaps it is not too much to hope that future editions will carry such a summary. However, the book is a mine of information as it stands and will be invaluable to those working in the field.

A. M. MARSHALL

### Lie Groups for Pedestrians

By Harry J. Lipkin. Second edition. Pp. ix+182. (Amsterdam: North-Holland Publishing Company, 1966.) 20 guilders; 40s.

THIS book is written for physicists, and will not satisfy those who seek an understanding of the mathematical properties of Lie groups and Lie algebras. The author assumes a previous familiarity with spin and isospin, with creation and annihilation operators and with other quantum mechanical concepts, and he develops the properties of Lie algebras largely by analogy. The book is suitable for those who seek a superficial understanding of the mathematics; it is not recommended for a serious theoretical student, who should have some understanding of the topological background (especially the idea of compactness), and of the relationship between Lie groups and Lie algebras. For an experimental physicist who simply seeks to understand particle classification schemes on a phenomenological basis, the book provides a painless introduction to the subject, working out details of many of the more familiar classification schemes. The author admits that the book is not comprehensive, and this should be borne in mind by the reader.

J. S. R. CHISHOLM

**Mixed Boundary Value Problems in Potential Theory**  
By Ian N. Sneddon. Pp. viii+283. (Amsterdam: North-Holland Publishing Company; New York: Interscience Publishers, a Division of John Wiley and Sons Inc., 1966.) 40 guilders; 80s.

READERS of this book are impelled along by a number of problems taken from mathematical physics which have the form of boundary value problems of mixed type. Functions, usually harmonic, have to be determined from the specification of the function on parts of the boundary of the domain and of its normal derivative elsewhere on the boundary. There are plane problems and axisymmetric problems including the problems of the charged disk and the charged spherical annulus from electrostatics, punch and crack problems from elastostatics, and slit problems from hydrodynamics. All these are variants on one theme.

Most of the book is devoted to the solution of the harmonic mixed boundary problem by means of dual integral equations and dual series representations. The author is an authority on the subject, and this is reflected in his presentation of the material.

The principal formulae used in the analysis are gathered for easy reference into one chapter, and the basic methods for solving dual integral equations are presented early in the book in connexion with the classical problem of the electrostatic disk.

Most of the dual integral equations studied have Bessel or trigonometrical functions as kernels, whereas the dual series are based on Bessel functions, trigonometrical functions, Legendre polynomials and Jacobi polynomials. Triple integral equations and series representations are

also considered, these arising when different conditions are imposed on three parts of the boundary of the domain.

The power of the methods is evident in the application in Chapter 8 to the electrostatic field effects of electrified disks, strips and spherical caps. Some interesting methods of obtaining bounds to the capacity of condensers formed from circular disks are given here.

This book is to be recommended to all students who wish to become familiar with current methods of research in the title topic.

E. E. JONES

### Theory of Automatic Control

By H. Takai. Translated by Scripta Technica, Ltd. Edited by E. J. Feakes. Pp. ix+315. (London: Iliffe Books, Ltd., 1966.) 75s. net.

CONTROL engineering and control theory have much relevance to other fields, so that it is worthwhile to spread the gospel outside the boundaries to other disciplines. One object, however, to the fact that it is always the same portion of the Bible which the preachers expound.

Nine of the ten chapters in *Theory of Automatic Control*, translated from the Japanese, deal with the well worn topic of continuous linear systems. Some of the standard sub-topics are discussed and treated quite competently as well, but no more thoroughly or more topically than any number of well established texts which already exist in English and which do not need translation. The tenth chapter is entitled "Nonlinear Automatic Control" but, along with a superficial treatment of non-linear systems, it discusses sampled data systems as if the phenomenon of sampling made it fit into this chapter.

In short, the necessity of translating in 1966 this text, published in Japanese in 1961, is not readily obvious.

PAUL ALPER

### The World of Learning

1966-67. Seventeenth edition. Pp. xiv+1578. (London: Europa Publications, Ltd., 1967.) 150s. net

THE seventeenth edition of *The World of Learning* differs from the sixteenth in that it is even larger and contains more detail on universities and academies in a number of countries which were covered only sketchily in the previous volume. The information on the Soviet Union has been expanded, and the learned societies and research institutes of Argentina, Austria, Japan, Mexico and Holland are now classified systematically. One new feature of the book is the additional information on the chief language of instruction where this is not at once obvious, and on the length of the academic year. More than 150 countries are dealt with in more or less detail: their universities and colleges, libraries, museums and art galleries, and their learned societies and research institutes are listed, as are the names of their leading scholars. The book also contains an international section on the aims and functions of Unesco, and on the various world scientific and cultural organizations. Anyone who deals with scientists outside his own country will find this book a valuable guide to the proliferating network of international science.

JOHN SPENCER

## OBITUARIES

### Professor C. F. A. Pantin

CARL FREDERICK ABEL PANTIN, who died on January 14, at the age of 67, made many distinguished contributions to invertebrate physiology.

After Tonbridge and Christ's College, Cambridge, he went to the Marine Biological Laboratory, Plymouth, in 1922, where he investigated the physiology of amoeboid movement. In 1929 he returned to Cambridge as lecturer

and fellow of Trinity College. He was then concerned with the physiology of the crustacean muscular system; he wrote a series of papers on the mechanism of excitation and inhibition. Later he made a very precise investigation of the mode of action of the nerve net and muscular system of Coelenterates, in particular in sea anemones. He discovered that the nerve net will only respond to a series of shocks, each of which elicits a single impulse in the net. Each group of muscles responds to a series of shocks of characteristic number and time interval. This enabled Pantin to explain the mechanism by which the animal's behaviour is controlled. He later investigated the functional anatomy of the nemertines, which were then little known in Britain. During this time he became reader in zoology, and succeeded Sir James Gray as professor in 1959.

Among his many general writings was a paper on identification and recognition, which was a presidential address to the British Association. Pantin was also active in the Royal Society, to which he was elected in 1937, and which awarded him a Royal Medal in 1950; he had been president of the Linnean Society, and in 1963 became chairman of the Trustees of the British Museum (Natural History). He was always interested in marine biology overseas, and was recently a very active chairman of the Nuffield Unit of Tropical Animal Ecology in Uganda. Zoology at Cambridge has benefited much from his great interest and experience in the teaching of science.

#### Professor R. M. Sievert

ROLF MAXIMILIAN SIEVERT died in Stockholm on December 3, aged seventy. He had been director of the Institute of Radiophysics of the Karolinska Institutet since 1937.

When Gösta Forsell, the founder of Swedish radiology, met Sievert, a physicist, in the United States, he persuaded him to start work in radiation physics and build up a hospital organization. In the early twenties hospitals did not standardize the amount of radiation which they administered to patients, and so Sievert and his assistants developed the Sievert condenser chambers to measure dosage. These were soon in use throughout the world. Dosage for the treatment of tumours was based on detailed measurements made on wax phantoms. Sievert was also keenly interested in radiation protection, and this resulted in his work on radiation biology, on which he became an authority.

Sievert realized the importance of international co-operation in the establishment of dose units and protection rules which were generally accepted in all countries. In 1928 he was one of the founders of the International Radiation Protection Committee, and he was later on the United Nations Scientific Commission on Radiological Protection; he was chairman in 1963-66. He was the first member with a natural science degree to be appointed professor of radiation physics at the Karolinska Institutet, which is a medical institute. In 1944 Sievert became a member of the Royal Swedish Academy of Sciences, and in 1959 of the Academy of Engineering Sciences. During the Second World War he had taken the initiative in the formation of a research institute for defence, and he was a member of the board until 1945. His administrative talent was also used in the planning of the Geophysical Research Institute in northern Sweden. His last years were occupied with plans for the National Institute of Radiation Protection, which was ready in 1965. Sievert remained active until the last days before his death, as a chairman of committees, and he continued to take an interest in the work of his institutions.

ARNE FORSSBERG

#### Dr. Paul White

PAUL WHITE, senior lecturer in applied mathematics in the University of Reading, died on January 7, aged sixty-two.

He was born and grew up in Lewisham, where he attended Colfe's Grammar School, and he went to Clare College, Cambridge, in 1922 as an Entrance Scholar. He gained a double first in mathematics and then a first in physics, and went on to undertake research in the Cavendish Laboratory under Lord Rutherford, working on the properties of  $\beta$ -particles and their passage through thin films. A contemporary of this time refers to "a great personal impression of brilliance". After obtaining a Ph.D., he went to Aberdeen as a Carnegie Teaching Fellow, where with G. P. Thomson he continued and extended his work to consider cathode rays.

In 1933 he went to the University of Reading. Work with Crowther and Liebmann on the interaction of X-radiation with biological colloids and on electro-osmotic effects stimulated an interest in statistics. This led to a lengthy and fruitful period of collaboration with a number of workers, particularly at the National Institute for Research in Dairying, where he provided the statistical basis for the biological work. He also worked with Bond on precise measurements of gas viscosity, and was a founder member of the British Rheologists Club. The demands of war-time teaching developed an unexpected competence in astrophysics and meteorology. After the Second World War, expansion of the University and the shortage of mathematicians led Paul White to transfer to the Department of Mathematics, with special responsibility for the development and teaching of applied mathematics. This marked the end of direct personal and individual research, but he continued to help many people in other disciplines with the mathematical aspects of their work.

White led a very full life in the University; he lived in St. Patrick's Hall, of which he was Senior Resident, sang in the Choral Society and coached an eight on the river—he always undertook the timekeeping of the Head of the River race with enthusiasm and meticulous attention. No account of Paul White's life could be complete without reference to his Christian faith and work. He was a truly devout man, an ardent and active Congregationalist and a staunch supporter of the Student Christian Movement. A lay preacher from his Cambridge days, he was always a welcome preacher in Congregational pulpits over a wide area.

K. W. ALLEN

#### Dr. C. W. Farstad

CHRISTIAN W. FARSTAD, who was awarded the Gold Medal of the Entomological Society of Canada last year, died at the age of sixty, on November 18, after an operation in a Toronto hospital. He had made many contributions to the control of insect pests; his sawfly resistant variety of wheat has been of particular value.

Born in Norway, Farstad went, with his family, to America, where he was educated. He obtained his B.Sc. and M.Sc. from the University of Saskatchewan and his Ph.D. from Iowa State College. During this time he began his career as an entomologist in 1929, when he became an assistant in field investigations of grasshopper and cutworms at the Dominion Entomological Laboratory at Lethbridge; in 1945 he became the laboratory's chief. In 1935 he took charge of the work on the control of wheat stem sawfly. By 1946 the first solid stemmed spring wheat variety resistant to sawfly, named "Rescue", was available to farmers. This came out of an accumulation of knowledge and experience built up by Farstad with his assistants; his expertise was recognized when he was made director of the Plant Protection Service in 1959. Here he led the organization which was able to identify the golden nematode on Vancouver Island and introduce control measures very rapidly in 1965.

Farstad further contributed to entomology with his activities in the Entomological Society of Canada, of which he had been president. He was also a Fellow of the American Society for the Advancement of Science.

# UGA: A Third Nonsense Triplet in the Genetic Code

by

S. BRENNER

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Two base triplets of the genetic code are known not to represent any amino-acid. It now appears that, in *Escherichia coli*, the UGA triplet of the bases uracil, guanine and adenine does not code for an amino-acid and is therefore also a "nonsense triplet".

Most of the sixty-four triplets of the genetic code<sup>1</sup> have been allocated to one or other of the twenty amino-acids. The two known nonsense triplets (UAA, *ochre* and UAG, *amber*) are believed to signal the termination of the polypeptide chain. The only other triplet so far unallocated is UGA, for which binding experiments give uncertain or negative results.

In this article we show that UGA is "unacceptable" in our system (*Escherichia coli* infected with bacteriophage T4) and present suggestive evidence that it is nonsense; that is, that it does not stand for any amino-acid. Theoretical arguments make it likely that there is no transfer RNA (tRNA) to recognize it. The reason for this apparent absence of function is not yet known. Neither is it known whether UGA is nonsense in other organisms.

Evidence that UGA may be nonsense in *E. coli* has also been presented by Garen *et al.*<sup>2</sup> They investigated the reversion of *amber* and *ochre* mutants in the alkaline phosphatase gene of *E. coli*. *Amber* mutants (UAG) reverted, as expected, to seven different amino-acids including tryptophan which is coded by UGG. *Ochre* mutants (UAA) reverted to six of these amino-acids, but not to tryptophan. This negative result makes it unlikely that UGA stands for tryptophan (see also Sarabhai and Brenner<sup>3</sup>) and suggests that it might be a nonsense codon.

*Mutant X655 contains UGA.* Much of our genetic work has been concerned with the left-hand end of the B cistron of the *rII* region of bacteriophage T4. We have made extensive and detailed investigations of this region which are being reported elsewhere<sup>4</sup>. The mutant X655 occurs in the middle of this region. In brief our proof that X655 contains the triplet UGA consists in converting it to an *ochre* (UAA), using mutagens the behaviour of which is already known.

X655 was induced from wild type by 2-aminopurine, and identical mutants are also found after treatment of wild type phage with hydroxylamine. This shows that it differs from an acceptable triplet by a G-C to A-T base pair change in the DNA. It is not suppressed by any *amber* or *ochre* suppressor (Table 1) and is therefore neither UAG nor UAA. The reversion properties of X655 are shown in Table 2. It is strongly induced to revert to *r*<sup>+</sup> by 2-aminopurine, as is expected, but there is no induction to *r*<sup>+</sup> by hydroxylamine. Thus the triplet in the DNA either contains no G-C pairs or, if it does contain one, it is connected to another unacceptable triplet by a G-C to A-T transition.

The triplet is in fact connected to UAA by a transition, because X655 can be converted to an *ochre* and this change is induced by 2-aminopurine (Table 2). The nature of the

transition is more precisely specified by the finding that the conversion to an *ochre* is induced by hydroxylamine and that the *ochre* triplet produced does not require any replication for expression. Using a previous argument<sup>5</sup> this result suggests that the change arises from a G→A change in the messenger RNA. Because X655 is not an *amber*, this proves that it contains the triplet UGA. To confirm that an *amber* at the site of X655 would be suppressed by *amber* suppressors the X655 *ochre* has been converted to an *amber* by mutation and its properties tested (Table 1).

Table 1. SUPPRESSION PROPERTIES OF X655 AND ITS DERIVATIVES

Mutant	Triplet	<i>Amber</i> suppressors				<i>Ochre</i> suppressors		
		<i>su</i> <sup>-</sup>	<i>su</i> <sub>I</sub> <sup>+</sup>	<i>su</i> <sub>II</sub> <sup>+</sup>	<i>su</i> <sub>III</sub> <sup>+</sup>	<i>su</i> <sub>B</sub> <sup>+</sup>	<i>su</i> <sub>C</sub> <sup>+</sup>	<i>su</i> <sub>D</sub> <sup>+</sup>
X655	UGA	0	0	0	0	0	0	0
X655 <i>ochre</i>	UAA	0	0	0	0	+	+	+
X655 <i>amber</i>	UAG	0	+	+	+	+	+	+

Phage stocks were plated on the following strains: *su*<sup>-</sup>, CA244; *su*<sub>I</sub><sup>+</sup>, CA268; *su*<sub>II</sub><sup>+</sup>, CA180; *su*<sub>III</sub><sup>+</sup>, CA265; *su*<sub>B</sub><sup>+</sup>, CA165; *su*<sub>C</sub><sup>+</sup>, CA167; and *su*<sub>D</sub><sup>+</sup>, CA248.

Table 2. REVERSION OF X655

	Reversion index (in units of 10 <sup>-7</sup> )			
	Spontaneous	2-Aminopurine	Hydroxylamine direct	Hydroxylamine after growth
to <i>r</i> <sup>+</sup>	4	312	5	6
to <i>ochre</i>	4	51	1,090	533

X655 was treated with 2-aminopurine and hydroxylamine as previously described<sup>4,5</sup>. Total phage was assayed on *E. coli* B and *r*<sup>+</sup> revertants on CA244 (*su*<sup>-</sup>). *Ochre* revertants were selected on CA248 (*su*<sub>D</sub><sup>+</sup>) and distinguished from *r*<sup>+</sup> revertants by picking and stabbing about 300 plaques into CA248 and CA244.

*Other occurrences of UGA.* In three cases we have been able to produce the triplet UGA by selected phase shifts in our region. When (+ -) phase shifts are made over the first part of the B cistron, the two phase shift mutants frequently do not suppress each other. We have shown<sup>4</sup> that these barriers to mutual suppression are due to the generation of unacceptable triplets in the shifted frame. One of these barriers, *b*<sub>1</sub>, has been identified as an *amber* and two others, *b*<sub>3</sub> and *b*<sub>4</sub>, as *ochres*. Three barriers, *b*<sub>2</sub>, *b*<sub>5</sub>, and *b*<sub>6</sub>, have now been identified as UGA by their base-analogue induced reversion to *ochres*. In each case the identification has been checked by converting the *ochre* to an *amber* at the same site.

Tryptophan is represented by the single codon UGG. It would therefore be expected to mutate by transitions to both UAG (*amber*) and UGA, and thus in such cases *amber* and UGA mutants should occur in close pairs. The *amber* mutant, HB74, which maps close to X655,

is an example of this. Genetic crosses between it, X655, and the *ochre* and *amber* derived from X655, show that HB74 maps identically to the *amber* derived from X655, as expected (Table 3).

Table 3. RECOMBINATION BETWEEN VARIOUS MUTANTS

	X655	X655 <i>ochre</i>	X655 <i>amber</i>	HB74	Triplet
X655	0				UGA
X655 <i>ochre</i>	0	0			UAA
X655 <i>amber</i>	+		0		UAG
HB74	+	0	0	0	UAG

The phages were crossed in *E. coli* B and the complexes irradiated with ultra-violet light to stimulate recombination (see ref. 4). In the Table, 0 means that  $r^+$  recombinants were not significantly above the reversion rate, which was between  $2$  and  $9 \times 10^{-7}$ ; in those experiments where positive results were obtained (+), the frequency was between  $2$  and  $6 \times 10^{-5}$ .

So far we have found the expected pairs consisting of UGA and an *amber* in two other cases. In the A cistron, a mutant X665\* is found with the *amber* mutant N97, and in the B cistron, N65 is paired with the *amber* mutant X237. Both N97 and X237 are likely to have arisen from UGG (tryptophan) which is confirmed by the finding that they respond only poorly to the *amber* suppressor *su*<sub>11</sub> which inserts glutamine<sup>5</sup>. Both X665 and N65 have been converted into *ochre* mutants, showing that they contain the triplet UGA. These *ochres* have also been converted to *ambers* at the same site. Mapping investigations, analogous to those in Table 3, are consistent with these allocations.

**Unacceptability of UGA.** There is very good evidence that the amino-acid sequence coded by the first part of the B cistron is not critical for the function of the gene<sup>4</sup>. It can be replaced by varying lengths of the A cistron using deletions that join the two genes. Moreover, an extensive (- +) frame shift can be made without noticeable effect on the function. Of the fifteen known base-analogue mutants in the region, thirteen are either *ochres* or *ambers*; one, HD263, is temperature sensitive and X655 is UGA. The extreme bias towards *amber* and *ochre* chain-terminating mutants confirms the dispensability of the region<sup>4</sup>. These results make it unlikely that the unacceptability of UGA in X655 and the three barriers results from the insertion of an amino-acid, and strongly suggest that it is nonsense.

In addition, the UGA mutant X665 in the A cistron has been combined with the deletion r1589 and has been found to remove the B activity of this phage. This is the test for nonsense originally used by Benzer and Champe<sup>6</sup>.

In all these cases, however, it could be argued that UGA might code cysteine, especially as the two known triplets for cysteine are UGU and UGC. If the B protein already contained a cysteine essential for its function the effect of UGA elsewhere might be to produce an S-S bridge between the cysteine inserted by UGA and the (hypothetical) essential one, and thus inactivate the protein. Nevertheless we regard this as unlikely for two reasons, one genetic and one chemical.

The genetic evidence concerns the anomalous minutes produced by certain (+ +) combinations in the B cistron<sup>4</sup>. In some regions of the first part of the B cistron combinations of two (+) phase shift mutants are able to grow to some extent on the restrictive host, *E. coli* K12. The plaques produced are minute, however, showing that the wild type phenotype is very far from being completely restored. A detailed analysis of one set of these combinations showed that minutes are obtained only from pairs of (+) mutants which straddle barrier *b*<sub>4</sub>. The presence of the barrier is obligatory because, if it is removed by mutation, the (+ +) doubles are unable to grow at all on *E. coli* K12. The minutes are clearly due to a phase error of one sort or another and the phase error is dependent on the barrier *b*<sub>4</sub> which we now know to be UGA. This result shows that UGA cannot be associated with any

normal amino-acid reading and points strongly to the conclusion that it is nonsense.

The chemical reason for UGA not coding for cysteine comes from the work of Khorana *et al.*<sup>7</sup>. They have shown that poly (UGA)<sub>n</sub> when used as a messenger in a cell-free system derived from *E. coli* induces the production of poly methionine (corresponding to AUG) and also poly aspartic acid (corresponding to GAU). No other amino-acid appears to be incorporated. In particular, no poly cysteine was found. For various reasons this evidence is not completely decisive, but it at least makes it unlikely that UGA is cysteine.

**Function of UGA.** It might be thought that the sequence containing UGA was nonsense because it was the signal for the beginning or ending of a gene (or operon). In other words, that it produced its effect during the synthesis of the messenger RNA on the DNA template of the gene. This explanation is highly unlikely because the effects of UGA depend on it being read in phase. The phenotypic effect of X655 can be removed when the mutant is placed in a (- +) shifted frame<sup>4</sup>, and the barriers *b*<sub>2</sub>, *b*<sub>3</sub> and *b*<sub>4</sub> are of course produced by phase shifts. That is, the base sequence UGA actually occurs at these places in the wild type messenger RNA but in such a way that it is out of phase when the message is read correctly. Because we have no reason to suspect that RNA polymerase synthesizes messenger RNA in groups of three bases at a time these results imply that the phenotypic effects of UGA must occur during protein synthesis.

It thus seems unlikely that UGA codes for any amino-acid, and in particular it does not appear to code for either cysteine (UGU and UGC) or tryptophan (UGG). The wobble theory of codon-anticodon interaction developed by one of us<sup>8</sup> makes the prediction that because of a wobble in the recognition mechanism at the third place of the codon no tRNA molecule can recognize XYA alone without at the same time recognizing either XYG or both XYU and XYC. Such theoretical arguments cannot be considered conclusive, but they certainly suggest that UGA is a triplet for which no tRNA exists. For this reason we think it unlikely that UGA produces the efficient termination of the polypeptide chain, but more direct evidence will be needed to establish this point.

**Conclusion.** We have thus established that in the phage-infected cell UGA is certainly "unacceptable" in the rII cistrons, although it remains to be seen whether this is true for other species. We have produced reasons why it is unlikely to code for any amino-acid. We are confident that there must be weighty reasons if even a single triplet is not used in the genetic code, because otherwise natural selection would have certainly allocated it to an amino-acid. At the moment we are inclined to believe that UGA may be necessary as a "space" to separate genes in a polycistronic message. It is possible to make a plausible theory for *E. coli* along these lines, but we prefer to leave the discussion of this until we have more experimental evidence to support it. This we are at present attempting to obtain.

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\* This is not a misprint for X655.

# Intracellular Potassium and Macromolecular Synthesis in Mammalian Cells

by  
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The surface properties of cells may affect their ability to synthesize large molecules. These experiments raise the question whether the intracellular potassium concentration affects the synthesis of proteins and nucleic acids.

SURPRISINGLY little is known about the connexions between the surface activities of animal cells and the control of cell growth. This article describes the results of some simple experiments which suggest one possible connexion: that between the regulation of intracellular potassium concentrations and the synthesis of macromolecules.

These experiments were an outgrowth of our previous work on the control of bacterial growth, which showed that for at least two organisms, *Escherichia coli* B and *Bacillus subtilis*, the concentration of intracellular potassium sets the rate of protein synthesis<sup>1,2</sup>. With bacteria, it was possible to change the concentration of intracellular potassium at will by using mutants that did not accumulate potassium normally. For the present experiments on animal cells, analogous mutants were not available, but cell potassium concentrations could be manipulated by adding amphotericin B to the medium—this drug attaches irreversibly to steroids in the cell membrane and produces graded leaks of small molecules<sup>3</sup>. The amphotericin B used was the Squibb product, containing 41 mg sodium deoxycholate for each 50 mg of amphotericin.

With the loss of cell potassium caused by amphotericin there was a parallel depression of the rates of synthesis of protein and DNA. If the amount of potassium in the medium was increased, nearly normal levels of cell potassium could be sustained in these leaky cells and the depression of macromolecular synthesis could be largely prevented. The results are consistent with the hypothesis that in animal cells, as in bacteria, the level of intracellular potassium controls the rate of macromolecular synthesis.

Sarcoma-180 (S-180) cells were cultured in suspension at 37° C in Eagle's spinner medium, supplemented with both essential and non-essential amino-acids and with 10 per cent horse serum<sup>4</sup>. Doubling time was 1 day and cell densities ranged from 3 to 6 × 10<sup>5</sup> cells/ml. In order to measure the kinetics of potassium influx, potassium-42 chloride was added to growing cell suspensions; samples were removed periodically and collected on SS 'Millipore' filters (3 μ pore size) under soft suction equivalent to 2 cm of water. Cells on the filters were rinsed briefly with Eagle's medium and the radioactivity retained on the filter was measured in an end-window counter. The steady state potassium content of normally growing S-180 cells was estimated by equilibrating the cells with potassium-42, centrifuging a concentrated suspension in a haematocrit tube, and determining the potassium-42

content and volume of the packed cells. The results were corrected for extracellular fluid trapped in the pellet, as determined by the content of <sup>14</sup>C-sucrose in the pellet. The potassium concentration of normal S-180 cells was 135 ± 20 mmoles/l. Measurements of intracellular specific activity showed that the amount of potassium-42 contained in cells in the steady state was proportional to the intracellular potassium content.

For measurements of macromolecular synthesis, <sup>14</sup>C-leucine, uridine or thymidine was added to the medium; samples were collected at intervals on 'Millipore' filters under soft suction, and the filters were rinsed briefly with Eagle's spinner medium without horse serum to wash off excess protein. When only a small amount of fluid remained above the filters, 5 ml. of cold 10 per cent trichloroacetic acid was added; after 1 min, the filters were sucked dry, washed briefly with water and the radioactivity retained was counted. This procedure adequately traps all trichloroacetic acid precipitable material on the filters.

**Reduced potassium levels after amphotericin B.** S-180 cells took up potassium-42 with a half-time of 25 min (mean of four experiments). In a typical experiment (Fig. 1), 1, 2 and 3 μg/ml. of amphotericin reduced the steady state concentration of potassium-42 to about 85, 40 and 30 per cent of the control level. No obvious change in cell volume occurred for about 2 h. After many hours and high concentrations of drug (3 to 30 μg/ml.), however, considerable damage was apparent; cells became swollen and permeable to trypan blue. A similar lag between potassium loss and haemolysis has been reported for erythrocytes exposed to amphotericin<sup>5</sup>.

Increase in permeability was also readily detected by measuring the efflux of potassium-42 from prelabelled cells. The response to the drug was prompt; as little as 1 μg/ml. more than doubled the efflux of potassium-42 within 5 min of the addition of drug.

**Protein, RNA, and DNA synthesis.** In most experiments, cells were incubated with amphotericin for 30–60 min to allow a new steady state concentration of potassium to be reached, and the incorporation of <sup>14</sup>C-leucine, uridine, or thymidine was then measured. At low concentrations of amphotericin, the rate of protein synthesis reached a new steady level at each concentration of drug (Fig. 2). After initial equilibration of cells with the drug for 60 min, the rate of synthesis in the presence of 1, 2 and 3 μg/ml. of drug was 80, 38 and 22 per cent of control. Incorporation of <sup>14</sup>C-thymidine into DNA was depressed



to about the same extent as incorporation of  $^{14}\text{C}$ -leucine. Thus the decrease in the intracellular level of potassium (Fig. 1) and the decreases in the rates of synthesis of protein and DNA were nearly proportional. At all concentrations of amphotericin (1–20  $\mu\text{g}/\text{ml}$ .) depression of RNA synthesis was only about half that of protein synthesis.

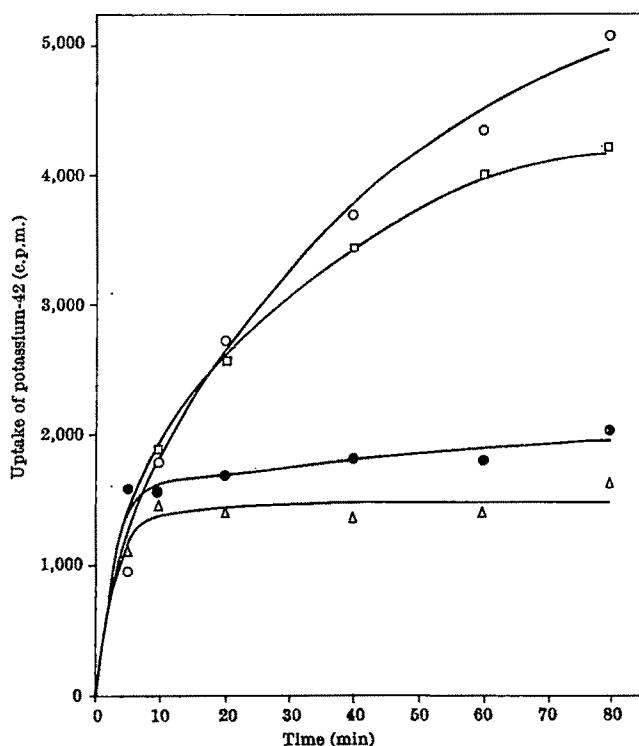


Fig. 1. Uptake of potassium-42 by S-180 cells after incubation for 60 min with amphotericin B, at either zero (○), 1 (□), 2 (●) or 3 (Δ)  $\mu\text{g}/\text{ml}$ .

**Reversal of inhibition of protein synthesis by media containing high concentrations of potassium.** These results showed that amphotericin produced both a loss of potassium and a reduced rate of protein and DNA synthesis. It was not clear, however, whether most of the depression of synthesis was due specifically to loss of potassium or to loss of other essential small molecules that may have leaked out.

Parallel experiments were therefore carried out in which incorporation of leucine (or thymidine) was measured in the presence of a modified Eagle's spinner medium, in which all of the sodium, except for the modest amount contained in the supplement of horse serum, was replaced by potassium, mole for mole. Fully substituted medium is referred to as 140 mmolar potassium Eagle's medium; normal Eagle's medium contains 5 mmoles of potassium per litre.

In preliminary experiments, it was found that 140 mmolar potassium Eagle's monolayer medium was toxic to S-180 cells. Cells in the corresponding 100 mmolar potassium Eagle's monolayer medium, however, proliferated almost as well on glass surfaces as control cells, and in 100 mmolar potassium Eagle's spinner medium the rate of leucine incorporation was about 85 per cent of the control value (mean of four experiments). Experiments on the reversal of amphotericin effects were therefore largely confined to media containing 100 mmolar potassium or less.

The typical experiment of Fig. 3 shows that amphotericin at 8.5  $\mu\text{g}/\text{ml}$  depressed protein synthesis after 60 min to 10 per cent of the control rate. In the presence of

100 mmolar potassium Eagle's medium, however, the same dose of drug depressed protein synthesis to only 60 per cent of the control rate. The reversal by high levels of potassium is striking, and similar results were repeatedly obtained over a wide range of concentrations of amphotericin. With low concentrations of amphotericin (for example, 2  $\mu\text{g}/\text{ml}$ .) and a medium rich in potassium, the rate of protein synthesis was depressed by less than 10 per cent of control values.

Loss of potassium was also produced by high levels of histones or polylysine<sup>6,7</sup>. Protein synthesis was strongly depressed by 100  $\mu\text{g}/\text{ml}$  of histone, but in the presence of 100 mmolar potassium Eagle's medium, the depression was small (Fig. 4). In other experiments, inhibition of incorporation of  $^{14}\text{C}$ -thymidine by amphotericin or by histone was similarly largely prevented by increasing the level of potassium in the medium. In a typical experiment, incubation of S-180 cells for 1 h with 100  $\mu\text{g}/\text{ml}$  of histone reduced incorporation of  $^{14}\text{C}$ -thymidine to 25 per cent of the control rate; but with cells suspended in 100 mmolar potassium Eagle's medium, histone reduced incorporation to only 80 per cent of the control rate.

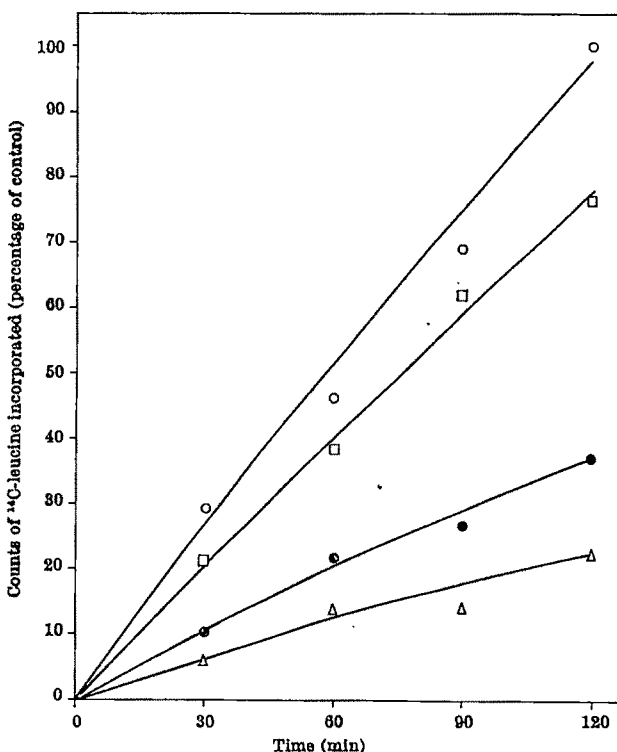


Fig. 2. Incorporation of  $^{14}\text{C}$ -leucine into protein by S-180 cells after incubation for 60 min with amphotericin B, at either zero (○), 1 (□), 2 (●) or 3 (Δ)  $\mu\text{g}/\text{ml}$ .

The following related results were also found: (1) the addition of 2 mmolar calcium chloride to the medium did not reverse the depressant effects of amphotericin; (2) deoxycholate, when added in amounts equivalent to that present in the preparation of amphotericin B, had no effect on macromolecular synthesis in intact cells; (3) amphotericin did not inhibit protein synthesis by direct action on polypeptide synthesis, as was shown by measurement of the incorporation of  $^{14}\text{C}$ -leucine in a microsomal system derived from rat liver; (4) measurements were made of the fraction of cells that became permeable to trypan blue after incubation with amphotericin; although cells had lost up to 50 per cent of their potassium after 2–3  $\mu\text{g}/\text{ml}$  of drug, less than 3 per cent of the cells had become permeable to trypan blue; the potassium loss

was not therefore due to total disruption of a sensitive fraction of the cell population, but was shared by the bulk of the cells; (5) amphotericin, in amounts up to 20  $\mu\text{g}/\text{ml.}$ , over a period of several hours, did not increase the rate of loss of prelabelled proteins; after many hours, however, or at higher concentrations of drug, leakage of protein was high (up to 50 per cent/h at 50  $\mu\text{g}/\text{ml.}$ ); (6) some of the experiments reported here were also carried out with *L*-cells in suspension culture; in these cells, amphotericin (5–10  $\mu\text{g}/\text{ml.}$ ) was found to inhibit protein synthesis considerably more than RNA synthesis; when protein synthesis was 15 per cent of the control rate, RNA synthesis was still 50 per cent; as with *S*-180 cells, the depression of macromolecular synthesis in *L*-cells by amphotericin was largely reversed by high concentrations of potassium; in two experiments, however, high levels of potassium did not reverse the depression of protein synthesis produced by histone (100  $\mu\text{g}/\text{ml.}$ ); (7) in preliminary experiments, ouabain at high concentrations (0.3–1 mmolar) was also found to reduce cell potassium and inhibit protein synthesis in *S*-180 cells; (8) doubling or halving the amino-acid content of Eagle's medium had no significant effect on the results, which indicates that the size of the amino-acid pool did not limit the rate of protein synthesis in leaky cells.

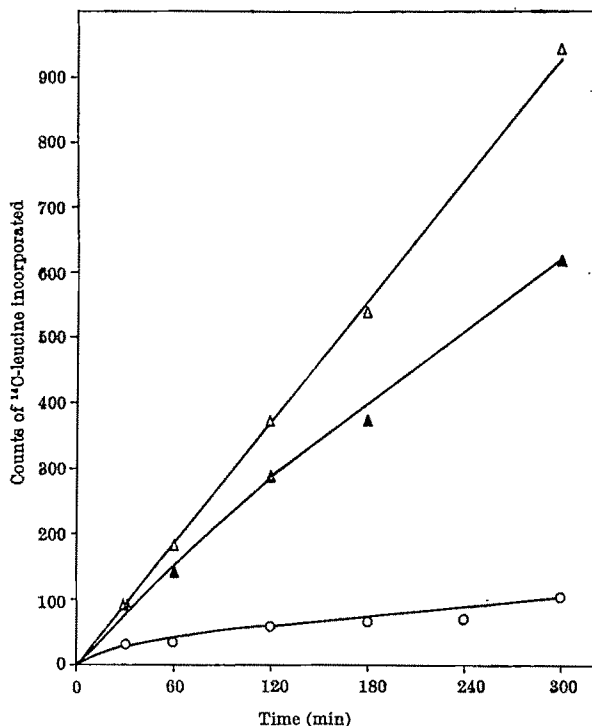


Fig. 3. Effect of high and low potassium media on protein synthesis after amphotericin B.  $\Delta$ , Control;  $\circ$ , incorporation in 5 mmolar potassium Eagle's medium, with 8.5  $\mu\text{g}/\text{ml.}$  of amphotericin B;  $\blacktriangle$ , 8.5  $\mu\text{g}/\text{ml.}$  of drug, but 100 mmolar potassium Eagle's medium.

Two inferences can be drawn from the results: (1) the depression of macromolecular synthesis in the presence of amphotericin B can be specifically reversed by increasing the level of intracellular potassium; and (2) only a small decrease in intracellular potassium is needed to produce a proportional decrease in macromolecular synthesis.

It must be stated explicitly, however, that the results do not distinguish between a direct effect of potassium levels on macromolecular synthesis, and indirect effects, such as inhibition of synthesis or utilization of ATP, or of glycolysis (see ref. 3 for the effect of potassium levels on glycolysis in yeast). Moreover, the loss of potassium

from mammalian cells is known to be largely compensated for by a gain in sodium, and these preliminary results could equally well be attributed to a change in the potassium/sodium ratio.

One practical result is of interest: of the sizable number of agents which have been found to depress macromolecular synthesis or growth of mammalian cells in culture, some may have as their common mode of action simply a loss of cell potassium.

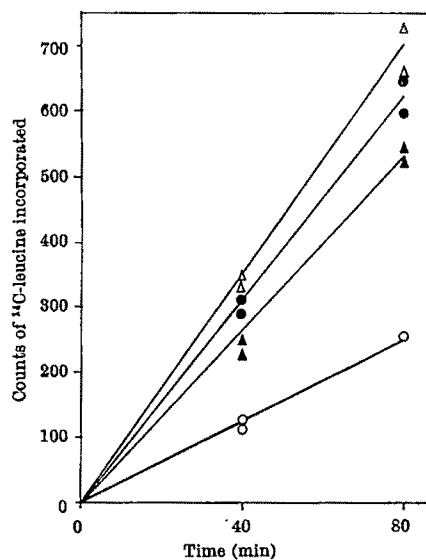


Fig. 4. Inhibition of protein synthesis by histone.  $\Delta$ , Control, cells in 5 mmolar potassium Eagle's medium, no drug;  $\bullet$ , cells in 100 mmolar potassium Eagle's medium;  $\circ$ , 5 mmolar potassium Eagle's medium plus 100  $\mu\text{g}/\text{ml.}$  of histone;  $\blacktriangle$ , 100 mmolar potassium Eagle's medium plus 100  $\mu\text{g}/\text{ml.}$  of histone. The histone used was the Sigma 3,000 fraction.

Although it is unclear whether the findings are relevant to cell growth within intact animals, some conjectures may be worth considering and testing experimentally. For example, cells in normal tissue, unlike those in tumour tissue, were recently found to possess low resistance junctions<sup>8</sup>. If potassium and sodium are involved in control of cell growth, such junctions may serve to equalize the cation concentrations throughout a communicating set of cells and thereby regulate the common rate of growth. In addition, normal and tumour cells differ in their growth responses to the solid substrate on which they may lie; normal cells in confluent monolayers exhibit a high degree of contact inhibition<sup>9</sup>. One possible explanation that has not been fully examined is that the nature of the cell surface and its contacts affects the leak or pump rate of monovalent cations, and that inhibition of growth on an unfavourable surface may be partly due to a decreased intracellular potassium/sodium ratio.

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# Nucleic Acid Synthesis in Seedlings

by

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As little as one part in  $10^7$  by weight of contaminating bacteria can alter the labelling of seedling nucleic acids with phosphorus-32. The bacterial nucleic acids are labelled in preference to those of the plant cells. With seedlings free of bacteria the labelling occurs at a slower rate. Systems involving the labelling of nucleic acids should be tested for bacterial contamination.

CONTAMINATING bacteria can contribute to the labelling patterns of the macromolecules of rapidly growing tissues. This has already been shown to be possible in the case of fertilized sea urchin eggs<sup>1</sup> and it is here illustrated with lettuce and radish seedlings. These seedlings have a normal bacterial flora which produces a labelling pattern similar to that previously reported to be characteristic of tissues from other seedlings<sup>2-5</sup>. The similarity in the labelling patterns does not necessarily mean that the earlier results also arise in whole or part from artefacts, but it does show the need to test as carefully as possible for the contributions of bacteria in future investigations of this type.

Bacterial contamination in seedlings is hard to eliminate. "Surface-sterilization" does not always work; for example, Green and Gordon found low levels of bacterial contamination and substantial levels of bacterial DNA synthesis in seedlings germinated aseptically from "sterilized" tobacco seeds<sup>6</sup>. The use of antibiotics in addition to surface-sterilization is not always effective or desirable. The bacteria also cannot be washed from the seedlings—a method which was effective with sea urchin eggs<sup>1</sup>.

The methods usually used to treat and to germinate seeds for labelling experiments are as follows: Two grams of lettuce seeds (Asgrow, 'Grand Rapids') or radish seeds (Burpee, 'Rapid Red') were rinsed in ethanol and then washed 20 min in 100 c.c. of neutral 1 per cent sodium hypochlorite and rinsed with sterile water. They were germinated for 3 days in sterilized covered glass trays lined with filter paper and wetted with 18 ml. of either sterile water or the antibiotic solutions described below. The last 2.5 days of germination were in darkness.

For labelling with phosphorus-32, trays of dark-grown seedlings were transferred under dim green light to a light-tight box and moved to the isotope laboratory where a neutral solution of carrier-free <sup>32</sup>P-sodium or ammonium phosphate (10 or more mc.) was injected by way of an inlet tube. The box was equipped with a tray-sized paddle on a sliding shaft so that the seedlings could be pressed down and wetted occasionally during the exposure. At the end of the exposure the box was opened to the light, and the plants were washed *in situ* with a stream of ice cold 2 mmolar carrier phosphate and with an aspirator for drainage.

Nucleic acids were extracted by homogenizing the seedlings at "high speed" for 75 sec in an 'Osterizer' in ten volumes of an ice-cold slurry of 1 per cent sodium dodecyl sulphate containing 0.02 molar *tris* buffer pH 8 (measured at 22° C), 0.1 molar sodium chloride and 5 mmolar mercaptoethanol. Eight additional volumes of redistilled phenol (containing 10 per cent aqueous 1 mmolar ethylenediamine tetraacetic acid (EDTA)) were added, the mixture was again blended for 15 sec, and mechanically shaken for 15 min in the cold room. The aqueous layer was separated by centrifugation and saved and the remainder was re-extracted with the detergent slurry. The aqueous layers were pooled, re-extracted with phenol, and mixed with 0.1 volumes of 20 per cent

potassium acetate pH 5.3 and 2.5 volumes of cold ethanol to precipitate the nucleic acids. After one night in the refrigerator, the precipitate was centrifuged and washed with 70 per cent ethanol. It was then redissolved in the detergent slurry and extracted once again with phenol, re-precipitated, washed, and dissolved in a few millilitres of 0.05 per cent sodium dodecyl sulphate in dilute *tris* buffer and stored frozen. The discarded solid residues from the initial phenol extractions of seedlings free from bacteria contained relatively little additional labelled RNA; 5 per cent more, at most, could be extracted with detergent and phenol at 60° C.

For bacterial assay, samples of seedlings from the isotope exposure step were homogenized in isotonic saline. Colony counts were made on diluted aliquots of the homogenate which were spread and incubated for several days at room temperature on nutrient agar.

Radish seedlings from seeds treated with hypochlorite were often "free from bacteria" (which is defined for these experiments as less than  $10^3$  bacteria/g) while untreated seeds yielded seedlings with about  $10^8$  bacteria/g. Even with hypochlorite treatment, lettuce seedlings grown in water contained more than  $10^8$  viable bacteria/g wet weight. Among the bacteria found in lettuce and radish seedlings were *Corynebacterium* sp., various pseudomonads, *Flavobacterium* sp. and *Bacillus macerans* (McCoy, E., personal communication).

Sterile lettuce seedlings could sometimes be germinated from seeds treated with hypochlorite in a standard antibiotic mixture composed of 100 U/ml. penicillin G and 'Mycostatin' and 100 µg/ml. streptomycin sulphate and 'Aureomycin' or in the standard mixture without 'Aureomycin'. Even under these conditions, lettuce seedlings were often contaminated with more than  $10^4$  resistant bacteria/g. Lettuce free from bacteria could, however, be grown consistently from seeds treated with hypochlorite that were leached overnight in several-hundred volumes of standard antibiotic mixture while being agitated by a stream of sterile air bubbles. These seeds yielded plants free from bacteria, even when they were germinated in water.

Thirty-three pulse-labelling experiments have been made with lettuce seedlings and nine with radish. Nineteen of the lettuce experiments involved treatment with antibiotics; however, bacterial contamination occurred in six of these.

Fig. 1a shows the gradient centrifugation pattern of an extract from lettuce seedlings contaminated with bacteria. There are large counts for the components insoluble in trichloroacetic acid near the top of the tube (to the left) and in two RNA components (I and II) sedimenting with and between the bulk light and heavy ribosomal RNA. In this separation <sup>32</sup>P-DNA underlies the left side of component I. <sup>32</sup>P-DNA has been identified in other gradients by its resistance to pancreatic ribonuclease and to mild alkaline hydrolysis as well as by chromatography. The sedimentation properties of components I and II resemble those reported for the RNA

labelled in 3 day old cotton seedlings<sup>5</sup> and components seen in the labelled RNA of soybean hypocotyls<sup>4</sup>. Component II resembles the heavy ribosomal RNA (bacterial) found to be labelled in contaminated sea urchin eggs<sup>1</sup> and in general heavy bacterial ribosomal RNA. Component I does not appear to sediment slower than the bulk plant light ribosomal RNA, and this may argue against the theory that it is of bacterial origin.

A peak of labelled RNA sedimenting in the position of component II was only observed in experiments made with contaminated seedlings. Fig. 1b shows that tissue grown in the presence of antibiotics, but containing  $7 \times 10^4$  viable bacteria/g, may still contain this peak.

Fig. 1c shows the sedimentation pattern of nucleic acids from lettuce free from bacteria and treated with antibiotic. Whenever nucleic acids were labelled in seedlings free from bacteria they showed, in addition to slowly sedimenting material, three rather flat sedimentation peaks: (1) a little faster than the bulk light ribosomal RNA; (2) with the heavy ribosomal RNA; (3) about a quarter farther down the tube than the bulk heavy RNA. The slowly sedimenting material is composed partly of non-nucleic acid impurities.

Fig. 1d shows a separation of the RNA from 1c which has been further purified by precipitation at 0° C with 2 molar lithium chloride to remove most of the DNA, transfer-RNA and slower sedimenting impurities. Recovery of material greater than 16S is essentially complete. Table 1 shows the nucleotide composition of components 1, 2 and 3 from the material separated in Fig. 1d. The hydrolysates of these fractions contained no large amount of any unexpected component; a separate experiment with a 'Dowex-1' column showed that the elution of nucleotide radioactivity precisely matched the ultraviolet absorption of the carrier nucleotides. (Nucleotides and nucleotide 2'-3' isomers were separated on a 20 cm column of 400-mesh 'Dowex-1  $\times$  8 Cl-' (Lot 5893-35) with a 600 ml. linear 0.02 normal hydrochloric acid gradient. The 2'-3' isomers of cytidine monophosphate and uridine monophosphate were not resolved. Most samples of 'Dowex-1' were not satisfactory for this separation.)

The results in Table 1 indicate that a high AMP content RNA may be present under components 1, 2 and, to a

Table 1

Per cent	C	A	U	G
Fraction 1	17.1	38.3	24.6	22.0
Fraction 2	17.3	38.7	24.6	24.3
Fraction 3	18.0	30.9	23.9	27.3
(Ribosomal RNA)	24.0	24.5	20.8	30.7

The base composition of the pulse labelled RNAs from lettuce seedlings free from bacteria as the percentage of recovered phosphorus-32 in each ribonucleotide. The sucrose gradient fractions of the RNA purified in lithium chloride shown in Fig. 1d were hydrolysed 18 h in 0.5 normal potassium hydroxide and desalted by the procedure of Strauss and Sinsheimer<sup>14</sup>. Nucleotides were separated electrophoretically by the method of Smith<sup>15</sup> and counted. It was calculated that the ribonucleotides together accounted for 99-101 per cent of the total hydrolysate phosphorus-32. The lettuce ribosomal RNA values were determined by chromatography on 'Dowex-1'; 3-day labelled ribosomal RNA was purified for this on a methylated albumin on kieselguhr column.

lesser extent, 3 in the form of a broadly dispersed peak on which is superimposed normal plant ribosomal RNA and another RNA (component 3) which could be a nuclear ribosomal RNA precursor as has been found in animal tissues<sup>7</sup>. The high AMP-RNA compositions resemble those reported for <sup>32</sup>P-RNA from soybean seedling hypocotyls<sup>4</sup> and roots<sup>8</sup> and from pea seedling roots<sup>9</sup>. RNA such as this has been called "DNA-like" by other authors<sup>4</sup>, despite its low UMP content. Another interpretation of the apparent high AMP content of these RNAs is that there could be non-uniform labelling of precursor 5'-nucleotides within the seedlings and also a non-uniform nearest neighbour frequency in the newly synthesized RNA. These together could give a non-uniform labelling of 3'-nucleotides released from the RNA by alkaline hydrolysis such that 3' AMP would have the highest specific activity<sup>10</sup>.

Figs. 1e and 1f show the sedimentation of pulse-labelled nucleic acids from contaminated and bacteria-free radish seedlings. Because the seeds were sterilized with only alcohol and hypochlorite it is clear that it is the absence of bacteria rather than the presence of antibiotics which causes an important decrease in incorporation and the disappearance of component II. Elimination of bacteria in lettuce or radish caused not only a loss of component II but also at least a seven-fold decrease in the labelling of RNA in the region of component I. This suggests that most of component I from contaminated seedlings is an artefact of bacterial origin and that only a part is genuine RNA (component 1) from the plant cells.

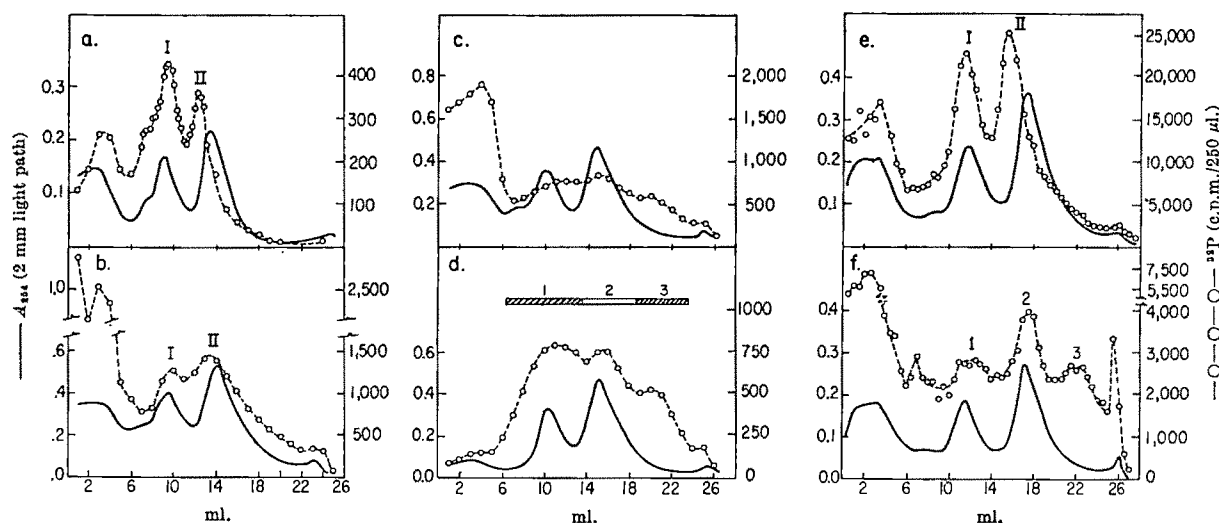


Fig. 1. Sucrose gradient centrifugation of <sup>32</sup>P-nucleic acids from seedlings. The top of each tube is to the left. (a) Contaminated lettuce grown from hypochlorite treated seeds and containing  $10^4$ - $10^5$  bacteria/g (on the basis of analysis of similarly grown samples). After a 20 min labelling 0.06 per cent of the fed phosphorus-32 was recovered in the trichloroacetic acid insoluble components of the extract. (b) Slightly contaminated from hypochlorite treated seeds germinated in "complete antibiotic mixture" and containing  $7 \times 10^4$  bacteria/g. 60 min labelling, 0.011 per cent recovery. (c) Lettuce free from bacteria from antibiotic leached seeds also germinated in the "complete antibiotic mixture". 60 min labelling, 0.008 per cent recovery. (d) Lithium chloride insoluble RNA from c representing 0.005 per cent of the fed phosphorus-32. (Fractions 1, 2 and 3 were pooled from identical gradients for base analysis.) (e) Contaminated radish from untreated seed and containing  $4 \times 10^4$  bacteria/g. 60 min labelling, 0.2 per cent recovery. (f) Radish free from bacteria from seeds treated with alcohol-hypochlorite. 60 min labelling, 0.07 per cent recovery. Separations were made at 2° C in the Spinco S15-25 rotor in a 4-20 per cent gradient of sucrose containing 0.05 molar pH 7.0 Tris buffer and, except in a, 0.05 molar sodium chloride. Gradients b, c and d also contained 0.5 mg/ml. polyvinylsulphate. Fractions were precipitated with carrier serum albumin and trichloroacetic acid for scintillation counting.

Components 1, 2 and 3 from radish were also found in samples purified by precipitation with lithium chloride; these were shown, as with lettuce, to have a high content of AMP.

The standard antibiotic mixture did, in fact, stunt seedling growth. Control experiments with sterile radish indicated that this resulted in a proportionate decrease in the incorporation of isotope. The antibiotics, however, did not cause any important changes in the sedimentation pattern or composition of the labelled RNA.

Other types of experiments with contaminated lettuce seedlings showed that some pulse-labelled RNA could be recovered in a ribosome-like particle which sediments more slowly than the bulk ribosomes and resembles the "nascent" cotton seedling ribosomes of Waters and Dure<sup>6</sup>. This RNA sediments as do components I and II of Fig. 1, but there is relatively more of component II. No component II is found, however, in the labelled RNA from ribosomes of uncontaminated seedlings and in the lettuce system it seems likely that the "nascent" particle is therefore an artefact derived from bacterial ribosomes.

Labelled seedling nucleic acids were also analysed by column chromatography on methylated albumin bound to kieselguhr by the method of Mandell and Hershey<sup>11</sup>. The results of a few of these analyses are shown in Fig. 2. The bulk light and heavy ribosomal RNA from plants could not be clearly separated with the steep elution gradients which I used; light and heavy ribosomal RNA from *Escherichia coli*, however, could.

Elution patterns of labelled extracts of contaminated lettuce seedlings varied somewhat from experiment to experiment (perhaps as a function of the type and extent of bacterial contamination), but a substantial amount of radioactivity always appeared with the first eluting shoulder of light ribosomal RNA. Component I in Fig. 2a is a clear example; components I and II are not always this clearly defined and radioactivity often fills in the valley between them. Even in experiments where these could not be sharply defined by chromatography, gradient centrifugation of the same samples showed the characteristic two peaks associated with bacterial ribosomal

RNA. When the separated components from gradient centrifugation were loaded on and eluted from methylated albumin columns together with carrier lettuce ribosomal RNA, the slower sedimenting one (I) ran largely ahead of the ultra-violet absorbing peak while the faster (II) eluted later. In this sense, components I and II seen in the centrifuge are the same as chromatographic components I and II. Figs. 2c and 2d show that lettuce (antibiotic treated) and radish (no antibiotics) seedlings free from bacteria have very little radioactivity eluting in the region of component I. There is also a relative decrease in the labelling of DNA.

The most salient feature of the chromatography experiments is that seedlings which are contaminated with bacteria always produce a main <sup>32</sup>P-DNA component which elutes in advance of the bulk DNA. This is never apparent when there is no contamination, and a very small amount of bacteria will produce it as is shown in Fig. 2b where the seedlings contained only  $7 \times 10^4$  bacteria/g. The association of this peak with bacterial contamination has also been confirmed by K. Šebesta with cucumber seedlings (personal communication).

Some <sup>32</sup>P-RNA also elutes in the region of the <sup>32</sup>P-DNA peak from contaminated lettuce seedlings. Caesium chloride buoyant density centrifugation has been used to separate both labelled RNA and bulk DNA from this labelled (bacterial) DNA, and so this peak does not contain RNA and DNA in the form of a "chemical association" such as reported in peanut cotyledons<sup>3</sup>. Non-uniform labelling of any chloroplast or other satellite DNA would be hard to detect by the methods used here if it caused (as is reported for tobacco<sup>9</sup>) only a several-fold increase in specific activity relative to the bulk nuclear DNA. This is because the satellite DNA is likely to be present to the extent of only 1 or 2 per cent of the total.

The contributions of bacteria are not linearly proportional to the extent of contamination—there is not a thousand-fold increase in labelled bacterial DNA or RNA when the population is increased from  $10^5$  to  $10^8$ /g plant tissue. A similar non-linearity of bacterial protein synthesis in contaminated chloroplast preparations has been

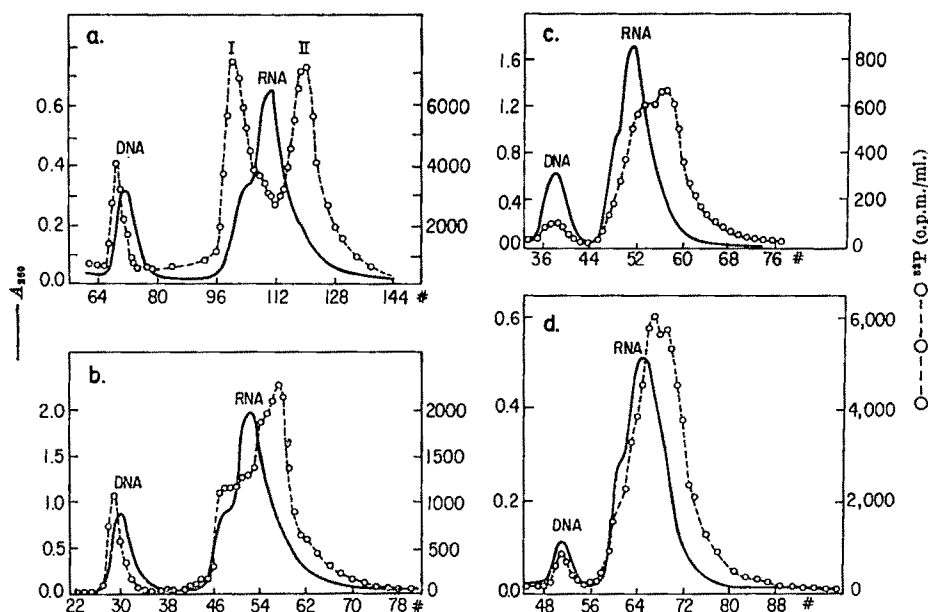


Fig. 2. The chromatographic separation of nucleic acid extracts from seedlings labelled with phosphorus-32. (a) Contaminated lettuce grown from hypochlorite treated seeds and containing  $10^5$ – $10^8$  bacteria/g. 0.2 per cent of the fed phosphorus-32 was recovered in a form insoluble in trichloroacetic acid in the crude nucleic acid extract after a labelling period of 20 min. (b) Slightly contaminated lettuce—the extract used in Fig. 1b. (c) Lettuce free from bacteria from seed treated with hypochlorite germinated in "complete antibiotic mixture" but without 'Aureomycin'. 40 min labelling led to a 0.002 per cent recovery of phosphorus-32 in the extract. (d) Radish free from bacteria from seeds treated with alcohol-hypochlorite. 65 min labelling gave 0.04 per cent recovery. Nucleic acids were loaded on to methylated albumin-kieselguhr and eluted with a linear 0.4–1.5 molar sodium chloride gradient (ref. 11); only the DNA and ribosomal RNA portions of the elution are shown.



reported<sup>12</sup>. This means that it is difficult to test whether or not a given component is bacterial on the basis of whether or not its labelling is decreased by a several-fold decrease in bacterial content, a practice which has been used to discredit the possibility of bacterial artefacts by other workers<sup>13</sup>.

I thank Mrs. Dorothy Lloyd for assistance and Dr. P. G. Heytler for his support and interest. I also thank Prof. E. McCoy and her students at the University of Wisconsin for identifying seedling bacteria and Dr. N. G. Anderson for a special sample of ion-exchange resin.

*Note added in proof.* D. A. Barber has recently also reported that bacterial contamination can profoundly alter phosphorus-32 incorporation into plants (*Nature*, 212, 638; 1966). His results show that bacteria compete with plants for limiting amounts of available phosphate. It seems likely then that bacterial contamination doubly obscures the nucleic acid labelling pattern in seedlings. Not only do rapidly labelled bacterial nucleic acids mask the more slowly labelled plant nucleic acids but also the relative rate of labelling of the latter is further decreased because the bacteria compete for the exogenous phosphorus-32.

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## Dual Localization of $\beta$ -Glucuronidase in Endoplasmic Reticulum and in Lysosomes

by

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Acid hydrolases are not exclusively lysosomal enzymes as indicated by the specific case of  $\beta$ -glucuronidase. This enzyme is a membrane protein found in the endoplasmic reticulum and in the lysosomes of the cell.

LYSOSOMES are organelles in a particular sedimentable fraction of tissue homogenates. They are characterized by a structure linked latency of their constituent acid hydrolases. A strong bias in favour of an exclusively lysosomal localization of acid hydrolases has developed because the lead salt technique for acid phosphatase demonstrates lysosomes only<sup>1</sup>. Non-lysosomal sites for these enzymes have scarcely been considered. Yet deDuve showed in 1955 (ref. 2) that only about 20 per cent of the total activity of another acid hydrolase,  $\beta$ -glucuronidase, could be found in the lysosomal fraction of centrifuged rat liver, while 40 per cent is present in microsomes.

The present article considers with regard to enzyme staining reactions the validity of non-lysosomal location of  $\beta$ -glucuronidase, contends that dual localizations (lysosomal and non-lysosomal) are to be expected for other acid hydrolases, and suggests a non-lytic function for these enzymes.

In the course of the metal chelate technique<sup>3-5</sup>, fresh tissue specimens (2 × 2 × 5 mm) were fixed in formol chloral hydrate solution for 18 h, washed in running tap water for 2 h, transferred to Holt's 0.88 molar gum sucrose solution for 2 days and then cut on the freezing microtome or cryostat at 7–10 $\mu$ . The sections were carefully placed so as to float freely on the surface of the substrate solution (1 section per ml. in a 16 ml. 'Pyrex' beaker). The Fishman-Baker substrate solutions<sup>6</sup> contained 15.25 mg of 8-hydroxyquinoline  $\beta$ -D-glucosiduronic acid in 0.1 per cent polyvinylpyrrolidone solution<sup>3</sup> at

pH 3.8. After 3 h at 4° C the beakers were covered with 'Parafilm' and incubated in a water bath at 37° C for 30 min. The surface deposit was then removed with filter paper, and the section transferred to distilled water and agitated gently.

The washed tissue was immersed in oxalate buffer for 15 min and briefly returned to distilled water. To convert the ferric iron to Prussian blue, the tissue was treated with an equal mixture of molar hydrochloric acid and 2 per cent potassium ferrocyanide for 15 min, rinsed in distilled water, transferred to a 1 per cent solution of warmed Knox gelatine, placed on a glass slide and allowed to dry in air. The sections were secured to the slides by treatment with 10 per cent formol-calcium for 10 min. Carmalum or neutral red were used as counterstains. The sections were dehydrated routinely and were mounted in 'Technicon'. Those tissues in which the ferric chelate was not converted to Prussian blue were mounted directly (Fig. 1a, rat liver) without dehydration.

In the electron microscope experiments, 40 $\mu$  sections of fresh mouse liver were cut on the cryostat and were fixed in 2.5 per cent glutaraldehyde buffered at pH 7.2 with 0.1 M cacodylate buffer for 30 min<sup>6</sup>. The tissues were removed from the fixative with glass rods, washed five times in 0.1 molar cacodylate buffer pH 7.2 for 10 min and incubated in the Fishman-Baker medium as described, first for 1.5 h at 4° C and then at 37° C for 45 min. After conversion of the ferric chelate to Prussian blue, the sections were cut into 1 mm<sup>2</sup> pieces, dehydrated through graded alcohols and embedded in a mixture of

methyl and butyl methacrylates. Thin sections were cut on a Porter-Blum ultramicrotome, and examined under the electron microscope (Fig. 1b). Boiled tissue sections were used as controls.

Negative reactions were found in sections of fixed rat liver, kidney and muscle previously heated in water at 100° C for 1 min or at 56° C in acetone for 1 h. The mixed substrate controls<sup>3</sup> using the glucosiduronic acids of phenolphthalein, menthol and bacalein showed no reaction at concentrations of 0.001 molar, 0.003 molar and 0.00018 molar, respectively. Saccharolactone inhibited about 50 per cent of the reaction at 0.001 molar concentration and almost all of it at 0.003 molar. The post-incubation azo-dye coupling technique, using naphthol *AS-BI*- $\beta$ -D-glucosiduronic acid<sup>7</sup>, was used at pH 4.5 using the method of Fishman and Goldman<sup>8</sup>.

Ferric chelate deposits were visible throughout the cytoplasm of the parenchymal liver cells (Fig. 1a) es-

pecially bordering the bile canaliculi, and in granules of Kupffer cells in a manner indistinguishable from the naphthol *AS-BI* azo dye pigments<sup>8</sup>. Under the electron microscope (Fig. 1b), the endoplasmic reticulum appeared as a main site of deposition of enzyme products. Lysosomes exhibited accumulations of dense material on their limiting membranes. The boiled tissue sections did not have enough contrast to be photographed.

In Fig. 2a the Prussian blue reaction product is located on the boundaries of the large lipid containing vacuoles and in the intervacuolar cytoplasmic spaces of the preputial gland, as is the 8-hydroxyquinoline-azo-dye pigment (Fig. 2b) product obtained in the absence of ferric ions and in the presence of fast dark blue *R*, and the pigment product of the post-coupling technique with naphthol *AS-BI*- $\beta$ -D-glucosiduronic acid as a substrate (Fig. 2c). These intervacuolar spaces are filled with elements of endoplasmic reticulum and Golgi apparatus<sup>9</sup>.

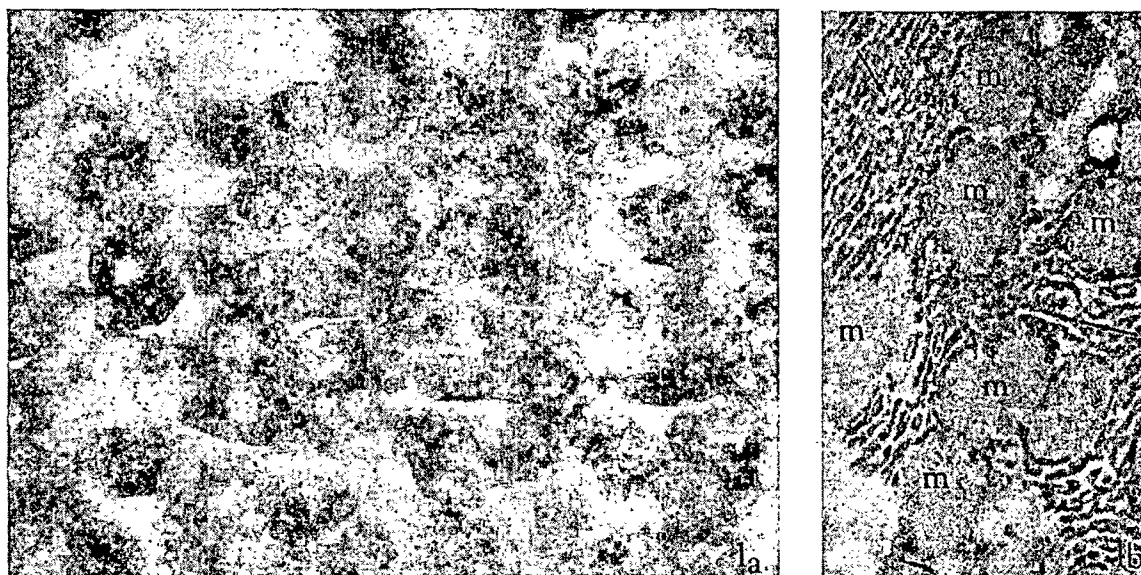


Fig. 1. Liver  $\beta$ -glucuronidase localization. *a*, Positive sites are visible as iron-8-hydroxyquinoline deposits unconverted to Prussian blue. ( $\times 600$ .) *b*, Ferric-8-hydroxyquinoline deposits converted to Prussian blue. Arrows indicate deposits on endoplasmic reticulum. *m*, Mitochondria. ( $\times 20,000$ .)

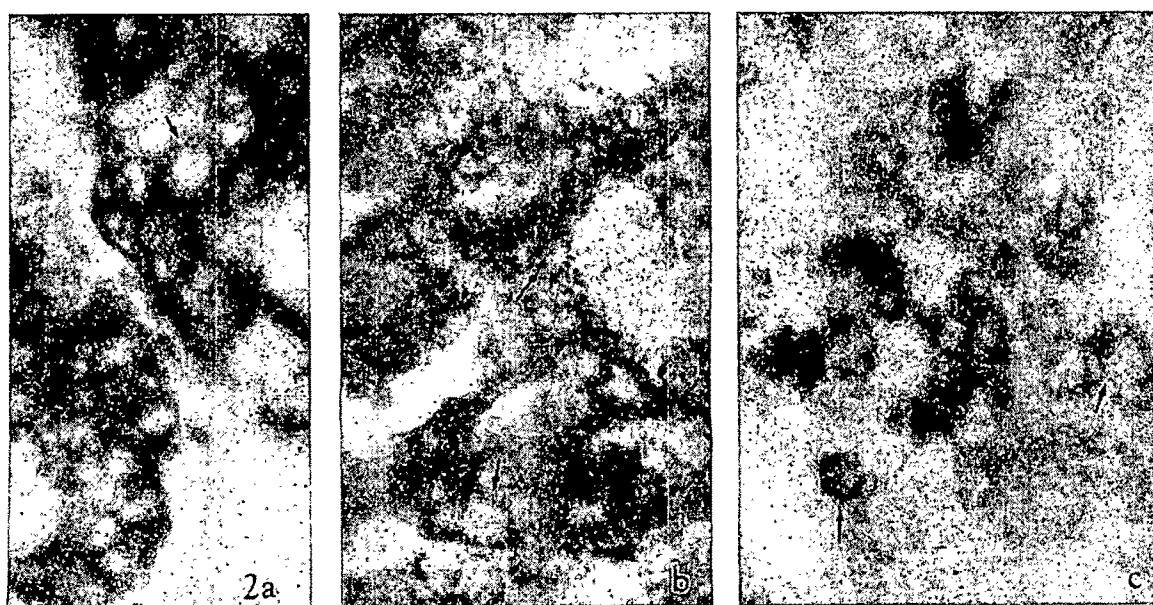


Fig. 2. Rat preputial gland  $\beta$ -glucuronidase visualized, *a*, by 8-hydroxyquinoline-iron chelate Prussian blue technique, *b*, by 8-hydroxyquinoline glucosiduronic acid-fast dark blue *R* simultaneous coupling reaction, and *c*, by naphthol *AS-BI*- $\beta$ -D-glucosiduronic acid-fast dark blue *R*, post coupling technique. Arrows point to membrane locations. ( $\times 1,200$ .)

Rat cartilage gives almost identical results with the metal chelate and with the azo dye methods (Fig. 3a and b) in that the limiting surfaces of the lipid droplets<sup>10</sup>, but not the lipid interior, carry the larger part of the  $\beta$ -glucuronidase activity of the cells<sup>11</sup>.

In the present investigation, two methods based on widely different principles have shown both lysosomal and non-lysosomal sites of  $\beta$ -glucuronidase activity. The non-lysosomal sites visible in the light microscope are interpreted as the endoplasmic reticulum sites of the electron microscope, an observation which agrees with the biochemical fractionation investigations of deDuve *et al.*<sup>2</sup> and especially of Van Lancker, who emphasized the dual lysosomal and microsomal localization of  $\beta$ -glucuronidase and acid phosphatase in regenerating rat liver<sup>12-15</sup>. Moreover, R. E. Smith has also demonstrated (personal communication) the presence of  $\beta$ -glucuronidase in endoplasmic reticulum and lysosomes in an electron microscope investigation of rat pituitary.

The most widely accepted cytochemical localization of acid phosphatase is exclusively in the lysosomes<sup>1</sup>. The absence of non-lysosomal sites may, however, be a consequence of enzyme inhibition both by formaldehyde or glutaraldehyde fixation and by lead ions in the incubation digests of the lead salt technique. Burstone<sup>16</sup> showed both a "peribiliary pattern of distribution and generalized cytoplasmic reaction" in rat liver with naphthol AS-BI phosphate and red violet LB.

In the case of non-specific esterase<sup>17</sup>, an exclusive peribiliary reaction in rat liver and a droplet reaction in rat kidney contrasts with the predominantly microsomal location found on differential centrifugation<sup>18</sup>. Shnitka and Seligman<sup>17</sup> and DeLellis and Fishman<sup>19</sup> were, however, able to demonstrate clearly both droplet and cytoplasmic activity in tissues that were not inhibited by prolonged formalin fixation or by potassium ferro-ferricyanide in the digest. Finally, esterase activity is present in the endoplasmic reticulum according to the *p*-rosaniline<sup>20</sup> and thiolacetic<sup>21</sup> staining methods.

On the basis of the considerations stated for  $\beta$ -glucuronidase, acid phosphatase and esterase, their lysosomal and non-lysosomal cytoplasmic locations are valid and can be expected to vary in a relative way to one another and also in response to factors such as the nature of the tissue, the species and the physiological and pathological status.

The intense staining of sites reflecting dual enzymorphological localization of acid hydrolases, although it accords with the high enzyme activity of tissue homogenate fractions, does not necessarily indicate the extent of *in vivo* activity, which is now regarded as slight. For example, in tissues maintained in organ culture, much, if not all, the  $\beta$ -glucuronidase activity, as tested in cryostat sections, is present as latent activity (Chayen, personal communication). Moreover, the evidence indicates an almost complete lack of metabolic hydrolysis of steroid glucuronides and sulphates<sup>22-25</sup>, and of adrenaline glucuronide (Drell and Clark, personal communication) which failed to elicit a pressor response in the pithed cocaineized cat.

On the other hand, body fluids such as salivary, gastric and intestinal juices, bile, vaginal and spinal fluids, and especially urine, contain non-latent  $\beta$ -glucuronidase and its substrates in aqueous solution, and these circumstances favour hydrolytic cleavage. Furthermore,  $\beta$ -glucuronidase in bile<sup>27</sup> and in intestinal secretions functions in the enterohepatic circulation of drugs and hormones<sup>26</sup> and, under pathological circumstances which produce a drop in pH, a localized area of intense hydrolysis of glucosiduronic acids may well occur in both body fluids and tissue.

The prominence of non-lysosomal sites of  $\beta$ -glucuronidase associated with endoplasmic reticulum suggests a structural role for the enzyme. The concept that enzyme proteins are ordered in a sequence and in numbers adequate to account for membrane structure is now the accepted

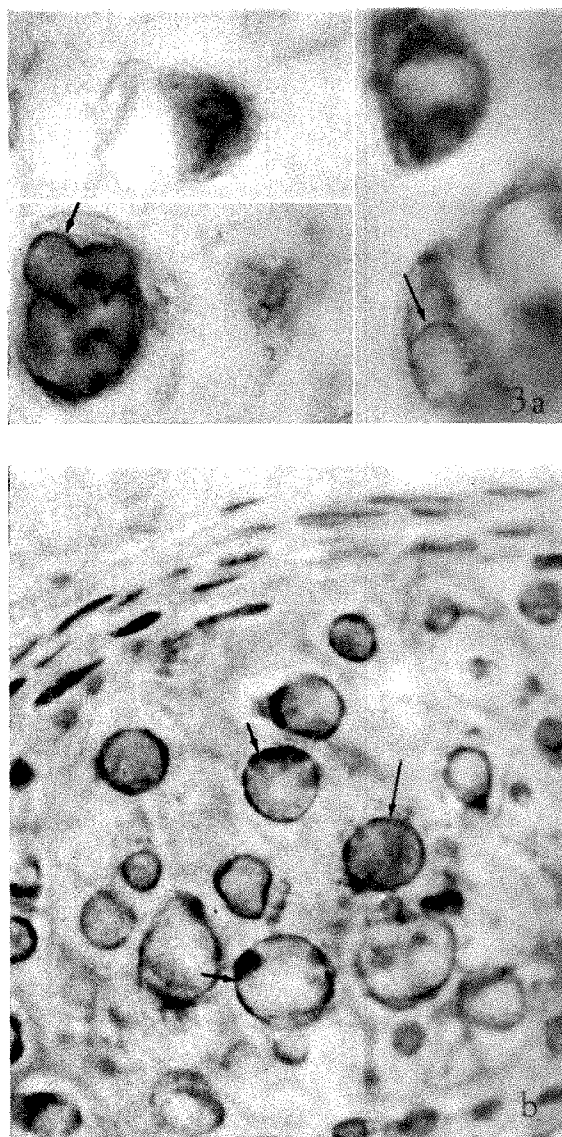


Fig. 3. Rat cartilage  $\beta$ -glucuronidase visualized by, a, 8-hydroxyquinoline-iron chelate Prussian blue technique and, b, by naphthol AS-BI- $\beta$ -D-glucosiduronic acid-hexazonium pararosanilin-post coupling technique. ( $\times 1,200$ .) Arrows point to membrane locations.

explanation of electron transport enzyme mechanisms in the mitochondrion<sup>28</sup>. By analogy, enzyme proteins such as  $\beta$ -glucuronidase, acid phosphatase, cathepsin, ribonuclease, deoxyribonuclease, glucosidase and sulphatase may be similarly ordered, perhaps in association with their respective macromolecular substrates and phospholipid to contribute to the structure of endoplasmic reticulum. It would follow that the fragments of endoplasmic reticulum in the cell cytoplasm could be collected in lysosomes by a process of "budding off" and this would account for the bewildering array of acid hydrolases in these organelles. This hypothesis does not require teleological explanations of their pathological intent. In addition, demands for endoplasmic reticulum formation such as occur in differentiating tissues, in regenerating liver, in neoplastic cell proliferation, in phagocytosis, in cells damaged by toxic agents and in the responses of target tissues to hormone would result in a heightened rate of production of structural membrane (catalytically inactive) proteins. Diluted homogenates of such tissues remove latency, and these solutions would now show striking increases in individual acid hydrolases assayed

under their respective optimal conditions for hydrolysis. These values could now be interpreted equally well as a reflexion of new endoplasmic reticulum or as the release of increased amounts of degenerated endoplasmic fragments with their acid hydrolase activity from lysosomes.

In conclusion,  $\beta$ -glucuronidase has been demonstrated in lysosomal and in non-lysosomal sites, the latter being predominantly endoplasmic reticulum. From a consideration of physiological data, we think that  $\beta$ -glucuronidase is essentially inactive in the cytoplasm of cells. In the absence of a credible generalized hydrolytic function for the enzyme *in vivo*, except for body fluids, and in view of the enzymorphological evidence presented, we suggest that  $\beta$ -glucuronidase may serve as a structural protein of the endoplasmic reticulum. The inferences which have been drawn about a similar role for other acid hydrolases may not only succeed in raising a legitimate doubt about the exclusively lytic role assigned to acid hydrolases in the cell but also present a working hypothesis which can be tested experimentally.

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## “Non-parallel Transport” of Enzyme Protein by the Pancreas

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Pancreozymin differentially enhances the secretion of trypsinogen and chymotrypsinogen by an *in vitro* pancreas. This may suggest selective secretion of these enzymes.

THE transport of large molecules from within cells to the outside, as in the secretion of enzymes by the pancreas, is thought to be largely independent of the properties of the molecule which is secreted. The view that zymogen granules release their contents into the lumen of the pancreatic duct much as if a mixture of gases was pouring out of a number of punctured balloons in a random fashion is now familiar, and was embodied in Babkin's theory that the proportions of different enzymes in pancreatic secretion are determined solely by the relative rates at which they are synthesized<sup>1</sup>. This theory implies that the mechanisms within cells which are responsible for the transport of protein from ribosomes to sites adjacent to the apical plasma membrane of the acinar cell are not selective for specific molecules, and, furthermore, that transport through the plasma membrane out of the cell is a random process which is also unselective. Much, but not all, of the available evidence supports Babkin's theory, but this report describes a process of enzyme secretion in the rabbit pancreas which is apparently selective.

New Zealand white rabbits (*Oryctolagus cuniculus*) of both sexes were fasted for 18–22 h before the experiments. Secretions were collected directly from the pancreatic duct either *in situ* or *in vitro*. Details of the biological preparations and techniques used have been given elsewhere<sup>2,3</sup>. *In situ* investigations were performed with the blood supply to the pancreas left intact, whereas the *in vitro* pancreas was bathed by a Krebs–Henseleit saline buffered with bicarbonate and gassed with 95 per cent

oxygen and 5 per cent carbon dioxide and maintained at 30° C. Collections of the secreted fluid were made at hourly intervals in tared tubes and flow was measured in mg/h. Esterase activities of trypsin and chymotrypsin were determined from the initial velocities of reaction of their hydrolysis of *p*-toluene sulphonyl-L-arginine methyl ester-HCl (TAME) and N-acetyl-L-tyrosine ethyl ester-H<sub>2</sub>O (ATEE) respectively<sup>4,5</sup>. Details of the analytical procedures have been given elsewhere<sup>7</sup>. The hourly enzyme activity output is expressed as units of activity secreted/h, where an activity unit/h is the number of  $\mu$ moles of substrate split/min/g of juice  $\times$  g of juice secreted/h.

Trypsinogen and chymotrypsinogen have similar tertiary structures, are of comparable molecular weight (approximately 22,000–24,000), and are even similar in their amino-acid sequences<sup>8</sup>. On the basis of their similar physical properties and apparent consanguinity we might expect them to be handled similarly by a transport system. Thus they are excellent test molecules for the investigation of transport selectivity. In addition, activity of both trypsin and chymotrypsin can be measured by ester hydrolysis, so that measurements of enzyme activities in secretion are based on the hydrolysis of similar bonds in reactions with the same stoichiometry<sup>4,5</sup>, which helps to decrease error in the comparison of the content of the two enzymes in secretion.

The duodenal hormone, pancreozymin, causes a massive release of granules containing protease from pancreatic acinar cells into duct lumina<sup>9</sup>, but, on the other hand, its effect on protein synthesis is probably of minimal quantita-



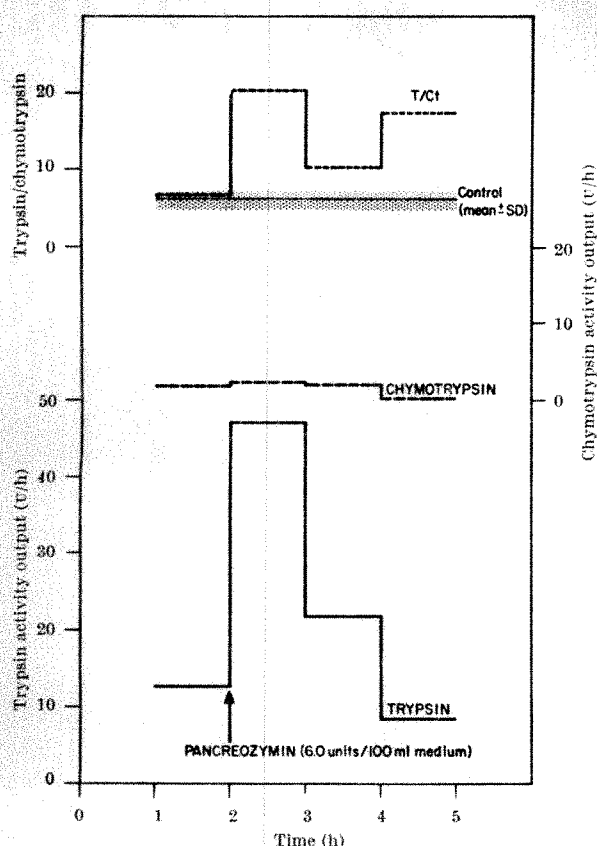


Fig. 1. The effect of pancreozymin on output of activity of trypsin and chymotrypsin by a rabbit pancreas *in vitro*. The shaded area indicates a standard deviation about the T/Ct ratio mean for control experiments.

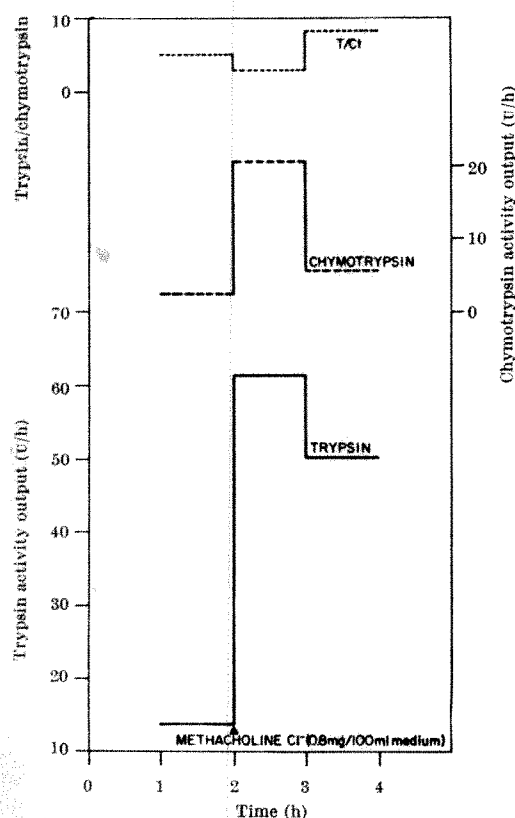


Fig. 2. The effect of methacholine chloride on output of activity of trypsin and chymotrypsin by a rabbit pancreas *in vitro*.

Table 1. EFFECT OF PANCREOZYMIN ON TRYPSIN/CHYMOTRYPSIN RATIOS OF RABBIT PANCREATIC SECRETION *in vitro*

Treatment	No. of experiment	Time after mounting (h)	Trypsin/chymotrypsin ratio $\pm$ S.D.
None	3	2	$7.0 \pm 1.2$
None	3	3	$6.6 \pm 1.1$
None	3	2	$6.1 \pm 0.6$
Pancreozymin	3	3	$15.8 \pm 6.0$

tive importance during the short time required to elicit zymogen release<sup>10</sup>. For this reason pancreozymin may be very useful for the investigation of zymogen transport without the difficulty caused by simultaneously varying significantly the rates of enzyme synthesis.

The *in vitro* rabbit pancreas preparation is also well suited for comparison of transport rates of exportable proteins, because trypsinogen to chymotrypsinogen ratios (T/Ct) in secretion are relatively constant and consequently the secretion of these enzymes relative to each other can be assessed with reasonable accuracy. The shaded area in Fig. 1 indicates one standard deviation about the ratio mean for control experiments ( $n=9$ ). Differences in the ratios from the second to the fifth hourly collections were not significant and the various hourly values were pooled to give the mean for the experiment ( $n$ ).

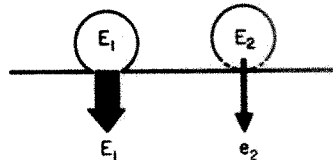
When pancreozymin (6.0 Ivy dog units of 'Ceeekin' (Vitrum)/100 ml. of water in the bath) was added to the solution bathing the pancreas, a great enhancement in secretion of trypsinogen (output) was observed while chymotrypsinogen in secretion was only slightly elevated (Fig. 1). As a result, the T/Ct ratio of secretion more than doubled (see Table 1) ( $P < 0.05$  (control and experimental preparations were compared for ratio differences from hours 2 to 3 using Cochran's approximation of the Behrens-Fisher test). If secretion of protein by the pancreas was not selective, then the T/Ct ratios would not have changed. T/Ct ratios of rabbit pancreatic secretion *in vivo* are quite variable, but a similar enzyme selectivity was observed in three anaesthetized rabbits ( $\Delta$ T/Ct: +22.0, +12.5, +5.9).

The presence in secretion of an inhibitor of trypsin or a possible chymotryptic inhibitor could alter the activities

### Soluble vs. Granular Transport



### Homogeneous Granule Competition



### Soluble Enzyme-Membrane Specificity

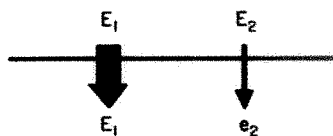


Fig. 3. Some transport pathways that could produce "enzyme-specific secretion" by pancreatic acinar cells.



of the enzymes and therefore had to be accounted for. To assay for enzyme inhibition we added known amounts of purified trypsin or chymotrypsin to samples of juice in which the enzymes were left as proenzymes and looked for diminished activity of the added enzyme standards. No diminution in the activity of the chymotrypsin standard was observed in any of the experimental conditions and alterations in the activity of the trypsin inhibitor could not account for the changes in ratio produced by pancreozymin.

A cholinergic stimulant, methacholine chloride, was also added to the *in vitro* medium (0.8 mg/100 ml. of fluid). Methacholine was a potent stimulus of the secretion of enzyme *in vitro* and the output of both trypsinogen and chymotrypsinogen increased after methacholine was added to the bath. The T/Ct ratio was more variable than in controls, but there was no consistent difference in the enzyme ratio between methacholine-treated and control preparations ( $\Delta T/Ct = +2.1 \pm 4.0$  S.D.;  $n = 3$ ).

If the selective enzyme secretion in these experiments was not the result of changes in the relative velocities of synthesis of trypsinogen and chymotrypsinogen, then some aspect of enzyme transport was modified. An altered intracellular transport preference probably cannot explain the phenomenon, for the amount of enzyme movement from the ribosomes into secretory granules would probably be quite small in comparison with the amount of enzyme "released" from the cell on stimulation with pancreozymin. This would be even more so in zymogen-filled acinar cells of rabbits which had been fasted for 18–22 h before the experiment. Selectivity, therefore, most likely occurs in the "release" phase or in transport of protein from the zymogen granules or cytoplasm into duct lumina. Such an effect could be explained in several ways.

For example, there could be competition for plasma membrane sites by zymogen granules containing different enzymes, although what the basis of such a distinction could be is not clear. An alteration in the rates of enzyme secretion from within zymogen granules as compared with secretion of enzymes free in the cytoplasm could give the observed results if the T/Ct ratio in the zymogen granules were different from that in the cytoplasm (Fig. 3). Some exportable enzyme is present apparently free in the cytoplasm<sup>11</sup>, although we do not know if these enzymes can be secreted directly or must be incorporated into secretory granules first. Yet another possibility is that the enzymes themselves compete for transport sites on either the plasma or zymogen membranes or both with one enzyme being favoured over the other. It remains to be seen whether or not this phenomenon is just an oddity or has more general implications concerning the ways in which large molecules are secreted.

This work was supported by grants from the U.S. Public Health Service and a basic science research grant from Smith, Kline and French Laboratories. I thank Mr. Weldon Lloyd for technical assistance.

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## Conversion of Non-immune Cells into Antibody-forming Cells by RNA

by

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RNA extracted from the spleens of mice immunized with sheep red blood cells converts some spleen cells from non-immunized mice to form antibody. The substance responsible for this conversion is RNA rich material with a coefficient of sedimentation between 8S and 12S. There may be specific recognition sites for this RNA on a few competent recipient cells which respond by producing antibody.

EXTRACTS of ribonucleic acid from macrophages exposed to antigen stimulate the formation of antibody in lymph node cultures<sup>1,2</sup>. The nature of the transfer substance is at present being investigated by several research groups. Askonas and Rhodes<sup>3</sup> and Friedman *et al.*<sup>4</sup> have suggested that antigen is involved; Fishman and Adler (personal communication) separated the reactivity of RNA-antigen complexes from RNA alone.

Previously, we reported that RNA extracted from the spleens of mice immunized with sheep red blood cells converted a small proportion of non-immune mouse spleen cells to form sheep cell haemolysins<sup>5,6</sup>. In general, sheep cell haemolysins are IgM (19S) globulins. The reaction was specific for RNA from immunized mice and was non-competitive over a wide range of RNA concentrations. The active material was very sensitive to ribonuclease, but was resistant to pronase, trypsin, and amylase. In this report, we present additional characteristics of the system: the effect of 5-fluoro-2-deoxyuridine; the kinetics of RNA synthesis after exposure to antigen; and the inhibitory effect of partially degraded RNA from mice immunized to

sheep red blood cells on the conversion of normal cells by non-degraded RNA.

RNA was extracted from the spleens of mice on day 4 after intraperitoneal injections of 0.1 ml. of a 25 per cent suspension of sheep red blood cells on days 0 and 3. The hot phenol technique<sup>7</sup> was followed as previously described<sup>8</sup>. Briefly, the spleens were quickly frozen on dry ice and then disrupted by high speed homogenization in an acetate: phenol mixture containing bentonite, sodium dodecyl sulphate, and 8-hydroxyquinoline. After precipitation and several washes with 66 per cent ethanol, the RNA was separated by sucrose density gradient centrifugation. Previous experiments indicated that the active RNA was in the 8–12S fraction. RNA from the 8–12S fraction was added to 0.3 molar sodium acetate and precipitated with two volumes of absolute alcohol. The RNA was used on the day it was isolated.

Spleen cells were obtained as indicated from immunized NIH or B6A.F1 mice by teasing the spleens apart in cold medium 199 (ref. 8). The cells were dispersed further by gentle pipetting, allowed to settle briefly and the super-

natant consisting mostly of single cells was removed. The nucleated cells were counted in a haemocytometer.

The number of cells producing sheep cell haemolysins was determined by the plaque assay method described by Jerne<sup>9</sup>. Medium 199 was added to purified agar to a final concentration of 0.7 per cent immediately before use. It was maintained at 45° C in 2 ml. portions. Each agar tube was allowed to cool to 38°–40° C before the addition of 0.1 ml. of 10 per cent sheep red blood cells (SRBC) followed by up to 0.5 ml. of spleen cells for analysis. After mixing, the agar-cell suspension was quickly poured over 0.7 per cent agar-199 base in a 10 cm Petri plate. The preparation was incubated at 37° C 2–3 h before the addition of 1.5 ml. of 25 per cent guinea-pig complement. After incubation for a further 30 min the plates were ready for examination. Plaques, which were frequently small, were identified under low power ( $\times 10$ ) magnification. Each plaque was symmetrically round and had an identifiable cell at its centre.

The pellet obtained after precipitation and high speed centrifugation of the 8-12S fraction was dissolved in medium 199. To minimize variation between individual animals, a pool of cells was prepared from the spleens of non-immunized mice. After the cell count had been determined, portions from this pool were suspended in 1.0 ml. medium 199 and were incubated after addition of 1.0 ml. RNA for 15 min at 37° C in a water bath equipped with a shaking device. Equal portions of cells were taken as controls from the same pool, and were incubated in medium 199 for the same period in the same total volume. After incubation, the cells were plated without further treatment and the proportion of cells producing sheep cell haemolysin was determined as previously described.

5-Fluoro-2-deoxyuridine (FUDR) acted as an adjuvant when administered in small doses<sup>10</sup> with antigen. A greater proportion of spleen cells forming haemolysins was found in mice injected with 1.0  $\mu$ g FUDR and SRBC than in mice receiving the same number of SRBC alone (Fig. 1). In an analogous manner, a greater proportion of spleen cells from non-immunized mice receiving 1.0  $\mu$ g FUDR (without SRBC) was converted to antibody-forming cells by RNA than were the same number of spleen cells from non-immunized mice not injected with FUDR (Fig. 2). No significant increase in the proportion of cells spontaneously forming haemolysins was found in animals receiving this dose of FUDR.

The rate of incorporation of uridine labelled with tritium into RNA was accelerated in the spleens of mice after injections of SRBC (Table 1). As early as 2 h after the

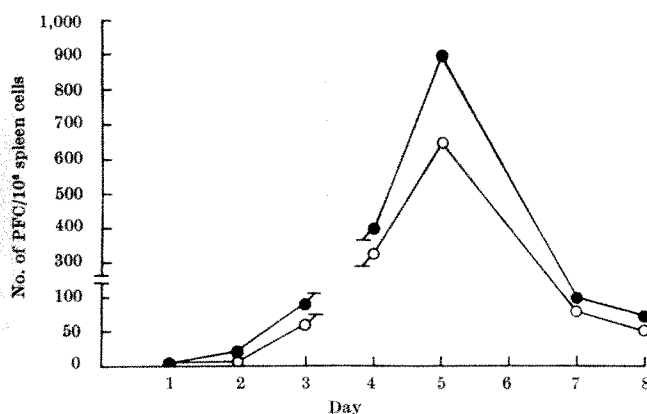


Fig. 1. Plaque formation in the spleens of mice after FUDR and SRBC. *NIH* mice were injected intraperitoneally with 0.1 ml. 25 per cent SRBC with (●) or without (○) 1.0  $\mu$ g FUDR. At varying times thereafter, the proportion of plaque-forming cells (PFC) in a pool from the spleens of four mice was determined by the plaque assay method. Groups exposed to FUDR and SRBC had significantly higher numbers of plaques than those exposed to SRBC alone  $P < 0.01$ . (Note. Variations from experiments reported in Tables 3 and 4 and Fig. 1 were not different from one another, hence the pooled estimate has been used throughout. We thank Dr. H. Andrews for performing the statistical analyses.)

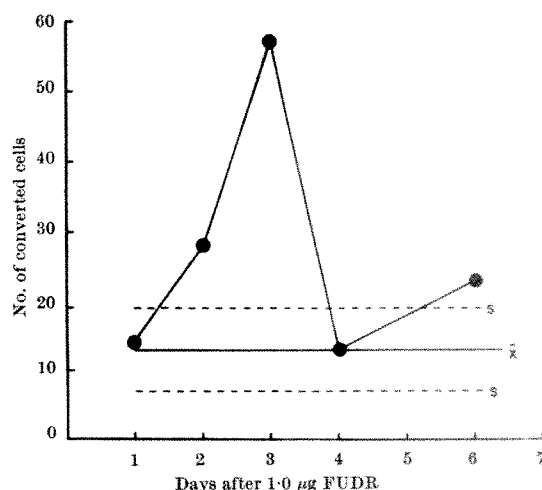


Fig. 2. Plaque formation by spleen cells from mice receiving FUDR (without antigen) after incubation with RNA from immunized mice. *NIH* mice, a non-inbred strain, were injected with 1.0  $\mu$ g FUDR (control mice were not injected). At varying times thereafter, 100  $\times 10^6$  cells prepared from their spleens were incubated with RNA extracted from the spleens of mice 4 days after immunization with SRBC. RNA from the 8-12S fraction of a sucrose density gradient was used. After incubation, the proportion of plaque-forming cells was determined by the plaque assay method. The points, averages of two independent determinations, represent the total number of plaque-forming cells found minus the number of plaques forming spontaneously in the same number of cells not incubated with RNA. The solid horizontal line represents the average number ( $\pm$  S.D.) of spleen cells from non-immunized mice not injected with FUDR converted to form antibody by RNA from the 8-12S fraction.

intravenous injection of antigen into *B6A/F1* mice (control mice received isologous mouse red blood cells) RNA synthesis was increased as measured by the specific activity of total splenic RNA after a 60 min pulse of tritium-labelled uridine before the animals were destroyed. This accelerated rate of incorporation of precursor occurred over a period of 72 h, but by 96 h after the injection of antigen it had returned to control levels. Separation of the RNA by density gradient centrifugation revealed a progressive increase in the rate of labelling of the 8-16S fraction (Fig. 3). These data confirm results recently reported by Mach and Vassalli<sup>11</sup>.

Table 1. RNA SYNTHESIS IN THE MOUSE SPLEEN AFTER ANTIGEN

Hours after antigen	Specific activity immune/control	Antibody-forming cells per 10 <sup>6</sup> spleen cells
2	1.64	0.9
24	1.12	0.8
72	2.47	54
96	1.02	470
120	1.02	112

*B6A/F1* mice received 0.1 ml. of 25 per cent SRBC intravenously, control mice received isologous mouse cells intravenously. Sixty minutes before they were killed, each mouse received 50  $\mu$ c. of tritium-labelled uridine in 0.1 ml. and the specific activity of the RNA extracted from the spleens was determined by counting in a liquid scintillation counter.

RNA from the 8-12S fraction was found to be relatively resistant to digestion by ribonuclease when this was attempted either in medium 199 or in physiological salts (Earle). In the presence of calcium (II) and magnesium (II), RNA has a higher degree of secondary structure<sup>12</sup>, which is reflected in the hyperchromicity obtained after heating RNA from the 8-12S fraction (Fig. 4). More biological activity remained after addition of ribonuclease when RNA was dissolved in medium 199 than when ribonuclease was added to RNA dissolved in sodium chloride and EDTA (Table 2). As measured by density gradient centrifugation, more large molecular weight components remained after RNA from the 8-12S fraction was treated with ribonuclease in physiological salts than in sodium chloride and EDTA.

Because the reaction was non-competitive, we postulated<sup>5,13</sup> that recipient spleen cells probably had specific

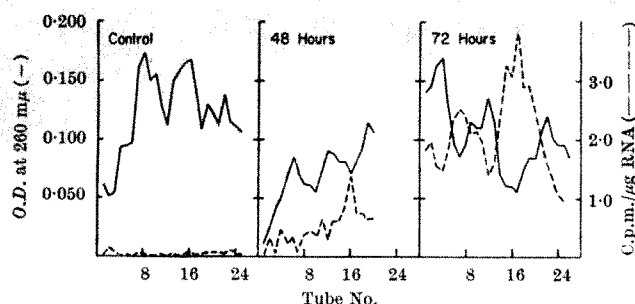


Fig. 3. Patterns of uridine incorporation into RNA after antigen. RNA was extracted from the spleens of mice at varying times after the intravenous injection of 0.1 ml. of 25 per cent SRBC and an intravenous injection of 50  $\mu$ c. tritium-labelled uridine in 0.1 ml. 30 min before the animals were killed. After separation of the total cellular RNA by density gradient centrifugation, the specific activity of each fraction was determined by precipitation with trichloroacetic acid before collection on membrane filters and by counting in a liquid scintillation counter.

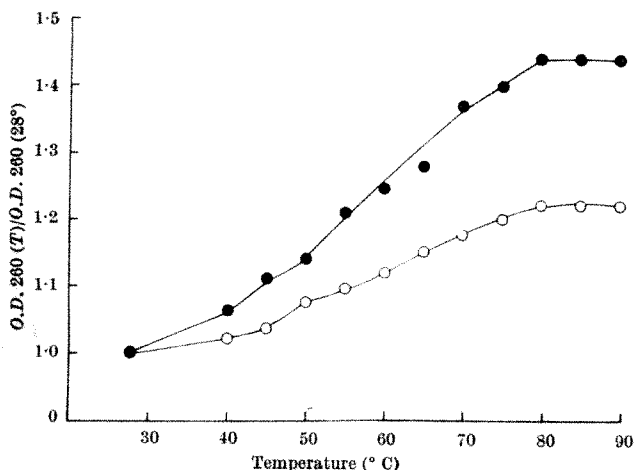


Fig. 4. Hyperchromicity of RNA from the 8-12S fraction after heating. About 160  $\mu$ g of RNA from the 8-12S fraction was dissolved either in medium 199 or in 0.15 molar sodium chloride containing  $2 \times 10^{-4}$  molar EDTA. Optical density at 260 m $\mu$  was determined in a Zeiss spectrophotometer equipped with a flow through heating device. ○, — Calcium (II) and magnesium (II); ●, + calcium (II) and magnesium (II).

recognition sites for RNA or an RNA-antigen complex. To test this hypothesis, two series of experiments were performed in an attempt to saturate these sites before the addition of biologically active material. Previous incubation of non-immune mouse spleen cells with minimally digested RNA from the 8-12S fraction of immunized mice inhibited the conversion of potentially active cells when non-digested RNA was later added (Table 3). This reaction was specific because previous incubation of non-immune spleen cells with digested or non-digested RNA from animals immunized with *Escherichia coli* or diphtheria toxoid failed to inhibit the conversion of cells to form sheep cell haemolysins by RNA from mice immunized with SRBC (Table 4).

Table 2. CONVERSION OF NON-IMMUNE CELLS BY ENRICHED\* IMMUNE† RNA AFTER INCUBATION WITH RIBONUCLEASE‡

Non-immune cells treated with	No. of plaques/10 <sup>6</sup> cells
166 $\mu$ g immune RNA	126
166 $\mu$ g immune RNA in medium 199 after 0.018 $\mu$ g RNase for 5 min	108
166 $\mu$ g immune RNA in sodium chloride after 0.018 $\mu$ g RNase for 5 min	54
(Non-immune cells alone)	50

\* Enriched RNA, RNA from the 8-12S fraction.

† Immune RNA, RNA extracted from the spleens of mice immunized with sheep red blood cells.

‡ B6A.F1 mice were injected intraperitoneally with 0.1 ml. of 25 per cent SRBC on days 0 and 3; RNA extraction was on day 4. After centrifugation in a sucrose gradient (7-20 per cent), the RNA in the 8-12S fraction was adjusted to 0.3 molar sodium acetate and was precipitated with two volumes of absolute alcohol. One portion of the precipitate was redissolved in medium 199, another portion in 0.15 molar sodium chloride containing  $2 \times 10^{-4}$  molar EDTA. Ribonuclease (boiled before use) was added to both portions for 5 min at room temperature with shaking. After incubation, the digests were added to  $150 \times 10^6$  non-immune spleen cells in medium 199 for 15 min at 37°C with shaking. From each group,  $100 \times 10^6$  cells were examined.

Table 3. CONVERSION OF NON-IMMUNE CELLS BY ENRICHED\* IMMUNE† RNA AFTER INCUBATION WITH DIGESTED ENRICHED\* IMMUNE† RNA‡

Non-immune cells with	No. of plaques/10 <sup>6</sup> cells
Experiment 1:	
230 $\mu$ g immune RNA	168
230 $\mu$ g immune RNA after pretreatment with digested immune RNA	77
230 $\mu$ g digested immune RNA	102
(Non-immune cells alone)	87
Experiment 2:	
200 $\mu$ g immune RNA	46
200 $\mu$ g immune RNA after pretreatment with digested immune RNA	15
200 $\mu$ g digested immune RNA	26
(Non-immune cells alone)	17

$S_d = 0.050$ ,  $L.S.D. = 0.110$ .

$P < 0.01$  for groups incubated with immune RNA and non-immune cells alone (see Note to Fig. 1).

\* Enriched RNA, RNA obtained from the 8-12S fraction.

† Immune RNA, RNA extracted from the spleens of mice immunized with sheep red blood cells.

‡ B6A.F1 mice were injected intraperitoneally with 0.1 ml. of 25 per cent SRBC on days 0 and 3; RNA extraction was on day 4. After centrifugation in a sucrose gradient (7-20 per cent) the RNA in the 8-12S fraction was adjusted to 0.3 molar sodium acetate and was precipitated with two volumes of absolute alcohol. One portion of the precipitate was redissolved in medium 199, another portion in 0.15 molar sodium chloride containing  $2 \times 10^{-4}$  molar EDTA. Ribonuclease (boiled before use) was added to a final concentration of 0.018  $\mu$ g/ml. to that portion dissolved in sodium chloride and, after incubation, the digest was incubated with  $150 \times 10^6$  non-immune spleen cells in medium 199 for 15 min at 37°C with shaking. At the end of this period, an aliquot of the portion of RNA dissolved in medium 199 was added and the mixture incubated for an additional 15 min.

Control incubations were for 30 min and  $100 \times 10^6$  cells from each group were examined.

Earlier, my colleagues and I reported that RNA extracts from mice immunized with sheep red blood cells converted a small proportion of non-immune cells into cells producing sheep cell haemolysins. The phenomenon, although consistent in replicate tests, was complicated by the fact that only a rather small proportion of non-immune spleen cells responded to the RNA extracts. About fifty in  $10^6$  cells responded, which means, of course, that in experiments in which the number of spontaneously appearing plaques in cell populations from non-immunized animals exceeds fifty in  $10^6$  cells (background), the total number of plaques found after exposure to RNA may be less than double the background. This problem, however, may not be technical in nature because a series of recent investigations indicated that the proportion of spleen cells competent to respond to a given antigen is limited<sup>14,15</sup>. These estimates were supported experimentally by Nakano and Braun<sup>16</sup>, who found a non-random distribution of mouse spleen cells responding to heterologous red cells in pieces of mouse spleen prepared and analysed after immunization. They associated these observations with a limited number of clones capable of forming antibodies to a given antigen. Friedman and Young<sup>17</sup> adapted the Jerne plaque assay system to an analysis of spleen slices and provided direct support for this conclusion by demonstrating the presence of a limited number of foci of cells producing sheep cell haemolysins in the spleens of immunized mice. Both

Table 4. CONVERSION OF NON-IMMUNE CELLS BY ENRICHED\* SRBC IMMUNE† RNA AFTER INCUBATION WITH DEGRADED OR UNDEGRADED RNA FROM HETEROLOGOUS IMMUNE‡ RNAs§

Non-immune cells treated with	No. of plaques/10 <sup>6</sup> cells
310 $\mu$ g sheep cell immune RNA	96
310 $\mu$ g sheep cell immune RNA after pretreatment with 160 $\mu$ g <i>E. coli</i> immune RNA	100
310 $\mu$ g sheep cell immune RNA after pretreatment with 160 $\mu$ g digested <i>E. coli</i> immune RNA	115
160 $\mu$ g digested <i>E. coli</i> immune RNA	53
(Non-immune cells alone)	66
Diphtheria toxoid	
200 $\mu$ g sheep cell immune RNA	71
200 $\mu$ g sheep cell immune RNA after pretreatment with 62 $\mu$ g diphtheria toxoid immune RNA	79
62 $\mu$ g diphtheria toxoid RNA	42
(Non-immune cells alone)	31

$S_d = 0.050$ ,  $L.S.D. = 0.110$ .

$P < 0.01$  for groups incubated with immune RNA and non-immune cells alone (see Note to Fig. 1).

\* Enriched RNA, RNA obtained from the 8-12S fraction.

† SRBC immune RNA, RNA extracted from the spleens of mice immunized with 0.1 ml. of 25 per cent sheep red blood cells.

‡ Heterologous immune RNA, RNA extracted from the spleens of mice immunized with  $10^6$  heat killed *E. coli* or 0.1 ml. diphtheria toxoid as indicated.

§ B6A.F1 mice were injected intraperitoneally with 0.1 ml. of 25 per cent SRBC, or with  $10^6$  heat killed *E. coli* in 0.1 ml., or with 0.1 ml. diphtheria toxoid (Diffo) as indicated on days 0 and 3; RNA extraction was on day 4. Experimental conditions were as described in previous tables.

Nakano and Braun, as well as Friedman and Young, showed that separate and distinct clones responded to different antigens. The number of presumed stem cells and the number of clones derived therefrom, with the capacity to respond to sheep red blood cells, must be limited even in the case of a complex antigen as sheep red blood cells.

Despite the limited number of stem cells, the proportion of potentially competent cells derived from them might be increased without the production of antibodies by certain adjuvants administered without antigen. The results obtained with FUDR, administered without antigen (Fig. 2), are consistent with this hypothesis.

Normally the immune response is accompanied by the division of antibody-forming cells<sup>18</sup>. It has been reported that RNA from immunized animals causes a generalized stimulation of cell division in lymphoid tissue<sup>19</sup>. This may explain the observation that the peak increase in the proportion of cells from mice treated with FUDR converted by RNA (Fig. 2) preceded by 2 days the maximum proportion of antibody-forming cells after the injection of antigen (Fig. 1).

What is the nature of the converting or activating substance in the RNA extracts from immunized animals? This material(s) originates either in macrophages or in lymphocytes or both. If it originates in macrophages, and the strain specificity of the response might support this supposition (Mithison (personal communication) found that the activation of antibody production by lymphocytes from non-immunized mice after exposure to macrophages from immunized mice was strain specific), it is probably either pure RNA or an RNA-antigen complex. (Despite the extraordinary sensitivity of the active material to ribonuclease, and its resistance to inactivation by proteolytic enzymes and amylases, the participation of a trace of antigen cannot definitely be ruled out. On the other hand, the finding of recognizable antigen fragments in active RNA preparations by others<sup>4</sup> does not establish the necessary participation of these fragments in the responses which we investigated.) The specific blocking of the reaction by preparations treated with enzyme, therefore, may represent an interference with the recognition by lymphocytes either of a polynucleotide sequence or of an antigen. Alternatively, if the activating substance originates in lymphocytes, then it probably is a messenger RNA and the blocking reaction might represent a specific interference with the transfer of messenger from lymphocyte to lymphocyte. In either event, the data strongly

suggest the presence of specific recognition sites on the competent recipient cells.

The presence of specified binding sites on recipient lymphocytes is predictable from a model of antibody formation which requires the co-operative efforts of two cell types for antibody formation<sup>13</sup>. This model, akin to intercellular regulations by hormones<sup>20</sup>, postulates that macrophages transfer an activating or informative material to a small number of competent lymphocytes. To react specifically to the stimulating material, these recipient cells must have recognition sites for the product of the macrophages. Moreover, if the transfer material is to "find" the few competent recipient cells, it must be produced in excess. This expectation is in agreement with our finding that RNA from the 8-12S fraction of the gradient, which was exclusively active in converting cells to form antibody<sup>6</sup>, is synthesized at an accelerated rate within 2 h after administration of antigen.

Investigations are in progress to define further and to elucidate the precise role of the active material in the immune response.

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## Death of *Saccharomyces cerevisiae* in Aerosols

by

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The addition of inositol to the yeast *Saccharomyces cerevisiae* protects the yeast—when suspended in air—against death from desiccation, ultra-violet light and X-rays.

PREVIOUS work has shown that the rate at which deaths of bacterial cells and viruses occur in aerosols is strongly influenced by the relative humidity of the air in which the micro-organisms are suspended. Not only does the relative humidity of the air alter the response of micro-organisms to desiccation alone, but to both ultra-violet light and X-rays. In general, aerosolized bacterial cells and viruses are more resistant to desiccation and ultra-violet light

when they are treated at relative humidities above 60 per cent but show their maximum sensitivity to X-rays when irradiated in air above 70 per cent relative humidity. In addition, the vitamin *i*-inositol has been shown to protect air-borne bacteria and viruses against damage caused by desiccation alone as well as by ultra-violet light and X-rays. As a result of these investigations, it was suggested that the deaths of micro-organisms in aerosols from desiccation

and ultra-violet light resulted from the removal and re-orientation of water molecules associated with the structure of large conjugate macromolecules. It was assumed also that many of the indirect effects of X-rays were caused by the ionization of these structural water molecules rather than free water<sup>1</sup>. The vitamin *i*-inositol possesses six hydroxyl groups on a cyclohexane nucleus potentially capable of forming hydrogen bonds similar in strength and structure to those formed by water molecules. The protective action of this compound was therefore considered to result from the ability of its hydroxyl groups to replace structural water and from the stability of its cyclohexane nucleus. This compound was found to prevent the inactivation of intracellular vegetative phages and the lysis of lysogenic bacteria when cells were desiccated and irradiated with ultra-violet light. In addition, in the concentrations required to afford protection to cells, *i*-inositol has been shown to inhibit protein synthesis, apparently by preventing the manufacture of specific mRNA<sup>2</sup>. The latter finding suggested that inositol combines with a molecule responsible for the coding of mRNA and it is thought that this combination renders the molecule more stable to desiccation, ultra-violet light and X-rays. In earlier work it was suggested that the reason why some microbial cells were more able than others to withstand desiccation and irradiation was because they contained higher concentrations of compounds similar to *i*-inositol. It seemed pertinent therefore to test this assumption through an investigation of the behaviour of a cell known to contain naturally large amounts of *i*-inositol. It is the purpose of this article to report findings with such a cell, the yeast *Saccharomyces cerevisiae* X841, supplied by R. K. Mortimer, of the University of California, Berkeley.

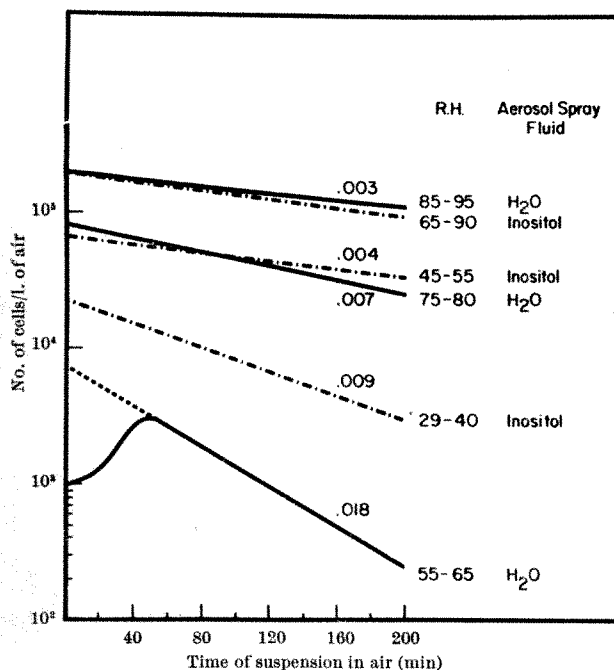


Fig. 1. The effect of relative humidity and inositol on the survival of *Saccharomyces cerevisiae* in aerosols.

Cells of *Saccharomyces cerevisiae* were grown on a wrist-action shaker for 48 h at 30° C in 200 ml. of Sabouraud liquid medium. The culture was removed in aliquots of 50 ml. and the cells washed once in sterile demineralized water by centrifugation. They were then resuspended in 10 ml. of demineralized water or a 5 per cent solution of *i*-inositol in water. The whole suspension was then transferred to a collision spray and the cells atomized

into a rotating drum<sup>1</sup> pre-set to a given level of relative humidity. Alternatively, aliquots of 1.0 ml. were deposited on 47 mm 'Millipore' filters. The filters plus the layer of cells, approximately ten to twenty cells thick, were then placed in a circular chamber and a steady flow of air of a given relative humidity passed over them. Approximately 2 h were required before the air in the chamber reached the desired level of relative humidity after the entry of the damp filters. The cells on the filters or those in the aerosol were irradiated with an X-ray tube operated at 200 kV and 20 m.amp to give a dose rate of 655 r./min at the positions of the filters and approximately 16 r./min in the drum. Following irradiation, the cells on the filters were washed off with 10.0 ml. of 0.85 per cent saline, serially diluted and 0.2 ml. of each dilution was plated into a medium consisting of 1 per cent yeast extract, 1 per cent peptone, 2 per cent dextrose and 2 per cent agar. Samples from the aerosol were taken using a liquid impinger<sup>1</sup> operating at 9.0 l./min and containing 10.0 ml. of saline. The cells were serially diluted in saline and plated as for those on filters. The extent of radiation damage was measured as a difference between the number of cells surviving desiccation alone and those remaining after desiccation and irradiation.

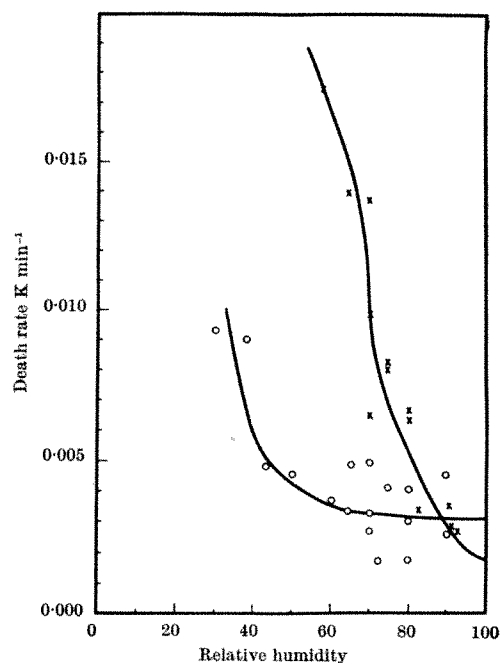


Fig. 2. The effect of relative humidity and inositol on the exponential death rate constant calculated from the slopes of the survival curves. ×, From water; ○, from inositol.

The initial experiments were conducted with cells deposited on filters. This was done because earlier work with the yeast had suggested that a dose of X-rays sufficient to produce cell deaths could not be delivered into the aerosol drum within a practical length of time. It soon became clear, however, that at humidity levels of below 75 per cent, numbers of the yeast cells died because of desiccation alone so that, with the long periods of time required to establish an equilibrium of relative humidity, variations in viable cell numbers following desiccation became greater than the variation caused by radiation. The experiments with the filters were therefore confined to values of relative humidity between 75 and 95 per cent. Within this range of humidity, few cells died as a result of the desiccation and the deaths which resulted from X-radiation appeared to follow a sigmoidal relationship with dose (Fig. 4). To destroy



approximately 90 per cent of the cells with X-rays required a dose of 70 kr. and the radiation sensitivity did not alter with changes in relative humidity between 75 and 95 per cent. Attempts to demonstrate a protective effect of *i*-inositol on the cells on filters against both drying and radiation damage failed. These results are in agreement with earlier findings with multiple layers of bacterial cells deposited on filters<sup>1</sup>. Related investigations on the effects of X-rays on air-borne bacteria revealed that such cells were unexpectedly sensitive to irradiation<sup>3</sup>. It seemed reasonable, therefore, to conduct experiments with aerosols of yeast cells first to determine whether they were more sensitive to X-rays than cells on filters and second to test the effects of relative humidity and inositol on their sensitivity.

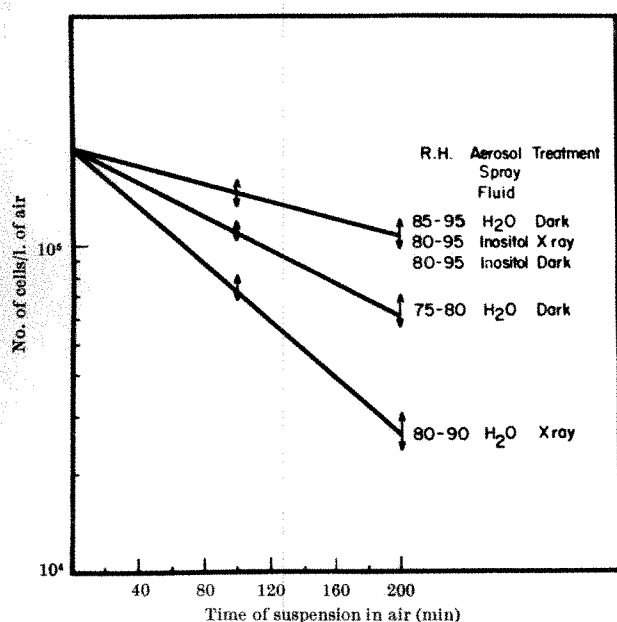


Fig. 3. The survival of *Saccharomyces cerevisiae* in aerosols with and without exposure to 200 kVp. X-rays at an average exposure rate of 16 r./min in the presence or absence of inositol.

The results obtained with the unirradiated (dark) aerosols of yeast cells demonstrated that they were extremely sensitive to drying. At relative humidities above 85 per cent, few deaths occurred, but as the relative humidities were lowered from 85 to 60 per cent, large numbers of cells died and the rate at which the deaths took place increased as the relative humidities decreased (Fig. 1). A large number of experiments were carried out of which only a few typical results are shown. At relative humidities below 85 per cent the rate of deaths of the cells appeared to follow two phases, an initial rapid rate of deaths taking place during the 10 min required to generate the aerosol and collect the first sample, followed by a period in which subsequent deaths took place more slowly and followed an exponential function with time. This initial rapid death rate is shown in Fig. 1 by the number of viable cells/l. of air in the initial aerosol samples. It can be seen that both the initial and secondary rate of deaths increased rapidly as the relative humidity was decreased. These findings were somewhat surprising, because they indicated that the inositol present naturally in the cells afforded them little protection against desiccation in aerosols. It was pertinent then to determine whether additional inositol would preserve their viability. This proved to be the case, because in the presence of inositol few deaths occurred above 60 per cent relative humidity and the rate at which they did so below this level was con-

siderably reduced (Fig. 1). In addition, the compound prevented both the initial and secondary deaths. Because the rates of deaths in both phases were dependent on the relative humidity and both were slowed by inositol, it is assumed that the mechanisms responsible for death in each case have at least one important factor in common. In Fig. 2, the exponential death rates obtained from individual experiments are plotted against relative humidity. The presentation of the results in this fashion shows clearly the marked increase in sensitivity of water-atomized cells as the relative humidity decreases below 85 per cent. This behaviour is different from that observed with bacterial cells<sup>1</sup> where the death rate remained approximately constant as the relative humidity was lowered from 90 to about 70 per cent and then rapidly increased as the relative humidity dropped to 40 per cent. The fact that added *i*-inositol almost completely preserved the viability of the dried cells down to 60 per cent relative humidity and offered considerable protection to the cells below this level (Figs. 1 and 2) suggested that it preserved the yeast cells through a mechanism similar to that by which it prevented damage to bacterial cells and viruses. At the same time, because the lipid-bound inositol naturally present in the yeast cell did not offer protection, it was reasonable to conclude that in order to exercise its protective ability, the inositol must be free.

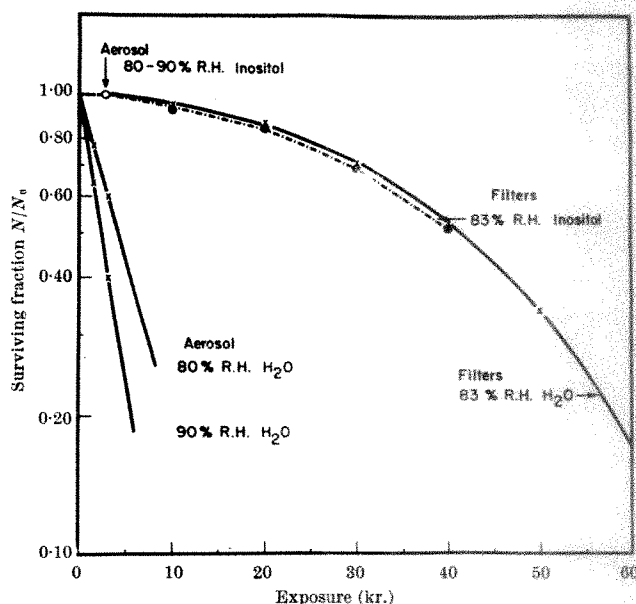


Fig. 4. The survival of *Saccharomyces cerevisiae* as a function of exposure to X-rays using aerosolized and filter-mounted cells. The death rate from drying has been subtracted to give the net effect of the radiation.

It has been shown in earlier work that bacterial cells both in aerosols<sup>3</sup> and on filters<sup>4,5</sup> displayed their maximum sensitivity to X-rays at relative humidity greater than 70 per cent. In view of these findings and because of the long periods of time required to deliver a given dose of X-rays into the drum, investigations into the effect of X-rays and inositol on air-borne yeast cells were carried out only at relative humidity values above 80 per cent. From the results there were two significant findings. First, yeast cells in aerosols were apparently completely protected from X-ray damage by added inositol (Fig. 3) and, second, air-borne yeast cells were very much more sensitive to X-rays than those treated on filters (Fig. 4). In Fig. 4 the results are shown as plots of survival versus dose, for both filter-dried and aerosolized cells. It can be seen that for the cells irradiated on filters the curve is of the sigmoidal (or multi-hit) type, whereas for the aero-

solized cells it is of the exponential (or single-hit) type (Figs. 3 and 4).

The foregoing results obtained with yeast cells in aerosols were similar to findings with bacteria and viruses both from the standpoint of their increased X-ray sensitivity and the action of *D*-inositol. The work suggests that natural inositol in the cell does not appear to endow it with added stability towards desiccation or X-rays. At this time it is not known why aerosolization changes the response of

microbial cells to radiation or why aerosolized cells are more easily protected by inositol than those desiccated on filters.

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## Apparent Phytochrome Synthesis in *Pisum* Tissue

by

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The increase of phytochrome in pea plants which have been irradiated with red light and then returned to the dark is related to the initial decrease of phytochrome (after irradiation) below a certain critical amount. The increase may result from the re-establishment of photo-reversibility in a fraction of the phytochrome present in a non-reversible pool.

THE phytochrome system almost certainly acts as a photoreceptor controlling the growth and flowering of plants grown in the light<sup>1</sup>. Most attempts to link phytochrome transformations with physiology, however, have been made on etiolated seedlings<sup>2-5</sup> because green tissues<sup>6</sup> do not readily lend themselves to work with the dual wavelength ratiospectrophotometer. Transformation investigations with etiolated seedlings that have been briefly illuminated with red light and returned to darkness have shown that the far-red-absorbing species of the pigment is labile<sup>2-5</sup>. Depending on the tissue examined, 50-80 per cent of the total phytochrome is destroyed, or at least rendered spectrophotometrically undetectable, as a result of the brief initial illumination. It seems likely, therefore, that in plants subjected to natural illumination the phytochrome in the tissue will be rapidly reduced to a very low concentration unless an additional mechanism is brought into operation. Some of the phytochrome might be conserved if a fraction of it were light-stable, or an adequate concentration might be maintained if synthesis of new phytochrome reached an equilibrium with the destruction of far-red-absorbing pigment. So far there is one reported instance of phytochrome which is light-stable<sup>6</sup> and none concerning phytochrome synthesis in non-etiolated tissue. Increases in total phytochrome accompanying growth of etiolated seedlings have been described in two reports<sup>4,5</sup>.

We have observed changes in the amount of phytochrome in pea plants that have been illuminated for various lengths of time with red light after an initial growth period of 7 days in darkness. Estimations of phytochrome were made with a dual wavelength spectrophotometer, using an external actinic light source and filters for the photoconversion of the pigment. The standard sample of tissue assayed consisted of 200 mg of segments cut from the epicotyl hooks of approximately twenty plants of *Pisum sativum* cultivar 'Alaska' (pea). The tissue was packed into metal cuvettes 6 mm in diameter to form a bed of material 1 cm in depth. The plant growth conditions, the *in vivo* assay, and the light sources and filters are described in detail in earlier publications<sup>2,3,7</sup>. Extracts of phytochrome were prepared by grinding in a chilled mortar 2 g of hook segments with 2 ml. of buffer and a small quantity of sand washed with acid. The resulting homogenate was filtered through glass wool and the filtrate centrifuged at 10,000*g* for 15 min. Two millilitres of the clarified extract was layered on the top of a 'Sephadex G-50' column which had been pre-equili-

brated with the extraction buffer, and eluted with the same buffer. Fractions of 2 ml. were collected and 1 ml. was taken from each fraction and run on to a 1 cm bed of finely divided, anhydrous calcium carbonate<sup>8</sup> in cuvettes of 1 cm diameter. The buffer solution was made from 0.3 molar potassium monohydrogen phosphate and potassium dihydrogen phosphate, pH 7.8, with 0.002 molar ethylenediamine tetraacetic acid dipotassium salt and 0.3 per cent mercaptoethanol. All the steps were conducted at 2° C under dim green safelights.

When pea plants grown in the dark, or excised segments of pea stems grown in the dark, are illuminated briefly with red light and then returned to darkness for 6-8 h, the total phytochrome falls to 45-50 per cent of the concentration in non-irradiated control tissue<sup>7</sup>. After 6 h all the remaining pigment is in the red absorbing form, and because this form appears to be stable the total phytochrome remains constant for at least 24 h. If the tissue, however, is pretreated with a succession of exposures to red light which last 15 min (so that the phytochrome falls to a low concentration) and then returned to darkness, the total phytochrome does not reach a constant concentration, but actually increases. This phenomenon is illustrated in Fig. 1. In this discussion, the term "new" phytochrome refers to the increase in total phytochrome above the minimum level measured after the light pretreatment. All the new phytochrome is of the form which absorbs red.

The values for the change in the change of the optical density ( $\Delta(\Delta O.D.)$ ) obtained by *in vivo* spectrophotometry are in part the results of amplification caused by dispersion of the measuring beam as it passes through the tissue<sup>8</sup>. It seemed possible that our findings were the result of increased light-scattering in the repeatedly illuminated tissue because of the formation of refractive bodies such as plastids or proplastids during the ensuing dark period. The increases observed *in vivo*, however, are matched by increases in extractable phytochrome (Fig. 2).

When light pretreatments were so arranged that the minimum phytochrome concentration in the tissue varied from 5 to 45 per cent of that in non-illuminated controls, it soon became apparent that significant increases in phytochrome in the subsequent dark period were observed only if the phytochrome fell below a certain critical concentration. So far the data suggest that the critical concentration is sharply defined at between 16 and 22 per cent of the initial concentration. Moreover, there is some indication that the amount of new phytochrome

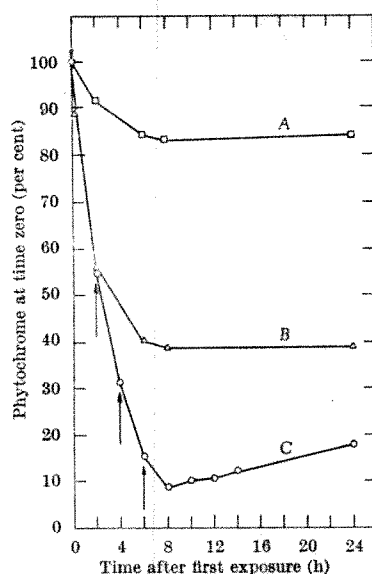


Fig. 1. Phytochrome levels in hook segments given various light pretreatments. Values are percentages of those in plants grown in the dark at h 0. Red light treatments of 15 min duration started at times indicated by arrows. Terminal bud removed before light treatment. A, Dark control; B, one red exposure; C, four red exposures.

synthesized in a 24 h dark period following illumination is closely related to the amount by which the phytochrome falls below the critical concentration—an amount designated the critical deficit. It can be seen in Fig. 3 that the relationship between the critical deficit and the amount of new phytochrome appearing in a tissue is approximately linear. This relationship has the effect of equalizing the amount of phytochrome in tissues which have received different illumination schedules and have then been returned to darkness. For example, five light (dark) cycles of 15 min light and 1 h 45 min dark reduced the phytochrome concentration in samples of hook tissues to  $\Delta(\Delta O.D.) \times 10^3$  of 40.0/g, whereas three light (dark) cycles of 15 min light and 1 h 45 min dark reduced the level to 100.0/g; in a 24 h dark period following these

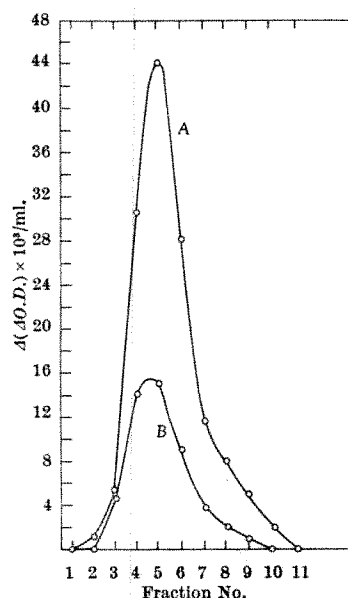


Fig. 2. Elution of phytochrome extracts from 'Sephadex G-50'. Each fraction was 2 ml. Samples were taken from tissue 24 h (A) and 2 h (B) after a pretreatment in which the tissue received four cycles of 15 min light, 1 h 45 min dark. Assay of the tissue *in vivo* taken for extraction: A,  $\Delta(\Delta O.D.) \times 10^3$  157.5/g; B,  $\Delta(\Delta O.D.) \times 10^3$  62.5/g.

schedules the phytochrome concentration rose to 160.0/g in each instance.

The onset of apparent phytochrome synthesis was dependent on time only to the extent that the rate of destruction of far-red absorbing pigment (which reduces the total phytochrome) governed the minimum time in which the critical concentration could be reached. By illuminating the plants with three different light-dark schedules we have been able to induce a similar critical deficit at 4, 6 and 24 h, and in each case the rate of appearance of new phytochrome was similar (Table 1).

Table 1. PHYTOCHROME LEVELS IN APICAL HOOK SEGMENTS AFTER VARIOUS LIGHT PRETREATMENTS

Light (dark) pretreatment	$\Delta(\Delta O.D.) \times 10^3/g$			
	h 0	h 2	h 6	h 8
4 cycles, 15 min light, 5 h 45 min dark	112.5	69.0	84.5	100.0
4 cycles, 15 min light, 1 h 45 min dark	135.0	74.0	105.0	120.0
4 h, red only	100.0	54.0	—	98.0

h 0 refers to the time immediately after the last and h 2 to a time 2 h after the last exposure to red light. Tissue cut from decapitated plants growing in vermiculite.

The cells in the tissue described here were mostly immature and were capable of appreciable increases in size<sup>9</sup>, whereas the cells at the base of the second internode matured rapidly after illumination with red light and so had a limited potential for further growth<sup>10</sup>. Samples of tissue from this latter region showed no apparent phytochrome synthesis when taken from peas which had received a light pretreatment which had induced synthesis in the hook region of the third internode. This result suggests that apparent synthesis is associated only with developing tissue. It has been possible, however, to separate synthesis from actual growth in hook segments. In these experiments 5 mm segments were cut from the stems of intact plants just before the fourth illumination in an inductive light schedule and placed in Petri dishes. The growth, apparent phytochrome synthesis and total protein content of control segments were compared with these characteristics in segments where cell expansion was inhibited by the inclusion of 2 per cent and 8 per cent mannitol in the standard incubation medium. Table 2 shows that these treatments did not significantly affect the appearance of new phytochrome even though 8 per cent mannitol almost completely inhibited segment elongation. Conversely, treatment of excised segments with  $10^{-4}$  molar naphthalene acetic acid inhibited the synthesis of new phytochrome without decreasing segment growth (Table 2). A more detailed account of this last, unexpected result, together with data on the effect of other inhibitors and growth regulators, will be published elsewhere.

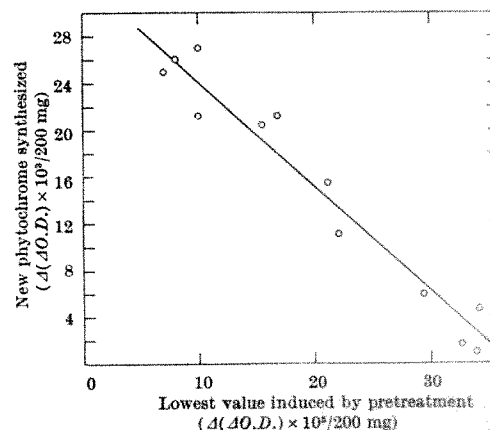


Fig. 3. The amount of new phytochrome synthesized in apical hook segments in the 24 h following the last illumination as a function of the lowest level of phytochrome induced by illumination pretreatment.

Table 2. PHYTOCHROME SYNTHESIS IN EXCISED BOOK SEGMENTS INITIALLY 5 MM LONG TREATED WITH MANNITOL AND NAPHTHALENE ACETIC ACID

Treatment	Elongation (mm)	New phytochrome $A(\Delta O.D.) \times 10^3/g$	New phytochrome: protein $A(\Delta O.D.) \times 10^3/mg$ protein
Experiment P-42			
Control	3.98	30.0	1.48
2 per cent mannitol	1.68	42.5	1.85
8 per cent mannitol	0.40	27.5	1.10
Experiment P-40			
Control	4.70	55.0	2.61
$10^{-4}$ molar naphthalene acetic acid	4.91	12.5	0.63

Light pretreatment in both experiments consisted of four cycles of 15 min light, 1 h 45 min dark. Tissue excised from intact plants between light exposures 3 and 4 and then kept for 24 h in darkness in Petri dishes containing 10 ml. of 0.1 molar potassium dihydrogen phosphate and potassium monohydrogen phosphate buffer pH 6.3 plus 0.4 per cent sucrose, with and without additives indicated. Total protein determined by biuret assay as described by Briggs and Siegelman (ref. 4).

What is the nature of the process that we have called apparent phytochrome synthesis? Several facts make it seem unlikely that it represents *de novo* synthesis of the entire chromoprotein. The process is relatively insensitive to progressive excision of the tissue, and proceeds almost as rapidly in short apical segments as in detached entire epicotyls, and almost as well in the latter as in intact plants. In excised segments it is unaffected by the presence or absence of sucrose in the incubation medium, although this factor certainly limits growth. In addition, a preliminary survey indicates that concentrations of chloramphenicol and puromycin that strongly inhibit growth fail to inhibit the appearance of new phytochrome. Though conclusive data on this question will come only from studies on the incorporation of labelled amino-acids, these observations suggest no close relationship between *de novo* protein synthesis and apparent phytochrome synthesis.

The dependence of apparent phytochrome synthesis, whatever its precise nature, on the reduction of the phytochrome content below a critical concentration requires further consideration, even though this is necessarily speculative at present. It may be useful to postulate the existence of two fractions of phytochrome in the cell, only one of them being the usual, photoreversible fraction measured in the assay. The other fraction would be closely related but irreversible and thus unmeasurable. This irreversibility might be a result of binding, structural modification or even a disassociation of the holochrome into chromophore and protein. Such an irreversible or unmeasurable fraction of phytochrome has been proposed before<sup>2</sup> for other reasons. Though there is no direct evidence for it at all, it would provide a basis for two alternative explanations of the present results.

The first explanation suggests that the critical concentration is the point at which these two fractions of phytochrome are in equilibrium. In plant material grown in the dark and tissue which has received only one red light

exposure, the measurable phytochrome fraction is greater than the critical concentration and thus no synthesis is observed. When the phytochrome is brought below the critical concentration by repeated exposure of the tissue to red light, irreversible phytochrome is released or rendered photoreversible. The rate of release is dependent on the critical deficit. A second possibility starts with the assumption that apparent destruction of far-red absorbing pigment represents a step in the formation of the irreversible fraction. When the latter reaches a critical concentration, release or reconversion of the measurable form begins. In this scheme the necessity for the measurable phytochrome to be reduced below a critical concentration would only be an apparent one, the real prerequisite for apparent synthesis being the "destruction" of a sufficient quantity of far-red absorbing pigment.

With either scheme in operation it is predictable that at some point the rate of destruction of far-red absorbing pigment and the reappearance of new phytochrome from the irreversible fraction will reach equilibrium. Such a system might operate to maintain a constant concentration of phytochrome in the developing tissues of plants growing in the light.

The only other report of observations in any way comparable with these is that of Spruit<sup>11</sup>, who studied phytochrome transformations in pea plumules and concluded that synthesis of red absorbing pigment, rather than any reversion of far-red absorbing pigment to red absorbing pigment, followed a single exposure to red light. The tissue used included the leafy bud and thus contained appreciable amounts of protochlorophyll; though ingenious corrections for interference by this substance in the *in vivo* assay were made, it is by no means certain that they were successful. The very small amount of protochlorophyll in pea stem tissue has a negligible effect on phytochrome assays, and this effect should be eliminated by the extraction procedure.

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## Ray Thickenings in the Walls of Conifer Tracheids

by

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The walls of conifer tracheids tend to be thicker when they are adjacent to medullary rays. In most species the extent of thickening seems to be less in the late wood than in the early wood. Thickening tends to be more pronounced in older wood and in vigorous trees.

It has been observed that the tangential tracheid wall in a number of species of conifers tends to be thickened in the region of the ray crossings. Fig. 1 shows a radial microscope section of late wood in Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, where it can be seen that the

tangential walls of the tracheids are thicker where they cross the two medullary rays than they are above, between, and below the rays. In transverse section the thickenings are apparent in the relative massiveness of the tangential tracheid wall in files that happen to flank

a ray in the plane of the section. This is illustrated in Fig. 2, which shows a transverse microscope section of red pine, *Pinus resinosa* Ait.

These thickenings in the region of the ray crossings, of which there appears to be no account in the literature, were first noticed by me some years ago in samples of *Pinus radiata* D. Don. from East Africa, the United Kingdom, and Canada, and in three species of podocarp, *Podocarpus gracilior* Pilger, *P. javanicus* Blume, and *P. macrophyllus* D. Don. Measurements on transverse sections showed that in the samples of *Pinus radiata* the tangential walls of tracheids in files lying alongside rays, that is, in the region of ray crossings, were on the average 18.4 per cent, 20.8 per cent and 20.3 per cent thicker than the tangential walls of tracheids in files not flanking rays. Corresponding values in the three species of podocarp ranged from 4.1 per cent to 9.8 per cent.

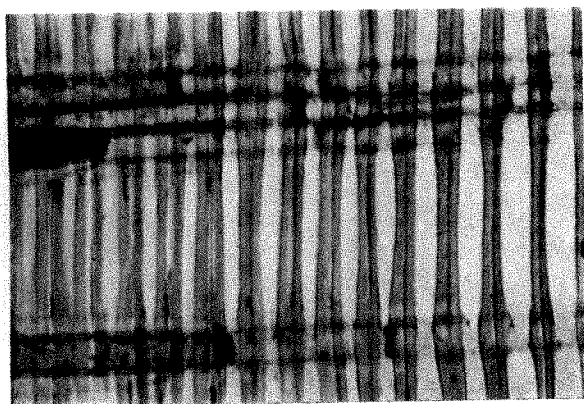


Fig. 1. Photomicrograph of a radial section of Douglas fir showing thickening of the tangential tracheid wall at the ray crossings. ( $\times c. 550$ .)

This has now been followed up by an investigation of a number of coniferous species. The results suggest that "ray thickenings", if they may be so called, are widespread in conifers, and that their relative size tends to increase from the pith outwards. The results also suggest that in certain species and in certain conditions the thickenings may make a significant contribution to the amount of cell wall substance present—thus influencing both the density of wood and its variability—and that they may be relevant to the behaviour of softwood fibres in the making of paper.

As in the earlier work, the measurements were made on transverse sections. On such sections the rays, because of their random distribution with reference to the vertical axis, are sectioned at various heights. Measurements of cell wall thickness in files contiguous to rays therefore give values which represent the average degree of thickening from top to bottom of rays, as opposed, for example, to the maximum degree of thickening such as could be obtained from radial sections.

Blocks for the preparation of transverse sections were taken, with no particular care as to their location, from hand specimens showing mature wood of the following species: balsam fir, *Abies balsamea* (L.) Mill, Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, loblolly pine, *Pinus taeda* L., long-leaf pine, *Pinus palustris* Mill. and another southern pine of unknown species. A single transverse section, 20  $\mu$  thick, was cut from each block and was stained with safranin and mounted in Canada balsam. On each section a single ring was selected at random and in twenty successive "ray" files, that is tracheid files contiguous to rays, the mid-point thickness of the common tangential tracheid wall in the first five cells in the early wood and in the last five cells in the late wood was measured under a microscope at a magnification of  $\times 850$  and using an eyepiece scale. Similar measurements were made in twenty control files, which were taken to be the file next but one to the ray file under examination. If the control file itself was found to flank a ray, the nearest suitable file was selected. The file immediately adjacent to a ray file was always avoided, however, in order to reduce the possibility of any inter-file effect on cell dimensions resulting from the mechanics of pseudo-transverse division.

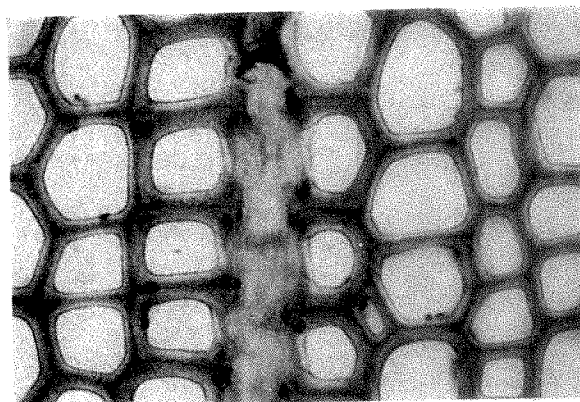


Fig. 2. Photomicrograph of a transverse section of red pine illustrating the relative massiveness of the walls of tracheids in files contiguous to a medullary ray. ( $\times c. 265$ .)

The average cell wall thickness in ray and control files is given in Table 1 with separate values, each based on 100 measurements, for early wood and late wood.

In order to arrive at an estimate of the amount of wood substance contributed by the ray thickenings, it was necessary to measure not only their size but also their frequency. The files between twenty-five successive rays in both the early wood and late wood of each ring were counted, and from the total of fifty counts the mean number of files between rays was obtained. From this value the ratio of ray to control files, expressed as a

Table 1. INCREASE IN TANGENTIAL TRACHEID WALL THICKNESS IN THE REGION OF RAY CROSSINGS

Species	Mean rings/in.	Position of sample in ring	Mean thickness—common tangential wall			Increase in wood substance due to thickenings	Increase due to thickening (%)
			Ray file ( $\mu$ )	Control file ( $\mu$ )	Difference as % of control (%)	Mean No. of files between rays ( $n$ )	
Balsam fir	5.8	E	3.64	3.53	+3.1	5.76	1.1
		L	7.97	7.24	+10.1		3.5
Douglas fir	14.2	E	4.77	4.28	+11.4	4.82	4.7
		L	12.62	11.61	+8.7		3.6
Loblolly pine	3.8	E	6.02	5.10	+18.0	7.14	5.0
		L	11.77	10.38	+13.4		3.8
Long-leaf pine	22.1	E	8.61	7.29	+18.1	6.52	6.6
		L	13.93	13.09	+6.4		2.6
Southern pine	3.3	E	6.96	5.88	+18.4	4.76	7.7
		L	8.44	7.58	+11.3		4.7

E, Early wood; L, late wood.



percentage, was derived in the knowledge that, of the mean number of files between rays, two files were ray files. A relative measure of the additional wood substance as a result of the presence of thickenings was then calculated by multiplying the mean percentage thickening by the mean percentage frequency of ray files. The results of these calculations are given in Table 1 together with the data obtained on ray frequency.

To investigate variation from the pith outward, measurements were made in the 5 year and 125 year ring from the pith in a fast and in a slow grown black spruce, *Picea mariana* Mill. (B.S.P.) showing 27.7 and 37.0 rings/in. respectively, and in the 1 year and 6 year ring of a fast and of a slow grown red pine, *Pinus resinosa* Ait., showing 5.8 and 8.7 rings/in. No measurements were made in the early wood of the younger rings, but otherwise the procedure followed and the data obtained were the same as described above. The results are given in Table 2.

Finally, in a single randomly selected ring of the fast grown red pine, measurements were made in twenty ray and twenty control files of the thickness of the first and of the last common tangential tracheid wall in the ring and at six predetermined points in between. The results are shown in Fig. 3, where the mean thicknesses of the wall of tracheids in ray and control files are plotted separately against distance across the ring expressed as a percentage of the width of the ring.

thickenings tended to increase from the pith outwards. In one black spruce the percentage thickening in the late wood increased from 1.6 per cent in the 5 year ring to 13.1 per cent in the 125 year ring, and in the other tree the increase was from 5.2 per cent to 7.4 per cent. The increase over 6 years of growth in one of the red pine was from 23.8 per cent to 31.3 per cent and in the other from 6.4 per cent to 20.9 per cent. A comparison of values at different distances from the pith but in rings of the same age showed that the thickenings tended to become more pronounced with rate of growth. This was particularly marked in the red pine where the percentage thickening in the late wood of the 6 year ring was found to be 31.3 per cent in the fast grown tree but only 20.9 per cent in the slow grown tree. Similar results were obtained in the black spruce, with the 125 year ring giving late wood values of 13.1 per cent compared with 7.4 per cent in the slow grown.

Of the various genera examined, *Pinus* tended to show the most pronounced thickenings. A comparison of early wood values showed that the most pronounced thickenings occurred in the unidentified southern pine (18.4 per cent) followed by long-leaf pine (18.1 per cent) and loblolly pine (18.0 per cent)—values close to those obtained in the early work on *Pinus radiata* where values from 18.4 per cent to 20.8 per cent were obtained. In the late wood, red pine tended to display the most pronounced

Table 2. VARIATION OUTWARDS FROM THE PITH IN THE SIZE OF RAY THICKENINGS

Species	Mean rings per inch	Age of ring	Position of sample in ring	Mean thickness—common tangential wall			Increase in wood substance due to thickenings	Mean No. of files between rays (n)	Ray files Control files × 100 (%)	Increase due to thickenings (%)
				Ray file (μ)	Control file (μ)	Difference % of control (%)				
Black spruce	27.7	125	E	4.43	3.90	+13.6	} 5.84	34.2	{	4.7
		125	L	8.70	7.69	+13.1				4.5
		5	L	5.00	4.92	+1.6				0.5
	37.0	125	E	5.03	4.47	+12.5	} 5.50	36.4	{	4.6
		125	L	8.81	8.20	+7.4				2.7
		5	L	4.47	4.25	+5.2				1.9
Red pine	5.8	6	E	3.91	3.39	+15.3	} 6.00	33.3	{	5.1
		6	L	6.16	4.69	+31.3				10.4
		1	L	4.53	3.66	+23.8				6.3
	8.7	6	E	4.14	3.80	+8.9	} 7.32	27.3	{	2.4
		6	L	6.12	5.06	+20.9				5.7
		1	L	3.49	3.28	+6.4				1.5

E, Early wood; L, late wood.

Ray thickenings were found in the tracheids of all the species examined. The most pronounced thickenings observed were in the late wood of the fast grown red pine where the tangential walls of tracheids were found to be 31.3 per cent thicker in the region of ray crossings than they were elsewhere. The least thickening observed was 1.6 per cent in the late wood of the 5 year ring of the fast grown black spruce.

In all the species examined except red pine and balsam fir, the thickenings were more pronounced in the early wood than in the late wood. In Douglas fir, for example, the increase in cell wall thickness at the ray crossings was 11.4 per cent in the early wood compared with 8.7 per cent in the late wood, while in the long-leaf pine the increase amounted to 18.1 per cent in the early wood compared with 6.4 per cent in the late wood. In the two red pine and the balsam fir, on the other hand, where the increase in wall thickness was more pronounced in late wood than in the early wood, the differences were even more marked. In the fast grown red pine the ray thickenings in the late wood were relatively twice as massive as those in the early wood (31.3 per cent compared with 15.3 per cent), while in the balsam fir the percentage thickening in the late wood was 10.1 per cent compared with only 3.1 per cent in the early wood.

The data obtained from the black spruce and red pine given in Table 2 showed that the relative size of the

thickenings (20.9 per cent and 31.3 per cent) with loblolly pine next at 13.4 per cent. The late wood of long-leaf pine, however, gave the relatively low value of 6.4 per cent. It could be argued that the tendency for the thickenings to be more pronounced in the pines is the result of high growth rates, associated either with the innate vigour of the genus or, in the case of the southern pines, with a favourable environment, but some evidence against this is provided by the three samples of *Pinus radiata* from East Africa, the United Kingdom and Canada, examined in the earlier investigation. All three samples showed much the same degree of thickening at the ray crossings, although they were grown in widely differing environments.

In calculating the relative amounts of additional wood substance attributable to the ray thickenings it was assumed, with some support from casual observation, that the radial walls in ray file cells were thickened to the same extent as the tangential and, further, that ray and control file cells were the same size in cross-section—that their mean radial diameters would be the same was beyond question, while a series of measurements on selected samples gave no indication of significant differences in their tangential diameters.

The results of the calculations, listed in the last column of Tables 1 and 2, showed that in all but the red pine and balsam fir the relative contribution of the ray thickenings

to the amount of wood substance present tended to be greater in the early wood than in the late wood. Thus, in the unidentified southern pine the increase in the amount of wood substance attributable to the thickenings was calculated to be 7.7 per cent in the early wood and 4.7 per cent in the late wood. On the other hand, in the fast grown red pine the late wood value was double that calculated for the early wood, 10.4 per cent compared with 5.1 per cent. The increase in wood substance was found to increase both with age from the pith and with growth rate. For example, a comparison of late wood values showed that 6 years' growth in the two red pine resulted in an increase from 6.3 per cent to 10.4 per cent in one tree and from 1.5 per cent to 5.7 per cent in the other, while in these same trees both early wood and late wood values in the sixth ring from the pith were markedly higher in the fast grown than in the slow grown tree—5.1 per cent and 10.4 per cent as compared with 2.4 per cent and 5.7 per cent (Table 2). The relations in black spruce were similar but not so marked. These increases appear to be caused more by the progressive enlargement of the thickenings from the pith outwards, as discussed above, than to increases in the file:ray ratio, a ratio which appears to remain comparatively stable throughout the life of the tree. For example, the mean number of files between rays in one black spruce was found to be 6.12 in the 5 year ring and 5.84 in the 125 year ring, with corresponding values of 5.42 and 5.50 in the other tree. In both red pine the ratios were slightly reduced in the course of 6 years' growth, from 7.56 to 6.00 in one tree, and from 8.44 to 7.32 in the other. This relative constancy in the file:ray ratio, which is a measure of the relative number of ray crossings, is of some intrinsic interest. It is apparently maintained in spite of marked changes in the absolute frequency of rays. Data obtained in another investigation in which measurements were made on transverse sections from the 10 per cent height level in twenty black spruce trees showed the mean ratio of files to rays changed from only 5.9 in the 5 year ring to 7.6 in the 135 year ring, although the mean number of rays/mm of ring circumference in these same rings decreased from 10.8 to 5.4 (unpublished data). The average tangential width of the tracheids in these trees, however, increased from  $15.3\mu$  in the 5 year to  $24.5\mu$  in the 135 year ring, following the trend observed in a number of species of conifers<sup>1-5</sup>. The increase in the tangential width of tracheids accompanying the decrease in ray frequency provides a partial if superficial explanation for the relative consistency of the file:ray ratio as observed on transverse sections. It must be remembered, however, that the frequency of rays as measured on this plane is influenced by both the number of rays/unit of tangential area, which tends in conifers to decrease from the pith outwards, and by the height of the ray which tends to increase<sup>1-5</sup>, so that the underlying factors which affect the file:ray ratio are of a complex nature involving a number of dimensional relationships.

In conclusion, it may be said that the presence of ray thickenings provides some basis for Myer's<sup>9</sup> claim that from species to species "(wood) density is directly proportional to an increase in ray volume", although Weinstein<sup>10</sup> quotes unpublished results of Koehler *et al.* for ash and Sitka spruce that would appear to refute Myer's conclusion. Certainly it would appear that the thickenings may in some circumstances exercise an appreciable effect on the pattern of variation in wood density across the ring. In the ring from which the data illustrated in Fig. 3 were obtained, the mean number of files between rays was 4.18, that is to say, nearly half of the tracheid files were contiguous to rays. Thus in about half the files the pattern of change in wall thickness followed the course indicated by the solid line in Fig. 3, while wall thickness in the remaining files followed the course indicated by the broken line. The presence of ray thickenings must therefore greatly widen the range of variation in cross-sectional

cell dimensions, and it may be that accuracy would be gained in comparative work on transverse sections if a distinction were drawn between tracheids in ray files and those in other files.

Finally, it can be suggested that, as the secondary tracheid wall is apparently laid down at the ray crossings at a rate different from elsewhere, a closer investigation of the thickenings might throw further light on secondary growth processes in the tree, while from a practical standpoint it is possible that the thickenings may not only influence the density of wood, as discussed above, but may be of some significance in the behaviour of softwood fibres in papermaking. On disintegration of the cooked chips such fibres tend to bend at points of structural discontinuity that appear to be associated with ray crossings<sup>11-13</sup>. It may be that these discontinuities consist, at least in part, of the ray thickenings that have been the subject of this paper.

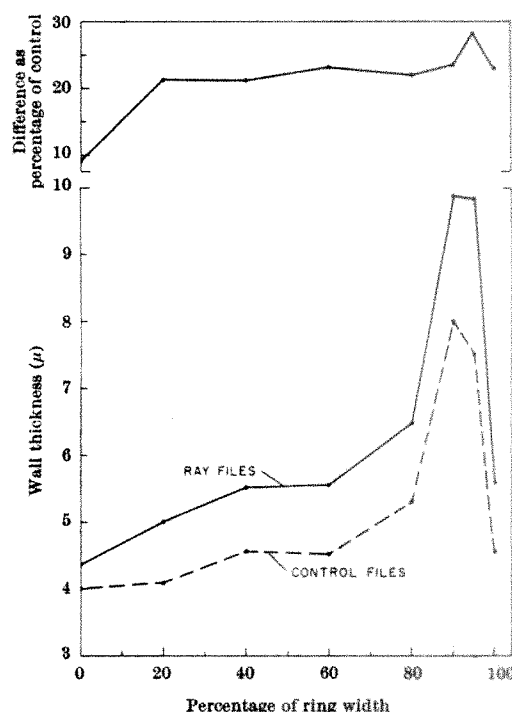


Fig. 3. Mean thickness of the tangential tracheid wall and ring width in a single ring of red pine: a comparison between the course of variation in "ray file" (—) as opposed to "control file" (---) tracheids.

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# Infra-red Spectra of Diamond Coat

by

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Natural diamonds are usually considered as having been grown in magma, the constituents of which are not easily determinable. An infra-red examination of a coated natural diamond indicates the presence of varying amounts of carbonate and water in the concentric rings of the coat region, which suggests that the diamond grew in a magma containing carbonate and water.

THIS article reports infra-red spectra of diamond coat which reveal differences in chemical composition between diamond and diamond coat. Diamond coat is the name given to the concentric layer structure that occasionally surrounds a central core of diamond. This type of diamond is especially prevalent in Congo stones and the coated diamond examined by us was a Congo.

Kamiya and Lang have reported that "the material of the diamond coat is also diamond, but is filled with dust-like matter, individual particles of which are generally of sub-micron size and are not clearly resolvable in the optical microscope". Custers<sup>2</sup> suggests that the foreign matter may be carbon. Seal<sup>3</sup> has examined one sample of diamond coat by electron microprobe and reports that most inclusions contain silicon and oxygen, although he found one inclusion which contained potassium and calcium. We believe we have identified the chief components of the foreign particles\* present in a coated diamond.

We measured the infra-red spectra of a number of areas of a 1.2 mm thick section of a typical coated diamond, using a Perkin-Elmer model 521 infra-red spectrophotometer with a 6-1 beam condenser.

A photomicrograph of this diamond section is shown in Fig. 1. Visual observation and infra-red spectra of this diamond indicate three distinct types of regions. Representative parts of these three regions are labelled on Fig. 1 and correspond to the spectra shown in Fig. 2. These regions are: *a*, the clear centre core; *b*, the clear, yellowish cracked area surrounding the core; and *c*, the region of the rings.

Fig. 2*a* shows the spectrum of the clear centre core of the diamond. This is typical of Type I diamond with strong impurity absorption from 1,000–1,400  $\text{cm}^{-1}$ †.

Fig. 2*b* shows a spectrum of a clear region between the core and the "rings" of the diamond coat. The spectrum differs from that of the core in that the usual 1,370  $\text{cm}^{-1}$  band is absent, and sharp bands are present at 3,107  $\text{cm}^{-1}$  and 1,405  $\text{cm}^{-1}$ . Thus the spectra indicate that the content of impurities in this region is different from that in the central diamond core.

Fig. 2*c* shows a spectrum of a region within the ring structure of the diamond coat. This spectrum differs from that of the core in that the 1,370  $\text{cm}^{-1}$  band is again absent and additional broad bands are present at 3,400, 1,640 and 1,430  $\text{cm}^{-1}$ . Narrower bands are also present at 1,000, 880 and 835  $\text{cm}^{-1}$ . The intensity of these bands in other selected narrow areas is greater in the darker regions

of the coat. The relative intensities of all six bands appear to remain constant throughout the diamond coat. The 3,107  $\text{cm}^{-1}$  band appears only weakly in the spectrum of the diamond coat; and the 1,405  $\text{cm}^{-1}$  band does not appear at all.

The intensity of the very sharp 3,107  $\text{cm}^{-1}$  and 1,405  $\text{cm}^{-1}$  bands may not be represented properly in Fig. 2*c*, however, because of the limited overall transmission of the diamond coat.

Angrist and Smith<sup>5</sup> have reported the absence of the 1,370  $\text{cm}^{-1}$  band in their spectra of diamond coat, as well as the presence of the 1,000  $\text{cm}^{-1}$  band. One of their spectra also shows the 880  $\text{cm}^{-1}$  and 835  $\text{cm}^{-1}$  bands, but they make no specific comment concerning them.

The spectrum of Fig. 2*c* (within the diamond coat) should be compared with the spectra (Fig. 3*a-c*) which are of sodium carbonate, sodium nitrate and hydrated ferric nitrate, respectively. It appears that the broad coat band at 1,430  $\text{cm}^{-1}$  and the narrow 880  $\text{cm}^{-1}$  band are due to carbonate impurity and the broad bands at 3,400  $\text{cm}^{-1}$  and 1,640  $\text{cm}^{-1}$  are due to water, probably water of hydration.

The ratio of the intensities of the 1,430  $\text{cm}^{-1}$  and 880  $\text{cm}^{-1}$  bands in the coat agrees within a factor of two with the ratio in sodium carbonate. The same agreement is found for the 3,400  $\text{cm}^{-1}$  and 1,640  $\text{cm}^{-1}$  bands of hydrated ferric nitrate and coat. These band intensity ratios can vary somewhat for various compounds, but the agreement shown here tends to support our assignment for these four absorption bands.

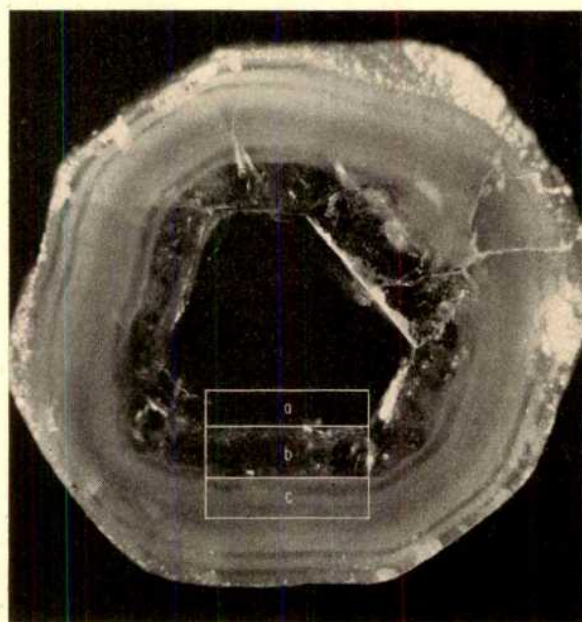


Fig. 1. Photomicrograph of a Congo coated diamond. The polished surface is a  $\langle 110 \rangle$  plane. ( $\times 30$ .)

\* The distribution of these particles causes the opacity of the rings in the diamond coat.

† Natural diamonds have been classified as Type I or Type II on the basis of their spectra. In the 2.5–50  $\mu$  (4,000–200  $\text{cm}^{-1}$ ) region of the spectrum, all diamonds have weak two phonon (1,900–2,600  $\text{cm}^{-1}$ ) and three phonon (2,800–4,000  $\text{cm}^{-1}$ ) absorption bands characteristic of the diamond lattice itself. Type II diamonds have no other absorptions for wavenumbers less than 1,600  $\text{cm}^{-1}$ , whereas Type I diamonds have additional absorption in the 1,000–1,400  $\text{cm}^{-1}$  and 480  $\text{cm}^{-1}$  regions caused by impurities. Actually the spectra of many diamonds fall intermediate between the defined types and one finds a nearly continuous gradation between types and further subdivisions such as Type 1*a*, 1*b*, 1*IIa*, and 1*IIb*.

We do not attempt to define the specific species responsible for the carbonate and water absorption bands except to say that they are probably present as a separate phase or phases and not as isolated ionic or molecular species in the diamond lattice. The bands are broad, unlike those of most isolated impurities. We believe it is this second phase that gives rise to the light scattering properties of the coat.

We also tried to identify the cation in the coat using an X-ray microprobe, but no metal impurities were detected. Using published data on calcium carbonate<sup>6</sup> in conjunction with our measured infra-red intensities, we estimate the concentration of carbonate ions at approximately  $10^{-18}$ /c.c., a factor of 10–100 below the limiting sensitivity of the X-ray microprobe.

We assign the sharp bands at 3,107 and 1,405  $\text{cm}^{-1}$  seen in Fig. 2b to carbon-hydrogen vibrations in the diamond.

An indistinct band at 1,360  $\text{cm}^{-1}$  and a definite sharper band at 835  $\text{cm}^{-1}$  have the same frequencies as the characteristic bands of nitrates, but such an assignment is very tentative.

It is interesting to relate the appearance of the diamond in Fig. 1 and its carbonate and water content to the possible environment in which this diamond grew. The clear centre core showing a typical Type I absorption spectrum indicates that "normal" conditions of growth existed at the time the core crystallized out of the magma.

At the boundary between the core (Fig. 1, region a) and the intermediate region (Fig. 1, region b) growth conditions evidently changed. Carbonates and water were probably present in the magma but were not incorporated into the inner regions because of the temperatures, pressures, and concentrations.

After the growth of the inner regions, the conditions for carbonate and water inclusion became more favourable and these environmental impurities could crystallize or be trapped in the growing diamond. It is possible that the concentration of these impurities in the melt increased at the boundary between the growing crystal and the melt

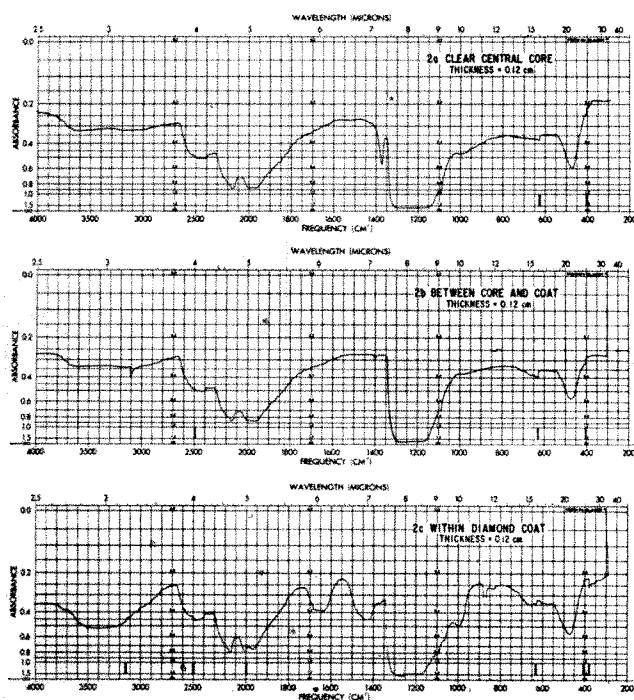


Fig. 2. Infra-red spectra of three areas of the coated diamond of Fig. 1. Thickness of 0.12 cm. Heavy dark vertical lines indicate positions of spectral features that are due to instrumental anomalies.

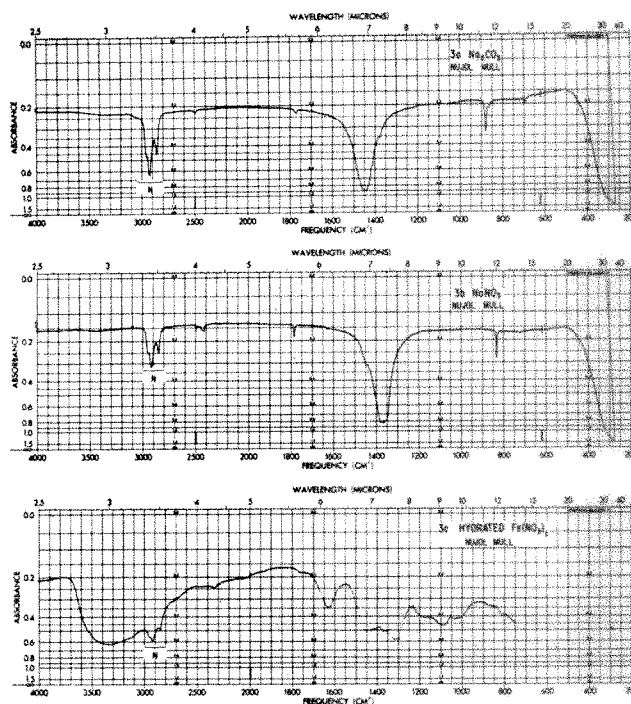


Fig. 3. Infra-red spectra of sodium carbonate, sodium nitrate, and hydrated ferric nitrate. The spectra were taken as Nujol mulls between salt plates. Heavy dark vertical lines indicate positions of spectral features that are due to instrumental anomalies. The only significant Nujol bands in the spectra are labelled by "N".

until nucleation of the other phases occurred. If this is the mechanism by which the coat was formed, it seems likely that this diamond grew from a phase rich in carbon rather than from a dilute solution of carbon in magma. The formation of rings may be due to alternate supersaturation and depletion of the melt in contact with the surface of the growing diamond. The possibility that the rings were caused by repeated changes of temperature and pressure cannot, however, be ruled out. It should be emphasized that the growth of the diamond itself continued while the impurities were being incorporated. The result gave rise to the "growth rings" as well as the varying amounts of impurities as seen by infra-red spectroscopy.

It is perhaps worth mentioning that the chemical groups responsible for the carbonate, hydrate, and CH bands contain elements that require the presence of only water and a metallic ion, together with diamond itself.

We believe that presence of the other phases during the growth of the diamond caused the formation of the finger-like structures in the coat found by Kamiya and Lang<sup>1</sup>. We cannot imagine how this finger-like structure could have been produced by precipitation of impurities which diffused in from outside an originally clear diamond after its growth. Furthermore, it is even more unlikely that a metal ion and water could both have diffused into the diamond to cause the hydrate and carbonate absorption bands of constant relative intensity throughout the coat.

Several theories have been put forward as to the constituents of the magma during diamond crystallization. Williams<sup>7</sup> gives many analyses of the kimberlite blue ground from which diamond is mined and finds calcium carbonate extremely common, and the carbonate content is very high for some analyses. He favours the secondary origin of the carbonate but says, "... on the other hand, some evidence has been such to tempt me to suggest primary origin for some of it". Rozhkov and Abrashov<sup>8</sup> in a study of kimberlite, have reported the presence of a carbonaceous crust surrounding a number of their diamonds. Our identification of carbonate impurities as



submicron precipitates in the diamond indicates that carbonates were indeed probably present in the magma out of which this diamond grew.

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## Eclipse Cycles and Eclipses at Stonehenge

by

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Was Stonehenge used to predict eclipses? Previous claims have assumed a high degree of sophistication on the part of the builders of Stonehenge, and that the number of Aubrey holes (56) is particularly significant. It may be that neither assumption is justified or necessary, and that if the builders of Stonehenge did predict eclipses, they did it in much simpler ways.

RECENTLY, Professor G. S. Hawkins has elaborated the suggestions of Stukeley<sup>1</sup>, Lockyer<sup>2</sup> and Newham<sup>3</sup> that alignments derivable at the ancient monument of Stonehenge have astronomical significance. He has proposed that many additional alignments between various stones and stone holes (mainly Stonehenge 1) and between the trilithons and certain sarsen arches (Stonehenge 111) were deliberately orientated to seasonal extremes of the rising and setting of the Sun and Moon<sup>4</sup>. Subsequently, he has suggested that Stonehenge can be used as a digital computing machine for predicting eclipses<sup>5</sup>. These ideas have since been popularized both in article<sup>6</sup> and book<sup>7</sup> form, and have aroused considerable interest and controversy. In particular, Professor R. J. C. Atkinson, the noted authority on Stonehenge, has been severely critical of the accuracy of Hawkins's alignments and of certain aspects of his archaeological interpretation<sup>8,9</sup>.

Hawkins's theory of the use of the 56 Aubrey holes to predict eclipses is based on the conjecture of a sustained 56 year eclipse cycle containing lunar periodicities which come "in a jumble of 19's and 18's, averaging two 19's to one 18 . . .". It is explicitly stated that the 19 year Metonic cycle<sup>10</sup> is a hitherto unrecognized eclipse cycle, overlooked previously "probably because it runs for only 57 years or so".

In fact, the Metonic cycle is related to phases of the Moon and arises because 235 lunations each of 29.53 days equal 6939.7 days, and 19 tropical years each of 365.2422 days equal 6939.6 days. After 19 years the phases of the Moon are repeated on the same calendar date; however, there is no relationship between the Metonic cycle and eclipses except for the accidental circumstances which will be described later.

Finally<sup>6</sup>, Hawkins reduced his theory algebraically to the commensurability of the eclipse year (synodic revolution of the lunar nodes) and the tropical year; that is, integers  $a$  and  $b$  were sought which came close to satisfying the equation

$$346.620 a = 365.2422 b$$

Certain of the resulting cycles, namely, those of 19, 37, 56, 74 and 93 years, were claimed to have special significance for eclipse prediction, because eclipses should repeat on the same calendar date for many periods before the Moon eventually moves outside the eclipse limits.

While we would agree with Hawkins that the foregoing expression ensures that the Sun, Earth and the lunar nodes are collinear, the fundamental requirement that the geocentric longitudes of the Sun and the Moon differ

by 180° appears to have been overlooked. In other words, none of Hawkins's cycles is an integral number of lunations, so the Moon is not at opposition after successive periods in these cycles. The present article is concerned with exploring the consequences of this unfortunate oversight.

In order to discuss actual rather than computed eclipses<sup>5</sup>, we have referred to the Nautical Almanacs to ascertain the calendar dates of lunar eclipses which occurred between 1855 and 1958 and the data are presented graphically in Fig. 1. It should be noted that since the elements of the Moon's orbit have not changed appreciably since 1500 B.C. the general pattern of eclipses at the time of Stonehenge is also represented by Fig. 1, although only about two-thirds of these eclipses would have been visible at Stonehenge. The exact computation of lunar eclipses is well known to astronomers, but we suspect that the broader comparative analysis of their periodicities presented here may have been overlooked in the past. Certainly, we have been unable to locate any graphical ephemeris similar to Fig. 1. These empirical relationships, however, are particularly relevant when discussing the astronomical attainments of ancient civilizations.

A number of fundamental features of lunar eclipses are at once apparent from Fig. 1. (1) Eclipses most frequently appear in groups of three, and each member of the triplet is separated by twelve lunations (354 days). Occasionally doublets occur, and very rarely quartets are observed. (2) There are usually two lunar eclipses each year, separated by six lunations. (3) Many genuine eclipse cycles can be immediately discerned—some of which are marked by bold lines. (4) A vertical line represents a cycle which gives eclipses on the same calendar date. It is conspicuous that such eclipses repeat every 65 years (in periods of 19, 19 and 27 years) and not every 56 years (19, 19 and 18 years) as claimed by Hawkins.

The important variables which determine the occurrence of eclipses are the relative positions of the Sun, Moon, Earth and the nodes of the lunar orbit. Since the nodes regress in a period of 18.61 years, a synodic revolution of the nodes takes rather less than a tropical year; in fact, it takes 346.62 days. The best known eclipse cycle is the Saros, which some authorities believe was known to the Chaldeans<sup>11</sup>. A Saros period is 18 yr 11 days (18 yr 10 days if five leap years intervene) and it arises because 223 lunations (6,585.32 days) and 19 synodic revolutions of the nodes (6,585.78 days) are nearly identical.



The Metonic cycle used by Hawkins is 1 Saros + 12 lunations (18 yr 11 days + 354 days = 19 yr). In Fig. 2 imagine a lunar eclipse predicted by a Saros cycle occurs with the Earth at position A, and the Moon at opposition exactly at its node. During the following 12 lunations the Earth will proceed to position B, but since 12 lunations are not equal to the period of rotation of the nodes, the nodes are no longer collinear with the Earth-Sun line. The question is, will an eclipse occur?

The mean daily relative motion of the Sun and the Moon's node is  $62' 19''$ , from which it follows that the Sun and the nodes separate by  $368^\circ$  in 12 lunations. There is therefore a difference of  $8^\circ$  between the Earth-Sun line and the nodes compared with the first eclipse. Similar considerations apply to the situation 12 lunations before the Saros eclipse. The condition for a lunar eclipse to occur with certainty is for the full Moon to lie within  $9^\circ$  of its node, but there is the possibility of an eclipse if the Moon is up to  $12.5^\circ$  from its node<sup>11</sup>. It follows that two or three (in exceptional cases four) eclipses can occur with a 12 lunation period between them; after this time the Moon has moved outside the eclipse limits (see Fig. 1). The same result is obtained if the initial eclipse were placed just inside the eclipse limits. Since the 19 year period differs from a quite accurate eclipse cycle by 12 lunations, it is clear that the number of eclipses that can occur in a cycle with this period is similarly limited.

Of the many eclipse cycles that can be seen in Fig. 1, only a limited number survive for more than a few periods. This is caused by poor commensurability in the expression

$$29.53059x = 346.62y/2$$

where  $x$  and  $y$  are integers. It is a simple matter to find integral values of  $x$  that give almost integral values of  $y$ .

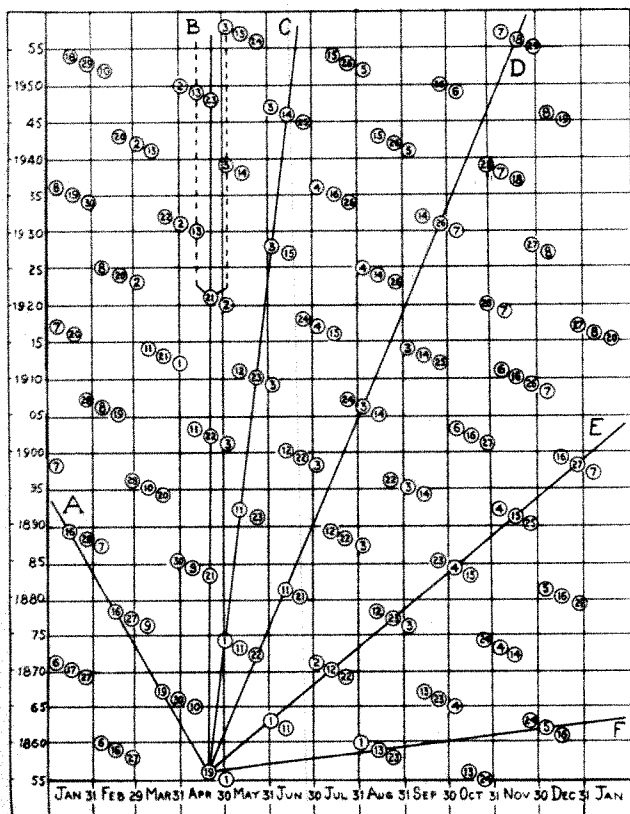


Fig. 1. Lunar eclipses (1855-1958). Key to diagram: each circle represents a lunar eclipse on the day of the month indicated. When an eclipse spans two calendar dates the earlier one is noted. The following illustrative eclipse cycles are marked by full lines (see text). A—135 lunations; B—804 lunations; C—223 lunations; D—311 lunations; E—88 lunations; F—41 lunations.

The incommensurability between lunations and synodic revolutions can be used with the eclipse limits to determine the number of periods for each cycle. The results are summarized in Table 1 for values of  $x$  up to 1,000. All cycles which survive for less than 10 periods have been eliminated (except the very important 41 lunation period). The convention used is to express errors as positive if the lunations are longer than the synodic revolutions of the nodes.

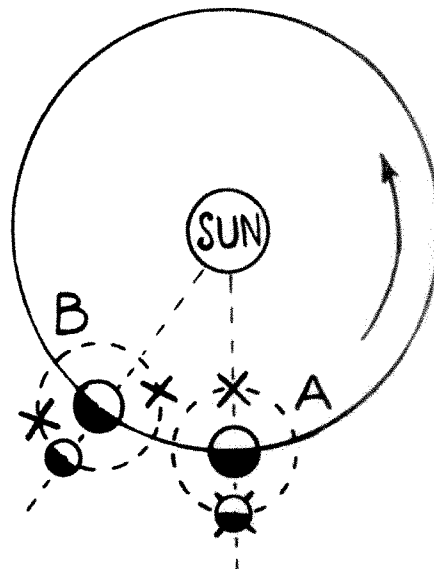


Fig. 2.

A remarkable feature that emerges is that all the eclipse cycles consist of the addition of small integral multiples of the two shortest cycles; for example, the Saros (223 lunations) is  $(3 \times 47) + (2 \times 41)$  lunations. The incommensurability of the 41 and 47 lunation periods are in the opposite sense and all other cycles are merely linear combinations of multiples of these cycles in which the additive error tends to zero (Table 1). Algebraically the cycles conform to the expression  $(41m + 47n)$ , where  $m$  and  $n$  are the integers,  $m = 0, 1, 2, \dots$  and  $m \leq n \leq 2m$ . The 358 lunation cycle is especially noteworthy as it survives between 12,000 and 17,000 years.

It emerges that some of the cycles listed in Table 1 were those used by ancient civilizations to predict eclipses, but it will be clear that the longer the period the less likely it would be for ancient peoples to discern a particular cycle. We have marked a few eclipse cycles in Fig. 1; the Saros (line C) is notable because each eclipse occurs close to the calendar date of the previous one. It may well be that this cycle was detected by early civilizations because its period is close to that of both the Metonic cycle and the regression of the nodes.

Line A (135 lunations) is the cycle used by the ancient Chinese as an eclipse predictor<sup>12</sup>. They were well aware of the Metonic cycle which they called the *chang*, and they also used a period four *changs* (76 years) called the *pu*. Their numerology was notable in that they recognized 81 lunations as an integral number of days, which when combined with the 135 lunation period gave a cycle of 405 lunations ( $m = 3, n = 6$ ) which is both an eclipse cycle and an integral number of days<sup>12</sup>.

In view of Professor Hawkins's attempts to find an eclipse cycle which gives eclipses on the same calendar date the 804 lunation cycle (line B) is of some interest. The repeating unit of 65 yr 2 days is made up of two 19 yr intervals and one of 27 yr 2 days. This is a true eclipse cycle because the incommensurability in the two 19 yr periods (that is,  $-16''$ ) is almost balanced by an error of

Table 1. ECLIPSE CYCLES

Lunations	Days	Syn. revs.	Days	Error (days)	Error (°)	No. of periods	
						Min	Max
41	1,210.7542	3.5	1,213.17	-2.4158	-2.519	7	9
47	1,387.9377	4.0	1,386.48	+1.4577	+1.5140	12	16
88	2,598.6919	7.5	2,599.65	-0.9581	-0.9951	18	25
135	3,986.6297	11.5	3,986.130	+0.4997	+0.5189	34	48
223	6,585.3216	19	6,585.78	-0.4584	-0.4761	37	52
311	9,184.013	26.5	9,185.43	-1.317	-1.367	13	18
358	10,571.951	30.5	10,571.91	+0.041	0.04258	428	587
493	14,558.581	42	14,558.04	+0.541	+0.562	32	44
581	17,157.272	49.5	17,157.69	-0.418	-0.434	41	57
628	18,545.210	53.5	18,544.17	+1.04	+1.080	16	23
763	22,531.840	65	22,530.30	+1.540	+1.599	11	15
804	23,742.594	68.5	23,743.47	-0.876	-0.910	19	27
851	25,130.532	72.5	25,129.95	+0.582	+0.604	29	41
939	27,729.224	80	27,729.60	-0.376	-0.391	46	63

\* Three unevenly spaced eclipses per period—see text.

+15.09° in the 27 yr 2 day period (334 lunations), so after the full period the relative movement of the nodes and the Earth-Sun line is only 0.91° and the cycle can repeat many times.

Also marked on Fig. 1 (dashed lines) are the manoeuvres necessary to obtain the 19, 19, 18 year sequence as described by Hawkins, although it is actually 19, 19, 18 years 11 days. It is inherently a very short cycle because there is no way to compensate for the error in the two 19 year periods. If it were not for the fact that his Stonehenge computer theory demands a 56 year cycle, a far more logical move would be to follow the 47 year sequence 19, 19 and (9 years minus 9 days), which gives the very good 581 lunation cycle (dashed line, Fig. 1). The error in the two 19 year periods is balanced almost exactly by that of the (9 year minus 9 day) period. Obviously the 804 and 581 lunation cycles are far superior in fulfilling the conditions sought by Hawkins.

Certain occasional irregularities in the 804 lunation cycle serve to underline the occupational hazards of making priestly predictions of lunar eclipses. Thus, the intervention of random groupings of eclipse doublets or quartets can increase or decrease the anticipated interval between eclipses on a particular cycle. For example, the quartet between 1908 and 1911 resulted in an extra eclipse on November 6, 1911, giving intervals of 19 and 8 years, rather than 27 years. Similarly, the more frequently occurring eclipse doublets caused eclipse omissions near May 24, 1937, June 1, 1882, and December 18, 1926, which resulted in 46 year periods (19+27) between eclipses. Prophecies in mid-October would have been particularly disastrous since successive eclipses expected on October 16, 1921, and October 18, 1948, did not occur, so that an interval of at least 65 years is involved.

It would seem safe to assume from the discussion so far that the Aubrey holes at Stonehenge were not constructed to predict eclipses on a 56 year cycle. Recently<sup>13</sup>, Professor Hoyle has suggested a most elegant and ingenious alternative way of using the Aubrey holes to predict eclipses, but the success of his method is not confined to circles of 56 holes. Hoyle regards the Aubrey circle as the ecliptic on which stones representing the Sun, Moon and the two lunar nodes are moved in such a way as to give approximately their periods of rotation. These periods are compared in Table 2 with corresponding values for other hypothetical circles, from which it is seen that 56 is not a unique number for this type of predictor. Viewed in the context of the limited astronomical knowledge of the Stonehenge society, the method appears to be an unlikely one because it is very sophisticated and in the calibration procedure, particularly, requires that the Stonehenge people were capable of storing rather subtle information for several periods of 18.61 years. Moreover, the method predicts every lunar and solar eclipse, but it can give no information as to which of these would be visible at Stonehenge; in fact, exclusion of an eclipse would be its only safe prediction.

Finally, we would like to propose a very simple method of predicting only those lunar eclipses visible at Stonehenge. It has been known from the third millennium B.C.

Table 2. NUMEROLOGICAL ECLIPSE PREDICTORS

No. of holes	Sun (period 365.25 d)		Nodes (period 18.61 yr)		Moon (period 29.53 d)*	
	Method	Period obtained	Method	Period obtained	Method	Period obtained
18	1 hole/20 day	360	1 hole/yr	18	3 holes/5 day	30
19†	1 hole/19 day	361	1 hole/yr	19	2 holes/3 day	28.5
36	1 hole/10 day	360	2 holes/yr	18	5 holes/4 day	28.8
37†	1 hole/10 day	370	2 holes/yr	18.5	5 holes/4 day	29.6
56	2 holes/13 day	364	3 holes/yr	18.67	2 holes/day	28.0
74	1 hole/5 day	370	4 holes/yr	18.5	5 holes/2 day	29.6

\* Since the Moon marker is adjusted opposite to, or coincident with, the Sun at each full Moon and new Moon respectively as seen from the Earth, the period required is the lunar month of 29.53 day, not the sidereal revolution of Moon (27.32 day) as Hoyle states.

† Obvious practical difficulties arise with odd numbers of holes since the Sun and Moon cannot be exactly opposite each other.

that for a lunar eclipse to occur the Sun and the full Moon must be diametrically opposed in the sky. If, in addition, the Moon rises a short time (up to 15–30 min, depending on the declination of the Moon) before the Sun sets, an eclipse of the Moon will occur that night and will be visible. If the time interval is longer, the eclipse will not be seen; if the full Moon rises after the Sun has set, the eclipse is already past, as Hawkins has mentioned. The even spacing of the Aubrey holes on an accurate circle<sup>14</sup> would enable it to serve as a giant protractor used by the observer standing at its centre to judge whether the setting Sun and rising full Moon were exactly opposite each other in azimuth. We believe that with practice such predictions could be made with considerable accuracy; the observer would be warned of the possibility of a forthcoming eclipse by noting how previous full Moons came progressively closer to a position in line with the Sun.

If this method of eclipse prediction were used, it could provide the reason for the construction of the many stone circles found in the British Isles for which no completely convincing purpose has been proposed. The advantage of the method is its simplicity, but it has the disadvantage that it is poor for predicting solar eclipses because of the difficulty of observing the Moon near conjunction. Since solar eclipses are rare events at any given spot, however, this is probably not a serious objection. It is clear that this type of predictor would work with any reasonably large number of holes, and we suspect that there are 56 Aubrey holes for quite different reasons, which will be discussed in a later communication.

We thank Dr. C. G. Barraclough, of the Department of Physical Chemistry, for his helpful comments.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Helium in the Galactic Disk B Stars

Greenstein and Münch<sup>1</sup>, and Sargent and Searle<sup>2</sup>, have recently shown that the atmospheric helium abundance in the halo and globular cluster B stars is low compared with that for normal B stars in the Galaxy. Greenstein, Truran and Cameron<sup>3</sup> have suggested that the low abundance may be the result of gravitational diffusion of helium from the atmosphere to a lower layer within the star. They consider that normally mixing currents set up by stellar rotation would counteract such diffusion. In contrast to galactic disk B stars, most of the halo stars appear to be slow rotators. This result is apparently confirmed by Sargent and Strittmatter<sup>4</sup>, who have discussed the weak helium line stars in Orion and conclude that they are intrinsically slow rotators, and that the anomaly is an atmospheric one.

In this communication we would like to report related results on 425 field B stars which suggest that the apparent helium abundance depends on the rotational velocity. The observational data are taken from Walker and Hodge<sup>5</sup>.

There is a significant positive correlation for spectral types B2 to B5 between the equivalent widths of the He I lines at wavelengths of 4388 and 4471 Å, and the values of the projected rotational velocity  $v \sin i$ , based on the half widths of the two He I lines. The correlation between the equivalent widths of H $\gamma$ , a measure of surface gravity, and  $v \sin i$  is always much smaller than that between the He I lines and  $v \sin i$  and not always of the same sign. The relevant correlation coefficients between the logs of the observed quantities are given in Table 1.  $W$  refers to equivalent width and the subscripts identify the line. For the He I lines we use the correlation between  $\log W_{4471}$  and  $\log (v \sin i)_{4388}$  to eliminate any effects of errors of measurement in  $W$  and  $v \sin i$  for the same line. The number of stars in each group is given by  $n$ . An example of the correlation for B4 stars is shown in Fig. 1.

After examination of the reduction procedure, we have concluded that systematic errors in estimating the level of the continuum or the line profile are not responsible

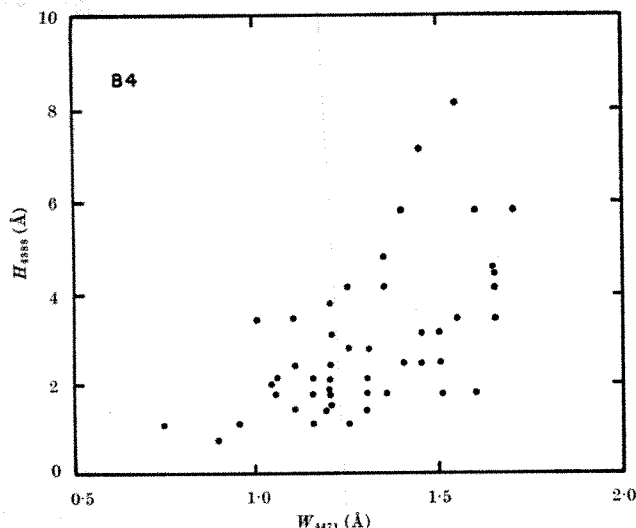


Fig. 1. The relation between the equivalent width  $W$  of the He I line at 4471 Å and the half-width  $H$  of the He I line at 4388 Å for B4 stars, showing the effect of rotational velocity, which determines  $H$ , on the strength of the He I lines.

Table 1. CORRELATION COEFFICIENTS FOR SPECTRAL TYPES B0 TO B5

Spectral type	$(W_{4471}, v \sin i)$ $r^*$	$(W_{H\gamma}, v \sin i)$ $r^*$	$n$
09, B0	0.05	0.12	46
B1	0.01	0.00	69
B2	0.26	-0.04	66
B3	0.32	-0.15	130
B3e	0.63	-0.36	23
B4	0.60	0.24	48
B5	0.29	0.02	66

$r^*$ , Correlation coefficient between the logs of the quantities in parentheses.

for the correlation between  $\log W_{4471}$  and  $\log (v \sin i)_{4388}$ . There is clearly a considerable scatter, since none of the correlation coefficients is very high. The distribution of points at all spectral types is, in fact, triangular (see Fig. 1), an effect which can be understood if the helium line intensities are sensitive to rotational velocity  $v$  but insensitive to  $\sin i$ . According to Greenstein and Münch<sup>1</sup>, the effects of changes in temperature and pressure over the surface caused by the rotation are expected to be small, and this is confirmed by our observation that the variation of the equivalent widths from type to type is less than the scatter within each type. We could improve the correlations considerably in the range 09 to B2 and at B5 by rejecting five very deviant points, but we prefer not to do this, since there is no other evidence that these stars are peculiar. These are, however, the only stars out of 425 which show low  $W_{4471}$  and high  $v \sin i$ .

The B3e stars were analysed as a separate group since the emission line stars have been thought to be rapidly rotating, and the observed variation of the equivalent widths of the He I lines with  $v \sin i$  would then reflect largely a variation with  $\sin i$  not  $v$ .

The frequency of B3e stars with low  $v \sin i$ , however, appears to be incompatible with a uniformly high  $v$  and a random distribution of  $i$ . Some of our B3e stars must therefore be intrinsically slow rotators. A similar conclusion is reached by Schild<sup>6</sup> in a study of the Be stars in  $\delta$  and  $\gamma$  Persei. The Be stars, therefore, give us no information about  $\sin i$  effects. The behaviour of the helium lines in B3e stars is, in fact, observed to parallel that of the normal B3 stars. Sharp line B3 and B3e stars have similar He I lines (Sargent and Searle<sup>7</sup>), but this cannot be used as evidence for the absence of  $\sin i$  effects. Other arguments such as those made previously must be used. Visual inspection of the original plates shows that other He I lines behave in the same way as those at 4471 and 4388 Å.

These results are qualitatively compatible with any mechanism for removing helium from the atmospheres of slowly rotating stars, such as that of Greenstein, Truran and Cameron, and consistent with the arguments of Sargent and Strittmatter that the abundance anomalies cannot extend far into the interior of the stars. Caution must therefore be exercised in comparing the helium content of the atmospheres of certain stars with the implications of some cosmologies concerning primordial helium abundance. A more detailed account of this research will appear elsewhere.

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## Detection of $\beta$ -Transitions in the Recombination Spectrum of Hydrogen near 9 cm Wavelength

THE detection in galactic emission nebulae of several of the hydrogen lines corresponding to transitions between levels with large principal quantum numbers has already been reported<sup>1-4</sup>. The lines were in the frequency range 8,873–1,424 Mc/s, corresponding to transitions in the range  $90\alpha$ – $166\alpha$ , using the notation suggested by Palmer and Zuckerman<sup>4</sup> in which  $n\alpha$  indicates a transition from level of quantum number  $(n+1)$  to  $n$ . A transition from  $(n+2)$  to  $n$  is indicated by  $n\beta$  (see Fig. 1a).

In predicting that hydrogen recombination lines should be detectable, Kardashev<sup>5</sup> calculated that  $\beta$ -transitions would be considerably weaker than  $\alpha$ -transitions, which are themselves weak. In recent observations of the intense HII regions, Orion-A and M17,  $126\alpha$  intensities of several degrees were recorded with the Parkes radio telescope. An attempt was therefore made to detect  $\beta$ -lines at nearby frequencies.

Two lines,  $158\beta$  and  $159\beta$ , were observed in the nebulae at frequencies near the calculated rest frequencies of 3,272.219 and 3,211.245 Mc/s, respectively; in the calculation the Rydberg constant  $R$  was taken as  $3.2880559 \times 10^{15}$  sec<sup>-1</sup>. The rest frequency of the  $126\alpha$  line was 3,248.713 Mc/s.

The half-intensity beamwidth of the 210 ft. telescope was 6 min of arc at about 3,250 Mc/s. The receiver consisted of a double side band mixer in front of a 48-channel recording system, with filters 37 kc/s wide spaced 33 kc/s apart. The overall system noise temperature with the telescope pointing at either Orion or M17 was 480° K, and when pointing at cold sky 300° K. The local oscillator was switched by 2.16 Mc/s from "signal" to "reference" frequencies. The first intermediate frequency was 30.43 Mc/s. Under these conditions, the  $158\beta$  line appeared in the signal band but, in addition, the  $159\beta$  line, 60.974 Mc/s lower in frequency, appeared in the image reference band as a negative deflexion.

The observational procedure was to record four or six profiles on source and the same number at a reference point  $\pm 12$  min away in right ascension. A single forty-eight point profile was recorded in 2 min of time. The

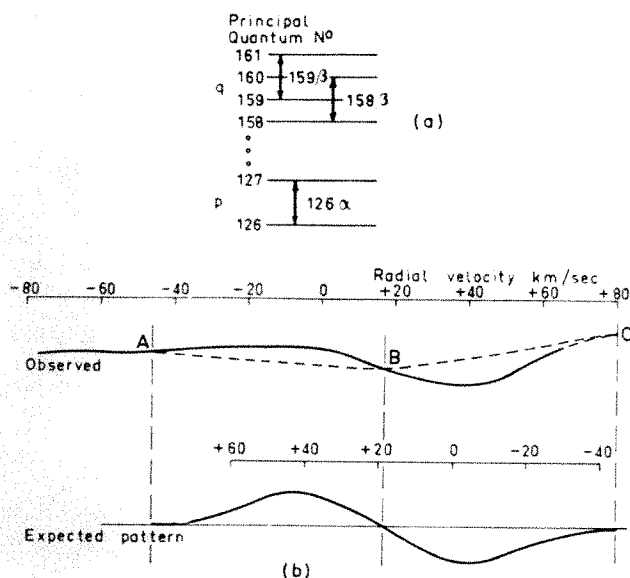


Fig. 1. a, The energy levels involved in the  $\alpha$  ( $\Delta n=1$ ) and  $\beta$  ( $\Delta n=2$ ) transitions. b, The observed profile for the combination of the  $158\beta$  (positive) and  $159\beta$  (negative) emissions in Orion A. The baseline is dashed. The expected pattern shown below was derived from the  $126\alpha$  line reduced by a factor of 0.2. The upper radial velocity scale refers to the  $158\beta$  line, the lower to the  $159\beta$  line. The ordinate is intensity.

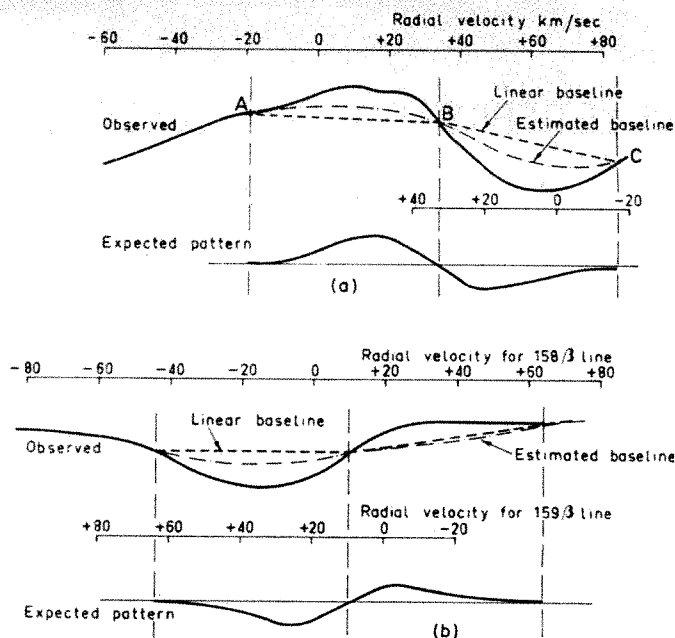


Fig. 2. The observed profiles for the combinations of the  $158\beta$  and  $159\beta$  emissions in M17. The expected patterns shown below were derived from the  $126\alpha$  line reduced by a factor of 0.16. The change of pattern from a to b was caused by retuning the receiver. The ordinate is intensity.

sequence of observations was such that changes in baseline as a result of changes in the zenith angle of the telescope and linear drifts with time were eliminated. Thirty-six sets of profiles were taken on each source. The intensity scales for the "on-source" and "reference-point" observations were different because of the different system temperature (see above). The scales were measured and corrections applied in the computer reductions.

The average spectrum as observed at intermediate frequency is shown in Fig. 1(b) for Orion A. For convenience, corresponding radial velocity scales (referred to the local standard of rest (LSR)), which are different for the two lines, are included: the upper for the  $158\beta$  and the lower for the  $159\beta$  transitions. The observed profile can be compared with the expected profile, which was obtained from the  $126\alpha$  observations<sup>4</sup> by suitably adjusting the frequency (that is, velocity) scales and by reducing the amplitude to be comparable with the  $\beta$ -amplitude. Now the radial velocities at points of zero intensity A, B and C are well established. The baseline between these points, however, is somewhat uncertain. The first approximation assumed straight lines from A to B and B to C. This proved sufficient in Fig. 1b, because such a baseline closely approximated one obtained from an observation of a point on the Crab Nebula (no hydrogen lines present) of continuum aerial temperature equivalent to that of Orion A.

Two profile patterns are shown for M17 in Fig. 2. The insertion of linear baselines is not realistic in these cases and suitably curved baselines are suggested following the obvious trends from observations outside the lines. But the validity of the line emission was demonstrated when a suitable change in local oscillator frequencies caused the sense of the S-shaped pattern to reverse from Fig. 2a to Fig. 2b. In making measurements from these profiles both the linear and the estimated baselines have been included, appropriately weighted and errors accordingly allotted.

Table 1 contains estimates of the central line intensity in terms of aerial temperature,  $T_{L\beta}$ , the ratio of the central line intensity to the continuum temperature  $T_c$ , and the ratio of the intensities of the  $\beta$  and  $\alpha$  transitions,  $I(158\beta)/I(126\alpha)$ , together with the radial velocity for Orion A and M17. The ratios are significantly higher for M17.

Table 1. SUMMARY FOR H-LINES 158 $\beta$ , 159 $\beta$  AT FREQUENCIES 3,272-219, 3,211-245 Mc/s

		Orion A	M17
$T_{1\beta}$	°K	0.8	1.2
$T_{1\beta}/T_e$	%	0.44	0.67
$I(158\beta)/I(126\alpha)$		0.13 ( $\pm 0.03$ )	0.22 ( $\pm 0.04$ )
R.V. (peak)	km/s	-4	+22

The expected intensity ratios of the  $\beta$  and  $\alpha$  transitions can be derived from the fundamental formulae of Menzel and Pekeris<sup>7</sup>. If the principal quantum number of the lower level of the  $\beta$ -transition is written as  $q$  and the corresponding number for the  $\alpha$ -transition as  $p$ , then from equations (1.2) and (1.3) of ref. 7, the ratio of the total emissions for small optical depths in both lines is given by

$$\frac{E_{q+2,q}}{E_{p+1,p}} = \frac{N_q}{N_p} \cdot \frac{f_{q+2,q}}{f_{p+1,p}} \cdot \left[ \frac{\nu_{q+2,q}}{\nu_{p+1,p}} \right]^3 \quad (1)$$

where the  $f$ 's and  $\nu$ 's are the oscillator strengths and frequencies of the transitions shown as subscripts. The populations  $N_p$ ,  $N_q$  of levels  $p$  and  $q$  are given by Boltzmann's formulae if local thermodynamic equilibrium (LTE) applies, that is

$$\frac{N_q}{N_p} = \frac{q^2}{p^2} \cdot e^{-(h\nu_{q,p}/kT)} \\ = \frac{q^2}{p^2} \text{ as } e^{-(h\nu_{q,p}/kT)} \approx 1 - 0.00035 \text{ for } T = 10^4 \text{ °K.} \quad (2)$$

In equation (1), we may rewrite

$$\frac{f_{q+2,q}}{f_{p+1,p}} = \frac{f_{q+2,q}}{f_{q+2,q+1}} \cdot \frac{f_{q+2,q+1}}{f_{p+1,p}} = \frac{0.113(q+2)}{(p+1)} \quad (3)$$

when the values of  $f_{n,n-1} = 0.18n$  and  $f_{n,n-2}/f_{n,n-1} = 1/8.8$  given by Kardashev<sup>5</sup> are used. Finally, if (2) and (3) are substituted in (1) and if the frequencies of the  $\beta$  and  $\alpha$  transitions are taken as equal (the average  $\beta$ -frequency 3,242 Mc/s is very close to 3,248, the  $\alpha$ -frequency), we obtain

$$\frac{E_\beta}{E_\alpha} \approx \frac{0.113q^2(q+2)}{p^2(p+1)} \quad (4)$$

$$\approx 0.224 \text{ for the } 158\beta/126\alpha \text{ transition} \\ \approx 0.230 \text{ for the } 159\beta/126\alpha \text{ transition.}$$

These numbers will also be the ratio of the central line intensities if the line widths are the same as is the case for Doppler broadening.

Table 1 shows that the observed central intensity ratio for M17 is very close to the value given here, but that for Orion is significantly lower.

Recently, Goldberg<sup>8</sup> has directed attention to a dependence of the  $\alpha$ -line/continuum ratio on the continuum optical depth and to possible differences between the line excitation temperature and the electron temperature,  $T_e$ . The  $\beta/\alpha$  intensity ratio is useful because it is independent of optical depth in the continuum. So, to explain the present observations of Orion A, either there must be a departure from LTE or the broadening must not be solely Doppler. If only the former is important and the ratio of the population of level  $n$  to that in LTE is denoted by the usual symbol  $b_n$  ( $b_n = 1$  for LTE) then the ratio of  $\beta/\alpha$  intensities to that in LTE is given from Goldberg's equation (4) as

$$\frac{\{I(158\alpha)/I(126\alpha)\}}{\{I(158\alpha)/I(126\alpha)\}_{\text{LTE}}} \\ = \frac{1 - \frac{b_{160}}{b_{158}} \cdot e^{-h\nu_\beta/kT_e}}{1 - \frac{b_{127}}{b_{126}} \cdot e^{-h\nu_\alpha/kT_e}} \quad \text{and} \\ \approx \frac{b_{160} - b_{158}}{b_{127} - b_{126}}$$

under conditions envisaged by Goldberg. The lower  $\beta/\alpha$  ratio for Orion would then imply that the  $b_n$  relation-

ship was flattening off at large  $n$  in a similar way to Seaton's<sup>9</sup> theoretical curve (IV), calculated for  $T_e = 10^4$  °K and an electron density of  $10^4/\text{cm}^3$ .

Our observations<sup>6</sup> of the H126 $\alpha$  line in Orion A have shown, however, that the line width is appreciably greater than might be expected from an extrapolation (proportional to frequency) of the observations<sup>1,2</sup> of the 109 $\alpha$  and the 94 $\alpha$  lines. Thus for Orion A, broadening effects other than Doppler are significant. (The H126 $\alpha$  width in M17 was closely proportional to frequency.) The full amount of the correction for broadening in Orion A is expected to come out of a projected series of measurements of, say, the 158 and 159 $\alpha$  intensities. Such information may shed light on the true population distribution at high quantum numbers.

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## PLANETARY SCIENCE

### Transient Lunar Brightening

MIDDLEHURST has examined<sup>1</sup> the relationship between transient lunar brightening and solar activity as measured by the incidence of sunspots (compare ref. 2). Working with a sample of 125 lunar events, she found no correlation between lunar brightening and the mean sunspot number for the month during which such an event occurred. Hence, she suggests that the reported events must be attributed to some physical phenomenon other than luminescent emission induced by enhanced solar wind bombardment or any other direct consequence of solar activity.

Most of the reported events appear to be of short time duration (less than 1 h), and it would seem more appropriate to make such a statistical investigation in terms of a parameter indicating the variation within a time interval much shorter than 1 month. Specifically, an experiment on *Mariner II* by Snyder *et al.*<sup>3</sup> showed that the velocity and energy flux of the solar wind are not strongly correlated with overall sunspot activity as determined by sunspot number and 10.7 cm radio noise emission but are convincingly and positively correlated with the magnitude of the planetary geomagnetic disturbance index  $K_p$ . This index measures the range of the variations in the geomagnetic field intensity within a period of 3 h and is scaled over the range 0- to 9+. The sum over 24 h, designated  $\Sigma K_p$ , is the sum of the eight values of  $K_p$  for a day and thus ranges from 0- to 75-. Internationally determined values of  $K_p$  are available for the past 34 years<sup>4</sup>.

The  $K_p$  index is therefore relevant for indicating the intensity of solar wind bombardment of the Moon. A relation between lunar eclipse brightness and the sunspot cycle, proposed earlier by Danjon<sup>5</sup>, is questionable, in view of my recent study<sup>6,7</sup> on the basis of a number of more reliable results from lunar eclipse photometry. Instead, it has been found that the eclipsed Moon seems to appear brighter when the eclipse occurs on a day of higher  $K_p$ . Thus, it is interesting to investigate the relationship between the occurrence of lunar brightening events and solar wind activity by way of the secondary parameter, the  $K_p$  index.



In a previous paper<sup>7</sup>, I have examined the values of  $K_p$  on the dates of about twenty recorded luminescence observations, but the number of dates included in the analysis does not seem to be sufficient to allow any convincing conclusion. The 3 h values as well as the 24 h sums of  $K_p$  indices are available without interruption since January 1932 (ref. 4).

The most recent catalogue compiled by Middlehurst and Burley<sup>8</sup> lists reports of 238 lunar brightening events, including 122 on known dates since 1932. Since the exact time of the observations is usually not known, the 24 h sums,  $\Sigma K_p$ , are used in the following analysis. A total of 366  $\Sigma K_p$  values are taken, one for the day of each event, one for the preceding day, and one for the following day. The frequency distribution of  $\Sigma K_p$  values on the event days is found by grouping the values into twelve classes, 0- to 5+, 6- to 10+, 11- to 15+, . . . , etc., with the exception that the final class includes all values greater than 55+. A second frequency distribution is found for the indices for the 3 day intervals, including each event day. Finally, a third frequency distribution is found by taking the  $\Sigma K_p$  values for each of the 31 days of every January between 1932 and 1966.

These three frequency distributions are shown in Fig. 1, with the area under each curve normalized to unity. The distribution for event days is shown by the solid line, and the distribution for the 3 day periods including the day before and after each event is shown by the dotted line. The broken line represents the distribution for 1,054 days in January of the past 34 years and is taken to represent the distribution of  $\Sigma K_p$  for a random selection of dates. The degree of fluctuation of the resulting distribution for each case may be compared by noting that the vertical shift of each point due to the difference of one day in each interval is 0.41, 0.14 and 0.05 of the ordinate unit (2 per cent) for the solid, dotted and broken lines, respectively.

I conclude from Fig. 1 that the frequency distribution of  $\Sigma K_p$  indices for days on which lunar brightening has been reported is not significantly different from the distribution for a random selection of days. Thus, in agreement with Middlehurst<sup>1</sup>, it appears that lunar brightening events are not related to solar wind activity.

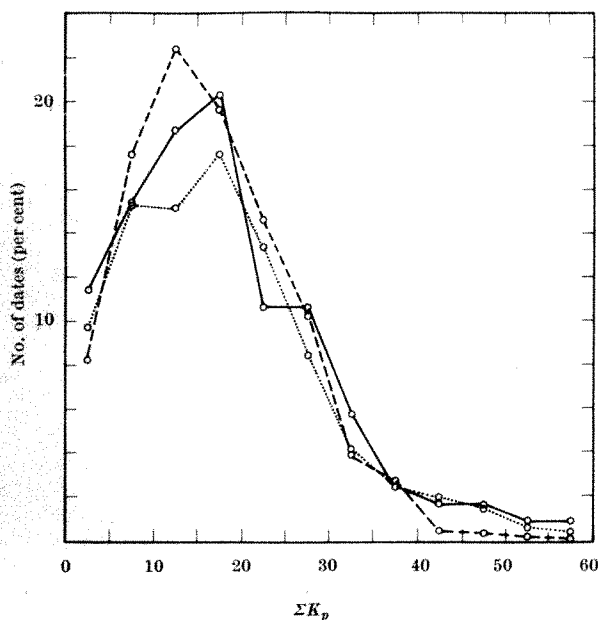


Fig. 1. The solid, dotted, and broken lines represent, respectively, the frequency distributions of  $\Sigma K_p$  values of the days of lunar brightening events, on the three days near each of the events, and on arbitrarily chosen dates.

An implicit assumption of the foregoing analysis is that lunar luminescence under solar wind bombardment, if indeed of observable intensity, is a prompt effect (compare ref. 9 for a discussion of the possibilities of energy storage and subsequent release at the advancing terminator).

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### Systematic Errors in the Palaeomagnetic Inclination of Sedimentary Rocks?

Van Andel and Hospers's<sup>1</sup> conclusion that an inclination error is present in sedimentary rocks throughout the geological column is considered highly premature for the following reasons. (a) Palaeomagnetic data from varves which have a depositional remanent magnetization (d.r.m.) for which it is suspected<sup>2</sup> there may be an inclination error are grouped with those from sedimentary rock types (for example, red beds) which have crystallization remanent magnetism (c.r.m.) for which an inclination error is most unlikely. (b) Certain pairs of data used by van Andel and Hospers are invalid for the purpose of their analysis, as shown later in this communication. (c) Their statistical method is not satisfactory in that they have to exclude data for which the inclination difference between lavas and sediments  $|I^*| - |I| = 0$ . The method is crude in that no account is taken of the magnitude of the inclination error for each data pair or of its accuracy.

Let us consider some important palaeomagnetic aspects of the data pairs listed by van Andel and Hospers<sup>1</sup> in their Table 1.

**Data pair 1.** The sediments (poles 202 and 203) are Cambrian, but the age of the Aberdeen gabbros (pole 201) ranges between 404 and 498 m.y. (Silurian-Ordovician)<sup>3</sup>. These data are therefore invalid, because they are probably different in age.

**Data pair 8.** The Carboniferous directions in Britain fall into two distinct groups<sup>4</sup>. In Table 1, where they are summarized, they are referred to as CI and CII. Directions CI may correspond to Triassic remagnetization<sup>4</sup>, so that it may not be valid to use entry 8 (a) as a test of the presence of inclination error. Thus entry 8 in van Andel and Hospers's Table 1 should be replaced by entry 8 (b) in my Table 1.

Table 1. SUMMARY OF BRITISH CARBONIFEROUS PALAEOMAGNETIC RESULTS

Entry No.	Direction group	Sedimentary rocks				Igneous rocks				$\Delta D$	$ I^*  -  I $
		N	D	I	a	N	D	I	a		
8 (a)	CI	4	35°	+29°	13°	1	47°	+38°	(10°)	12°	+9°
8 (b)	CII	4	15°	-36°	15°	4	25°	-30°	18°	10°	-6°

**Data pair 12.** The Siberian Triassic igneous poles (820 and 825)<sup>5</sup> differ significantly from each other. When directions  $D^*$  and  $I^*$  are calculated for each of these corresponding to the site of sedimentary formations 821, the sign of  $|I^*| - |I|$  is positive for igneous formation 821,

while this sign is negative for igneous formation 825 (see Table 2). These results contradict each other. Entry 12 in van Andel and Hospers's Table 1 is therefore invalid.

Table 2. PALAEOMAGNETIC RESULTS FOR THE TRIASSIC, SIBERIA

Entry No.	Ref.	Sedimentary data		Ref. No.	Igneous data		$D^*$	$I^*$	$\Delta D$	$ I^*  -  I $
		$D$	$I$		Pole					
12a	821	130°	+68°	820	60° N 133° E	155°	+79°	25°	+11°	
12b	821	130°	+68°	825	34° N 153° E	115°	+63°	15°	-5°	
12	821	130°	+68°	820+825	47° N 146° E	128°	+72°	2°	+4°	

**Data pair 14.** The igneous data (pole 1015) have too small a standard deviation ( $\delta = 4.4^\circ$  computed from precision  $K = 38.9$ )<sup>5</sup> to contain the full secular variation<sup>6</sup> of the Cretaceous geomagnetic field. Thus pole 1015 is most unlikely to be the true Cretaceous palaeomagnetic pole (that is, the Cretaceous pole of rotation) but is rather a non-axial virtual pole corresponding to the mean of a number of Cretaceous geomagnetic poles representing too short an interval of time for non-axial components of the field to have been averaged out. Thus the directions  $D^*I^*$  computed from pole 1015 are not Cretaceous axial dipole field directions and should not have been used by van Andel and Hospers in their analysis.

**Data pair 19.** The n.r.m. of the Akchagyisk and Apsheronsk stages (pole No. 1230) is only partly stable because it exhibits streaking<sup>5</sup>. This was corrected for by rotating the measured mean direction of magnetization along the great circle passing through it and through the present dipole field direction away from the latter. The magnitude of this correction was quite arbitrary and might well have been over-estimated. The effect of making it was to decrease the inclination of the measured mean direction, and the fact that this arbitrarily corrected value,  $I = 40^\circ$ , is less than that computed for the present axial dipole field is entirely meaningless. This pair of data is therefore invalid.

**Data pairs 21, 22 and 23.** In each of these pairs, the sediment is a varved clay the n.r.m. of which is known to be depositional and therefore not typical of the bulk of sediments studied palaeomagnetically.

The remaining palaeomagnetic data, the validity of which have not been contested and which are therefore still available for van Andel and Hospers's sign test, are listed here in Table 3. Of their twenty-three pairs they rejected three (Nos. 2, 3 and 4) because the inclinations  $I^*$  and  $I$  were of opposite sign (marked "/" in right-hand column). They rejected two more (Nos. 10 and 22) because the sign of the inclination error was zero, thus biasing their statistics in favour of their conclusion that an inclination error is present in sedimentary rock magnetic data. Even if following them we exclude these two data pairs, however, we still cannot reach any significant conclusions as indicated in Table 4.

Table 3

No. (van Andel and Hospers's Table 1)	Geological age	Area	Type of sediment	$\Delta D$	$ I^*  -  I $
1	Invalid				/
2		No comparison possible			/
3		" "			/
4		" "			/
5	Devonian	U.S.S.R.	Red beds	45°	-11°
6	"	Australia	" "	31°	-1°
7	"	U.S.A.	" "	10°	+14°
8	Invalid				
8(b)*	Carboniferous	U.K.	Various	10°	-6°
9	Carboniferous	Australia		29°	-20°
10	Permian	U.K.	Red beds	1°	0°
11	"	W. Europe	" "	15°	+2°
12	Invalid				
13	Triassic	U.S.A.	" "	18°	-14°
14	Invalid				
15	Eocene	U.S.A.	—	88°	-2°
16	Mio-Pliocene	U.S.S.R.	—	18°	+2°
17	"	U.S.A.	—	8°	-5°
18	Pliocene	U.S.S.R.	Brown and beds	2°	+12°
19	Invalid				
20	Quaternary	U.S.S.R.	—	11°	+5°
21	Pleistocene	Sweden	Varves	1°	+5°
22	Recent	Sweden	"	7°	0°
23	"	U.S.A.	"	5°	+11°

Table 4

Range in declination difference ( $\Delta D$ )	Total No. of pairs (N)	No. of pairs with positive inclination difference (n)	Probability $P^*$ (per cent)	Range of $\alpha$ for which $P^* > 5$ per cent
All sediments included				
$\leq 90$	14	7	60.8	2-12
$\leq 45$	13	7	50	2-11
$\leq 25$	10	7	17.1	1-9
$\leq 5$	3	3	12.5	0-3
Varves excluded				
$\leq 90$	12	5	30.8	2-10
$\leq 45$	11	5	30	1-10
$\leq 25$	8	5	36.3	0-8
$\leq 5$	1	1	50	0-1

\*  $P$  is the probability that of  $N$  pairs of data,  $n$  pairs will have a positive inclination error purely by chance. If the hypothesis of systematic flattening of the palaeomagnetic inclination in sediments were true,  $P$  would be smaller than a few per cent (5 per cent is often accepted in statistical work).

The presence of an inclination error has been shown in laboratory experiments on varved clays<sup>2</sup>; therefore, as a test of whether such an error is present in the n.r.m. of other kinds of sediments, the pairs of data containing results from varved clays should be set aside. This leaves twelve pairs of data (see Table 4).

Another objection to van Andel and Hospers's analysis is that they ignored the magnitude of the inclination difference  $|I^*| - |I|$ . Their method is incapable of taking account of pairs for which this difference equals zero, as already noted. A better approach using the few data presently available is to find the mean value of the inclination difference and its standard deviation. Using all pairs for which  $\Delta D \leq 25^\circ$ , we find that the inclination difference =  $+2.2^\circ \pm 2.2^\circ$ , with  $N = 12$ . This is not significantly different from zero, and data from varved clays for which the presence of an inclination error is physically plausible are included. Excluding the data from varves, the inclination difference is  $+0.9^\circ \pm 2.8^\circ$  ( $N = 9$ ), which is not significantly different from zero.

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### Evidence for Palaeomagnetic Inclination Error in Sediment

THE inclination error ( $\delta$ ) in sediment is the difference between the inclination of the ambient field and that of the remanent magnetization acquired by a sediment<sup>1</sup>. The error is reckoned positive (negative) if the inclination of remanence is less (greater) than that of the field. Inclination errors in nature may be studied by comparing observed inclinations in sedimentary rocks ( $I_o$ ) with that expected ( $I_e$ ) from studies of contemporaneous isotropic igneous rocks which are not subject to the error. There are two methods. Results from nearby localities may be compared directly (method 1), or results from widely spaced localities may be compared by calculating the expected inclination ( $I_e$ ) calculated from the palaeomagnetic pole derived from observations of igneous rocks with the observed inclination ( $I_o$ ) in sediments (method 2). Method 2 assumes that the Earth's field is a geocentric dipole. Both methods assume that the secular variation is averaged out so that only averages for formations covering many tens of thousands of years may be used. There are errors ( $\alpha$ ) in determining  $I_e$  and  $I_o$  so the inclination error is itself subject to error. Because of the statistical inhomogeneity of the palaeomagnetic data a formal calculation of the error in  $\delta$  would be unrealistic,

but a lower limit is set by the  $\alpha$  values. In simple observations of fossil remanence  $\alpha$  is usually about  $10^\circ$ , whereas after the application of magnetic or thermal cleaning techniques accuracies of about  $5^\circ$  can sometimes be achieved.

Van Andel and Hospers<sup>2</sup> have compiled estimates of  $\delta$  using method 2. For the most part they used "old" observations without magnetic or thermal cleaning. They apply a "sign" test to their data which indicates a systematic positive bias. An alternative procedure is to sum their  $\delta$  values and study their scatter. Nineteen values calculated by van Andel and Hospers have a mean of  $+2^\circ$ , the standard deviation of the individual values is  $10^\circ$ , and the error in the mean (95 per cent) is  $5^\circ$ . (The first entry in their tabulation is omitted because of the very large uncertainty in restoring the igneous rocks in question to their original attitude.) Van Andel and Hospers reject two of their results in which the differences between declinations of igneous and sedimentary rocks are large. Omitting them from the calculations, the mean now becomes  $+3^\circ$ , the standard deviation  $10^\circ$ , and the error  $5^\circ$ . The mean inclination error does not depart significantly from zero, and the scatter in the observations is accounted for by random errors in estimating  $\delta$ .

Table 1. INCLINATION ERRORS IN SEDIMENTS

	$I_e$	$I_o$	$\delta$
Late Carboniferous and Permian of Australia <sup>3,4</sup>	+81	+80	+1
Triassic of Australia <sup>5,6</sup>	-75	-82	-7
Triassic of Africa <sup>7,8</sup>	-47	-56	-9
Triassic of North America (Newark Series) <sup>9</sup>	+26	+24	+2
Core V16-57 (normal) <sup>10</sup>	-63	-65	-2
Core V16-57 (reversed) <sup>10</sup>	+63	+63	0
Core V16-134 (normal) <sup>10</sup>	-75	-71	+4
Core V16-134 (reversed) <sup>10</sup>	+75	+71	+4
Core V20-107 <sup>11</sup>	+62	+60	+2
Core V20-108 <sup>11</sup>	+64	+66	+2
Core V20-109 <sup>11</sup>	+65	+62	+3

A closer look at the problem may be made from data based on demagnetization studies (Table 1), many of which have been published after the sources quoted by van Andel and Hospers<sup>2</sup>. There are four results (method 1) from Palaeozoic and Mesozoic rocks (red beds and varves) with a mean  $\delta$  of  $-3^\circ$ . There are seven results (method 2) from deep ocean sediments which cover the past  $2 \times 10^6$  yr. The V16 data are separated into normal and reversed polarities. In the V20 data the inclinations are lumped together irrespective of sign. They may be compared with the inclination expected from an axial geocentric dipole field which is the approximate field configuration observed in igneous rocks of this age; their mean  $\delta$  is  $+1^\circ$ . The individual estimates are distributed randomly about zero and differ from it by amounts comparable with the error in estimation.

It is therefore concluded that the analysis of van Andel and Hospers does not demonstrate a systematic inclination error in sediments as they claim it did. Moreover, results based on demagnetization work, and on which, as a consequence, most reliance can be placed, show no systematic error.

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## Thickness and Viscosity of Etnean Lavas

THE thickness of a lava flow depends, in general, on three factors: the viscosity of the flowing lava; the angle of slope of the surface over which the lava is flowing; the local topography, that is, whether the lava is ponded in a depression or not. The thickness is seldom recorded by volcanologists, and even more seldom used as a tool (for example, in the interpretation of old volcanoes in which, owing to subsequent folding or tilting, the present attitude of the lavas may differ from the original attitude). No systematic study appears to have been made of the thickness and its relationship to the viscosity and angle of slope, and this communication, which records the results of a brief study recently made of the lavas of the Italian volcano, Etna, is a first attempt to explore this inter-relationship.

Etna was chosen for this study for the reasons that there are extensive spreads of young lavas on its slopes; these lavas are rather uniform in chemical composition (they are basaltic, near hawaiite: Rittmann, A., personal communication) and, it is supposed, in initial temperature and viscosity; eruptions take place quite frequently during which the viscosity can be determined; a great range of angles of slope is found, from  $0^\circ$  to  $>40^\circ$ ; there are numerous small quarries, road cuttings, and other sections in which the thickness of the young lavas may be measured. When the investigation was made, the 1966 eruption was still in progress, which allowed the viscosity of the lava to be determined.

There are certain difficulties in the measurement of a meaningful thickness of a lava flow. One is that most flows on Etna are compound, made of a number (sometimes a very large number) of separate flow-units; in this study the thickness of individual flow-units has been measured and recorded. Another difficulty is that lava channels which have been partially drained out are common on slopes steeper than about  $10^\circ$ , and the floors of such channels are much thinner than the levées on either side. For none of the eighty flow-units measured in this study is there any reason to suspect ponding of a lava in a topographic depression. All the measurements are on lavas on the southern and eastern slopes of Etna, nearly all of which are of aa type, and all are on young flows, most of which are historic lavas.

The graph in Fig. 1 shows the relationship between thickness and angle of slope. It can be seen that only on gentle slopes of less than  $7^\circ$  do lava flows more than 10 m thick develop, while no flow exceeding 5 m thick is found on a slope steeper than  $15^\circ$ . The considerable scatter of points on the graph, greatest at the low angle end, is probably a result of variations in viscosity. The viscosity of a flowing lava is least when it emerges from the vent: cooling, partial crystallization and loss of gases very quickly increase the viscosity and the resulting lava flow is correspondingly thicker.

The 1966 eruption of Etna, which began in mid-January, was still in progress in late May when this study was made. Lava was found to be flowing quietly from about five boccas (openings), and for the first few tens of metres below a bocca the lava typically flowed in a narrow stream 1-2 m wide between levées raised 1-1.5 m above the general level (Fig. 2). The angle of slope and velocity of flow were measured on eight different lava streams (see Table 1) and the viscosity derived from the formula

$$\eta = \frac{g \rho h^2 \sin \alpha}{3V}$$

where  $\eta$  = the viscosity in c.g.s. units (poises);  $g$  = 980.6 cm/sec<sup>2</sup>;  $\rho$  = density of flowing lava, taken as 2.0 g/c.c.;  $h$  = thickness of flow, in cm;  $\alpha$  = angle of slope, in degrees;  $V$  = velocity of flow, in cm/sec.

For the narrow streams just below the boccas, where the width of the stream is comparable with the thickness, and internal friction becomes significant, the formula



$$\eta = \frac{g \rho h^2 \sin \alpha}{4V}$$

is more appropriate (ref. 1).

The lowest values for viscosity were given by a pahoehoe flow which resulted when one of the levées of a lava stream a few metres below a *bocca* suddenly collapsed. The measurements were made hurriedly, but the value for viscosity that they give ( $0.4 \times 10^5$  poises) is believed to be more realistic than the  $0.7$  to  $0.8 \times 10^5$  poises given by the same lava stream where flowing between levées, in which the effect of internal friction by the walls may be greater than has been allowed for by the formula. In any event, the values recorded in Table 1 can be relied on only to give the correct order of magnitude of the viscosity.

Traced down the flow, the viscosity of a lava stream increases rather rapidly, and the highest value ( $1.5 \times 10^7$  poises) was measured on a flow-front about 500 m below the *bocca*. The viscosity at which flow virtually ceases (a flow rate of  $10^{-3}$  cm/sec, that is, rather less than 1 m/day, has been chosen) is plotted on the inset to Fig. 1 for different thicknesses and angles of slope, and lies between  $10^9$  and  $10^{11}$  for most Etnean lavas.

Three observations which have a bearing on the structure and thickness of lava flows were made on the 1966 lava field. One concerns the life and size of a flow-unit. Most of the *boccas* from which lava was flowing were

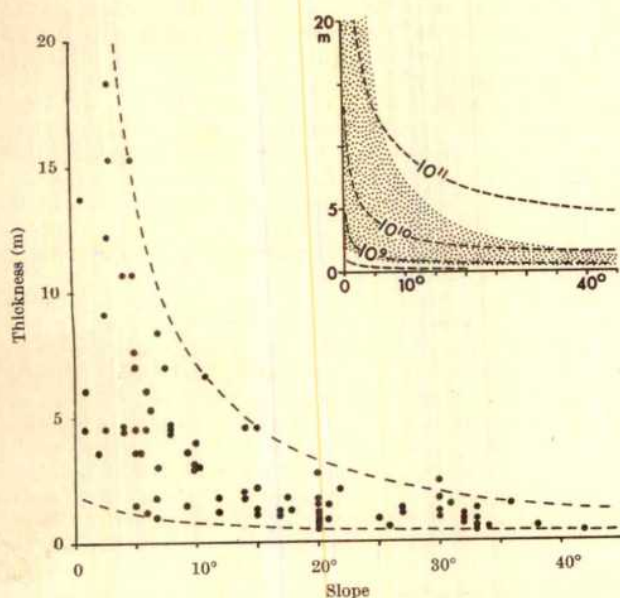


Fig. 1. The relationship between the thickness, and the angle of slope, of young basaltic lavas on Etna. Inset shows the viscosity in poises for which the rate of flow of the lava is  $10^{-3}$  cm/sec.

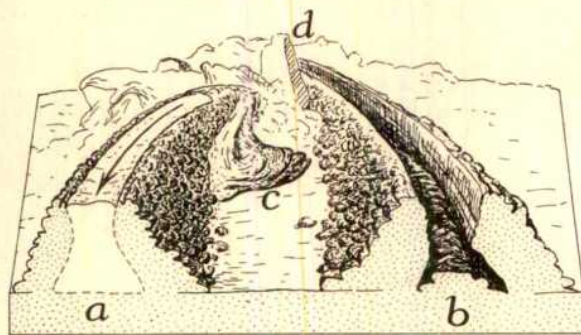


Fig. 2. Block diagram illustrating features on the 1966 Etna lava field. *a*, Lava stream in which the lava is flowing between levées standing about 1.5 m above the general level; *b*, abandoned, and largely drained-out, lava channel; *c*, pahoehoe flow-unit, too thin to be viable; *d*, older lava upraised at the time that the *boccas* feeding lava streams *a* and *b* were formed.

Table 1. VISCOSITY OF ETNA LAVA, MAY 1966

No. of lava stream	Approximate distance from <i>bocca</i> (m)	Timed distance (m)	Velocity $V$ (cm/sec)	Approximate thickness of flow $h$ (cm)	Angle of slope $\alpha$ (degrees)	Viscosity $\eta \times 10^{-4}$ (poises)
1	5	4.8	44	150	19	0.8*
1	10	3.3	47	150	28	1.1*
1	20	10.5	23	200	11	1.6*
2	2	6	6.7	120	14	2.6*
5	5	1	25	60	35	0.4*
7	2	3	11	120	8	0.9*
7	6	5	6.5	120	15	2.8*
8	5	3	20	120	12	0.7*
8	7	4	20	130	11	0.8*
8	1.5	3	21.5	120	12	0.7*
8a†	6	5	5.3 FF	30	20	0.4
8a†	10	4	6.1 FF	30	20	0.3
8a†	10	5	12.5	60	15	0.5
3	>50	1	1.7 FF	60	16	3.8
7	100	7	0.37 FF	70	22	3.2
8	100	4	11	120	10	1.5
4	>100	4	7	150	8	2.9
6	50	3	14.3	150	16	2.8
6	500	15	0.55 FF	150	33	150

\* Lava flowing in narrow channel (between levées), for which a different formula is used (see text).

† Pahoehoe flow resulting from burst levée.

FF, Flow front; otherwise all measurements are of the movement of the top surface of lava streams.

Flow 1 measured on May 22; 2-5 on May 24; 6 on May 27; and 7-8 on May 29, 1966.

secondary, being fed by lava tunnels the course of which could be traced by fumaroles at the surface. The *boccas* proved to be very impersistent. As one ceased to erupt lava, it was replaced by the appearance of another. No *bocca* erupting on the first day of observation was still erupting seven days later. Not only did the location of active *boccas* change, but also the lava streams from each *bocca* changed in position. During the period of observation, several streams were seen to be born, and as many were seen to die by ceasing to flow. It was estimated that about five substantial flow units, each with a volume of the order of  $10^3$  to  $10^5$  m<sup>3</sup>, were added to the lava field per day (the total rate of production of lava being about 1 m<sup>3</sup>/sec), plus a considerable number of smaller units. Thus the 1966 lava field must be built up of hundreds or even thousands of flow-units. Such compound flows are so common on basaltic volcanoes that they should be regarded as characteristic of basaltic eruptions.

A second observation was that there are certain minimum dimensions, best termed "minimum viable dimensions", for a lava stream that is able to flow for a considerable time (a day or more) and to reach a considerable distance from the *bocca*, as distinct from a stream that flows for only a short time and reaches only a short distance from the *bocca* before congealing. The minimum viable thickness on a slope of  $10^\circ$ - $20^\circ$  proves to be about 60 cm for the 1966 Etna lava, and the minimum cross-sectional area of a viable lava stream on the same slope about 1.2 m<sup>2</sup>. These minimum viable dimensions are for lava of viscosity close to  $4 \times 10^4$  poises; the viable dimensions are likely to be smaller for less viscous lavas, such as the basaltic lavas of Hawaii, and greater for more viscous lavas, such as the andesitic and rhyolitic lavas of other volcanoes. The minimum viable thickness for Etnean lavas on different slopes is probably given by the lower curve of Fig. 1 (assuming that none of the lava flow-units measured for the construction of Fig. 1 were impersistent, non-viable units, thinner than the minimum viable thickness).

A third observation was that a ropy, pahoehoe-type, lava surface was preserved only on flows within a few tens of metres of a *bocca*. The data in Table 1 suggest that the corresponding limiting viscosity is about  $2 \times 10^5$  poises; with higher viscosity, a surface of aa type developed instead.

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## THE SOLID STATE

## Formation of "Hollow Whiskers" from Metals by Reaction with Ferricyanide and Ferrocyanide

WHILE devising a microscopic test for the detection of small particles of metallic iron it was discovered that blue whisker-like structures (Fig. 1) were formed from iron particles or iron alloys in a 10 per cent (w/w) solution of acidified (0.06 N hydrochloric acid) potassium ferricyanide. Further microscopic examination of these structures revealed them to be hollow tubes. Considerable branching of these tubules was also evident, giving a general appearance not unlike that of fungal mycelium. Growth of these tubules proceeds at the tip, and the rate of growth, the diameter of the tubules, the amount of branching and shape of the tubules appear to be directly related to the concentration of ferricyanide and the type of iron alloy. With powdered iron, for example, in a 10 per cent concentration of acidic ferricyanide, the tubules vary from about 4 to 10  $\mu$  in diameter. They may grow outward from the iron particles to a distance of from 0.1 to 0.5 mm or longer within a second. In less than 30 sec most of the tubules have formed, many of them bent or looped, or both. At a ferricyanide concentration of 5 per cent (w/w) the tubules are more variable, larger in diameter, and grow much longer. At concentrations of 2-2.5 per cent the tubules grow more slowly, some of them forming a blue precipitate at the end which slowly drifts away giving the appearance of smoke coming out from a lengthening smokestack. At concentrations of 20 per cent (w/w) and higher, a few tubules may be seen but mostly blue hemispherical and spherical structures are formed on the surface of the iron. If the solution is then diluted, tubules may be seen to arise from these structures. The tubules formed from acidic ferrocyanide and iron appear to be lighter blue or green in colour.

Among the metals examined so far, tubules have been formed from zinc, cadmium, nickel, cobalt, lead, tin, chromium and copper with either acidic ferricyanide, ferrocyanide or both. The tubules from chromium and cobalt may often appear red or pink in colour while those from copper appear reddish brown with ferricyanide. In general, with the exception of cadmium and iron, the tubules are quite contorted and short (< 0.2 mm). Since copper is on the noble side of hydrogen in the electromotive series, it is necessary to make it anodic by means of an applied voltage, before the tubules appear.

Preliminary studies with some alloys in the form of wires have indicated that the tubules generally appear

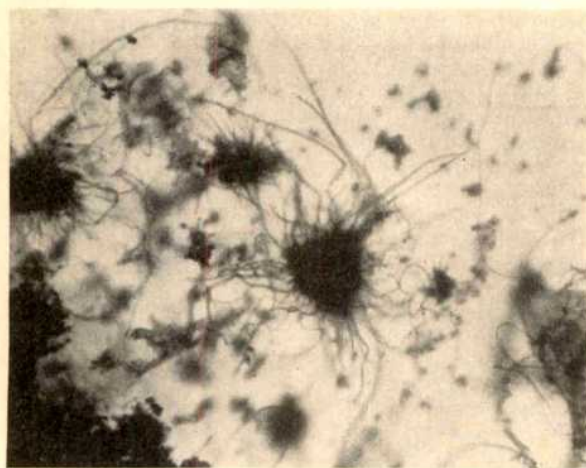


Fig. 1. Production of tubules from a particle of iron powder in an acidified (0.06 N hydrochloric acid) solution of 10 per cent potassium ferricyanide solution. ( $\times c. 135$ .)

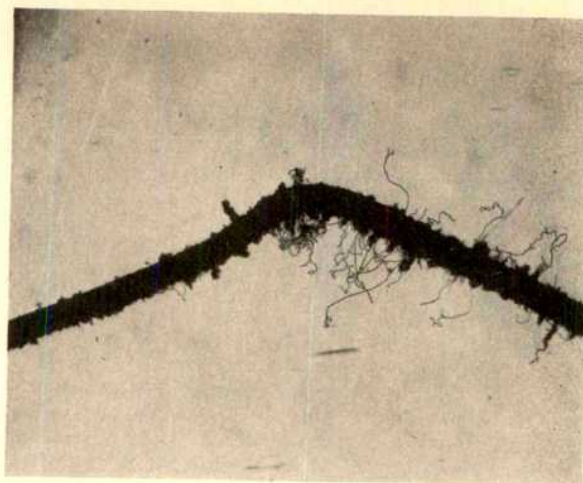


Fig. 2. Tubules formed from bend in nichrome wire in acidified 10 per cent potassium ferricyanide solution. ( $\times 10$ .)

to arise from portions of the metal which have been stressed by bending (Fig. 2). The various metallic components of the alloys, providing they react with the ferricyanide or ferrocyanide to form an insoluble complex, appear to produce their own distinct tubules as indicated by a difference in morphology and colour (Fig. 3). The concentration of reagents necessary for producing tubules appears to vary somewhat for each metal and for the same metal in different alloys.

In general, "hollow whiskers" or tubules seem to be produced by any metal which forms an insoluble complex with ferri- or ferro-cyanide. The following tentative mechanism for the formation of these tubules is suggested. When the acidified solution of ferricyanide comes in contact with the metal, the electrons from the metal,  $M \rightarrow M^{++} + 2e$ , react with the hydrogen ions to form atomic or molecular hydrogen at the surface,  $2e + 2H^+ \rightarrow 2H \rightarrow H_2$ . Removal of the electrons causes the metal to pass into the ionic form (the reaction is displaced to the right). The same results may be obtained (removal of the electrons) in a neutral solution of ferricyanide if the metal is made anodic by an applied potential. The areas where the metal forms ions may be called point anodes. When the metal ions first come into contact with the ferricyanide they react to form an insoluble complex and a hemispherical dome may be first formed. If the concentration of ferricyanide is sufficiently high, such domes are produced and indicate a membrane of the metal ferricyanide complex which is very "tough" and which resists the pressure of the ions coming from the point anode. If the concentration of ferricyanide is low the pressure of the ions is sufficiently great to overcome the lower membrane strength of the complex and the ions break through, leading to the formation of a hollow cylinder. More metal ions flow along the tubules to form a metal ferricyanide complex on coming into contact with the ferricyanide at the tip. The end result appears to be a series of metal ion tracks which originate from the many point anodes along the metal surface.

Such "hollow whiskers" but of greater size (0.2-0.4 mm in diameter and up to 90 mm in length) were observed in 1958 (ref. 1) from iron in rapidly flowing warm water (55° C) containing calcium bicarbonate in an amount insufficient for complete corrosion. Apart from the fascination of these "bio-like" structures, there appear to be many practical applications of this phenomenon which immediately suggest themselves. In metallurgy, it may provide a tool for quick determination, qualitative and possibly quantitative, of various elements in an alloy. By applying an increasing positive potential to the alloy, metal ions may be "pulled out" in succession in the characteristic form of their ion tracks or tubules. It may



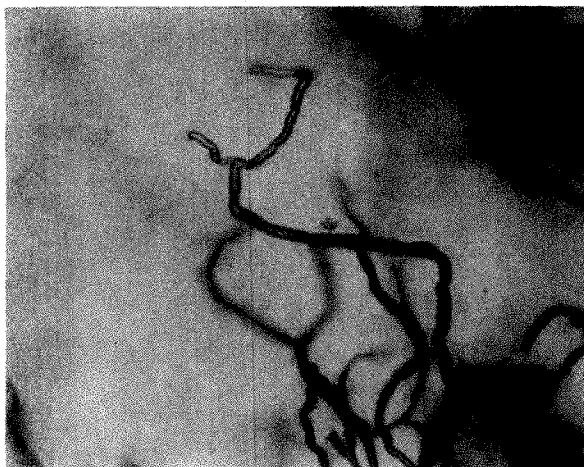


Fig. 3. End of tubules formed in acidified 10 per cent potassium ferri-cyanide solution. Tips are pink or light red, presumably due to a chromium ferri-cyanide complex, while remainder of tubule is blue-green due to the ferrous ferri-cyanide complex. ( $\times c. 135$ .)

also afford a valuable tool for the study of stress and stress cracking corrosion since the tubules appear to arise most easily at stressed points. A quick method for the determination of corrosion resistance of alloys may be afforded as well as a quick screening method for corrosion inhibitors. The voltage required for cathodic protection might also be rapidly determined by observing the potential at which whisker formation is prevented.

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## PHYSICS

### Extinct Radioactivity and the Discovery of a New Pleochroic Halo

BECAUSE of the cosmological implications of such a discovery<sup>1</sup>, considerable effort has been expended toward the detection of extinct radioactivity in crustal rocks and meteorites. An excess of fissionogenic xenon isotopes in the Fayetteville meteorite<sup>2</sup> and the presence of excess fission tracks in the Toluca meteorite<sup>3</sup> have in each case been considered possible evidence of spontaneous fission from the extinct nuclide plutonium-244. Although an earlier search for the neptunium series in nature failed to reveal its existence<sup>4</sup>, later results suggest that it may exist<sup>5</sup>.

The investigation of pleochroic haloes (minute regions of structural disorder which occur in various minerals as a result of prolonged alpha emission from microscopic radioactive inclusions) is usually related to the determination of the age of a mineral or to the question of the invariance of the decay constant over geological time<sup>6,7</sup>. Henderson also discussed extinct  $\alpha$  radioactivity relative to this phenomenon<sup>8</sup>, and haloes were found which could be attributed to various polonium isotopes. Previously, it was assumed that all polonium haloes originated from radioactive hydrothermal solutions containing equilibrium amounts of decay products from the uranium-238 series<sup>9</sup> and thus did not constitute evidence of extinct radioactivity from the cosmological point of view. A recent study of polonium haloes indicates this conclusion may not be valid<sup>10</sup>.

The purpose of this communication is to report the discovery of a new type (subsequently designated as type Y) of pleochroic halo which was found in Canadian Pre-Cambrian biotite. Two haloes of Y type have been found

in close proximity (Fig. 1), and the halo radii are about  $26.5 \pm 0.5\mu$  assuming a negligible correction for the size of the central inclusion. Combining previous measurements of the radii of haloes from uranium-238 and thorium-232<sup>11,12</sup> with my own results, a fairly accurate range-energy curve (Fig. 2) may be established for this mica. From Fig. 2 it is found that the Y haloes were produced by  $\alpha$  particles of approximately  $6.55 \pm 0.05$  MeV. These Y haloes exhibit a rather light coloration, thus implying that only a relatively small amount of  $\alpha$  activity existed originally in the central inclusion or that the parent nuclide decayed partially by  $\beta$  emission.

In attempting to ascertain the parent nuclide of the Y haloes it is natural to try to correlate the ring structure with that of the well known uranium or thorium haloes. In each case the progenitors of these haloes (that is, uranium-238 and thorium-232), however, are low energy  $\alpha$  emitters giving rise to small inner rings which are not present in the Y haloes. Furthermore, the development of the outer rings of the uranium and thorium haloes follows  $\alpha$  particle emission from the respective daughter products. Uranium and thorium haloes (Figs. 3 and 4) have been observed in the embryonic stage (where only the first ring is visible), the intermediate stage (where nearly all rings are visible), and the overexposed stage (where the inner rings are too dark to be discerned and only the outer rings are plainly visible). In none of these cases do the uranium or thorium haloes resemble the Y haloes, either with respect to halo radii or ring struc-

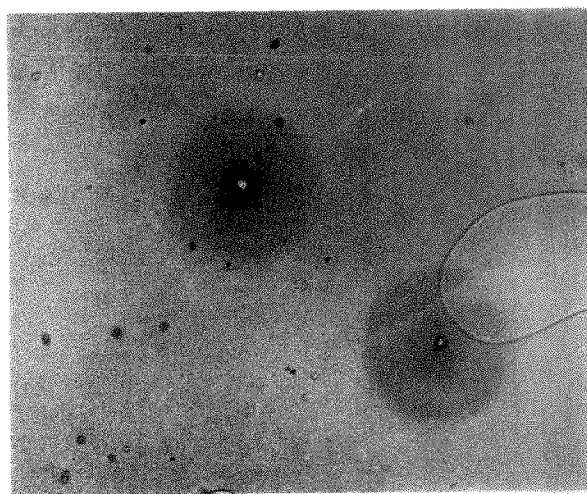


Fig. 1. Type Y halo ( $\times 375$ ).

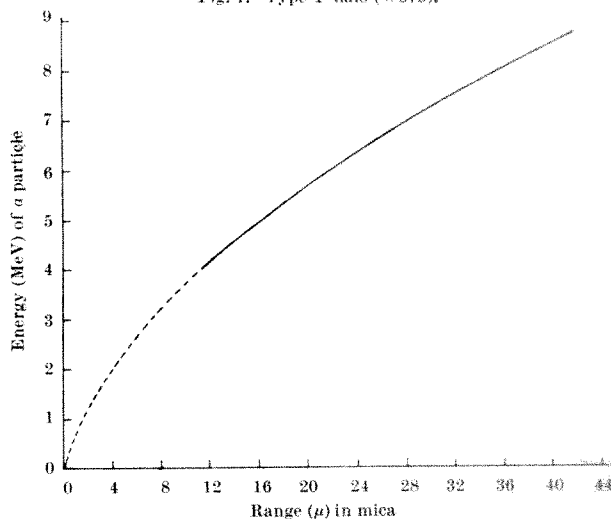


Fig. 2. Range-energy curves for Canadian mica. —, Experimental; ----, extrapolated.

ture. Also, by using the HF technique of etching with hydrofluoric acid for detecting fission fragment tracks in mica<sup>13</sup>, it can be shown that the central inclusions of the Y haloes do not contain any significant amounts of spontaneous fissionable nuclides such as uranium-238. An acid etch of this mica revealed no fission tracks which emanated from the central inclusions of the Y haloes whereas a cluster of fission tracks is always present in the uranium-238 haloes (Fig. 5).

The chart of the nuclides reveals a few  $\alpha$  emitters of approximately the required energy in the trans-uranium region (for example, einsteinium-252) and several in the trans-lead region (for example, radium-222, francium-211, polonium-217, bismuth-211). The emitters in the trans-uranium region may be ruled out for the same reason as were uranium-238 and thorium-232, namely, any halo which develops from the trans-uranium nuclides would exhibit many rings because of subsequent daughter  $\alpha$  decay. Some  $\alpha$  emitters in the trans-lead region may be excluded for the same reasons. Although it is not possible to identify the parent nuclide of the Y haloes unequivocally, it is felt that bismuth-211 represents a reasonable choice because of the  $\alpha$  energy of bismuth-211 and the fact that no other rings are to be expected from subsequent decay. If these haloes are caused by the  $\alpha$  decay of bismuth-211, they represent an actinium series analogue to the polonium haloes of the uranium-238 series. Polonium haloes from polonium-210 (Fig. 6), polonium-214 and polonium-218 have been found<sup>19</sup> independent of

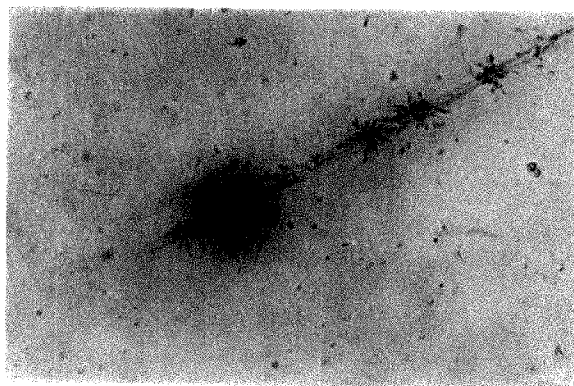


Fig. 5. Fission tracks from embryonic uranium halo.

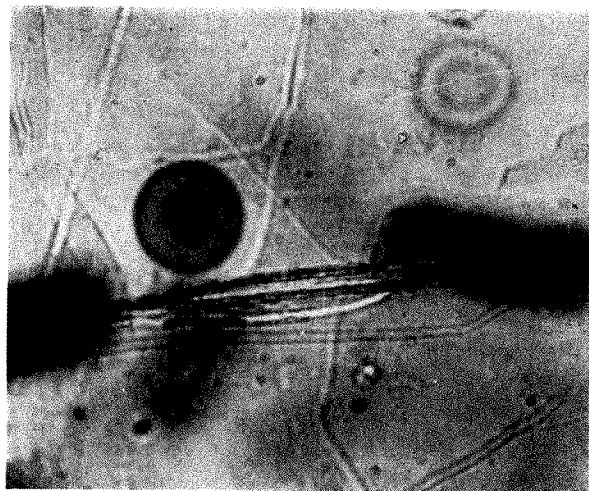


Fig. 6. Polonium-210 halo near conduit ( $\times 375$ ).  $\pi = 18.6\mu$ .



Fig. 3. Uranium halo ( $\times 375$ ).

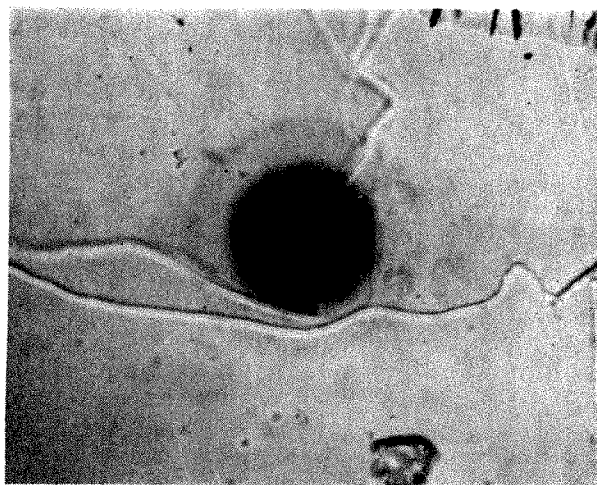


Fig. 4. Thorium halo ( $\times 375$ ).

uranium or thorium haloes, and in one case a ring from bismuth-212 occurs in a polonium halo. As was the case for the Y haloes, an acid etch of the polonium haloes revealed the absence of fission tracks from the central inclusions. To obviate the possibility that some metamorphic heating event had annealed the fission tracks in the mica, polonium haloes and uranium haloes were irradiated with a thermal neutron flux of  $10^{17}$  neutrons/cm<sup>2</sup>. Subsequent etching of the mica revealed a vastly increased number of fission tracks in the uranium haloes and no tracks whatsoever from the polonium haloes.

What relation, if any, these new haloes bear to the recently reported giant pleochroic haloes<sup>14</sup> is not known at this time. There seems to be little doubt, however, that the  $\alpha$  radioactivity responsible for the formation of the giant haloes and the Y haloes corroborates the evidence for extinct radioactivity of short half-life previously found in polonium haloes<sup>10</sup>. Further evidence of  $\alpha$  radioactivity of unknown origin has now been found in the black Ytterby mica<sup>15</sup>. It is difficult to reconcile these various types of extinct radioactivity with the current theories of nucleosynthesis and crustal formation<sup>16,17</sup>. The recent discovery of haloes<sup>18</sup> in the Wolsendorf fluorite (due to the sequence of emitters francium-219, astatine-215 and bismuth-211) further substantiates the identification of the Y halo with bismuth-211.

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### Thermosolutal Convection: Observation of an Overstable Mode

THE character of small amplitude convection in a fluid which has been subjected to a stabilizing gradient of some solute and a destabilizing temperature gradient has been calculated by Gershuni and Zhukhovitskii<sup>1</sup>, Veronis<sup>2</sup> and Sani<sup>3</sup>. Gershuni and Zhukhovitskii considered an infinite vertical layer of fluid, in which the flow varied in only one (horizontal) direction, while the other authors considered a horizontal layer in which flow was two-dimensional. All these treatments show that convection can begin either as a steady motion or as infinitesimal oscillations, depending on the conditions. In the case of overstability, Sani predicts that the amplitude of oscillations would be very small, while Veronis suggests that they would readily lead to a steady flow.

The treatment of Gershuni and Zhukhovitskii is physically so unrealistic that the results are primarily of qualitative interest. Experimental verification of the calculations of Veronis and Sani is also hampered, though to a lesser extent, because both authors assume ideal conditions of hydrodynamic freedom, with temperature and concentration constancy, at both horizontal boundaries. Nevertheless, the physical cause of overstability in this system is that the heat and the solute have different diffusivities, so that a change of boundary conditions is unlikely to affect its behaviour qualitatively.

The theoretical treatments assume that the undisturbed temperature and concentration gradients are constant throughout the fluid. Although it is possible to come quite close to this thermal condition in experiments, the establishment of a steady diffusive flux of solute is difficult because of the impermeability of most materials of which the upper and lower boundaries might be constructed. Nevertheless, the slowness of the diffusion process allows the establishment of transient temperature and concentration profiles which are markedly non-linear and yet sufficiently static for experiments to be performed. Turner and Stommel<sup>4</sup> have described experiments in which a tank of water containing salt in a solution in which the concentration increased downwards was heated from below, resulting in the formation of convecting layers. Because the boundaries of the liquid were impermeable to salt, the concentration profile was a transient, non-linear one. Although few details were given, it seems likely that the temperature profile was also non-linear owing to transient heating and perhaps to heat loss from the side walls.

In an investigation of the onset of convection in the first, that is the bottom layer in a system similar to that of Turner and Stommel, an oscillatory effect has been observed which is presumably the overstability predicted by the theories mentioned above.

The liquid used was water, with sugar as the solute. Two thermocouples were formed at the junctions of fine horizontal copper and Constantan wires, near the centre and respectively 0.5 and 1.0 cm above the bottom of the liquid, which was 9.7 cm deep. The differential output of these thermocouples was amplified and displayed on a strip chart recorder. As heating progressed the temperature difference increased, and the record was kept on scale by adding progressively larger microvoltages from a Tinsley vernier potentiometer in series with the thermocouples.

The records obtained in such an experiment are shown in Figs. 1 and 2. Fig. 1 is a graph of concentration gradient versus depth, measured by a scanning schlieren method before heating began. The gradient of this graph, that is the second derivative of concentration with respect to depth, is nowhere sufficient to produce a significant change in solute distribution by diffusion during the experiment, so that Fig. 1 represents closely the solute distribution at marginal stability. Fig. 2 shows the temperature record near marginal stability, the temperature gradient indicated at this stage being approximately 2° C cm<sup>-1</sup> near the bottom of the liquid. The heating rate was increased slightly at the point marked A, while at that marked B the sensitivity of the recorder was reduced by a factor of 50. As marginal stability approached, the record became increasingly noisy. The appearance of a definite oscillation was followed by a rapid growth of its amplitude, and after about 12 min it became increasingly disordered. About the time that the oscillation became large, that is just after B, schlieren observation showed several small cells about 0.7 cm high at the bottom of the liquid. These cells were initially separate from one another, but the number increased with time to form a fairly continuous layer. The height of the cells increased slowly to about 1.0 cm 5 min after they were first observed, at which stage they were about 1.5 cm wide at the base.

The features of this effect which can readily be checked against theory are the temperature gradient required to

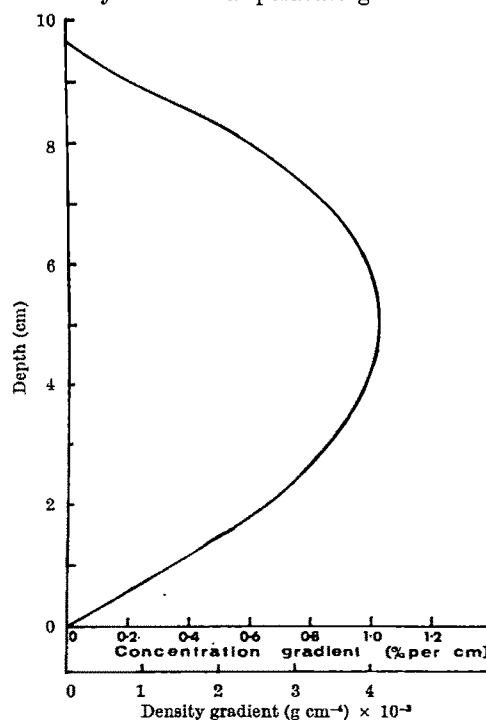


Fig. 1. Profile of the solute concentration gradient. The equivalent isothermal density gradient values are also shown.

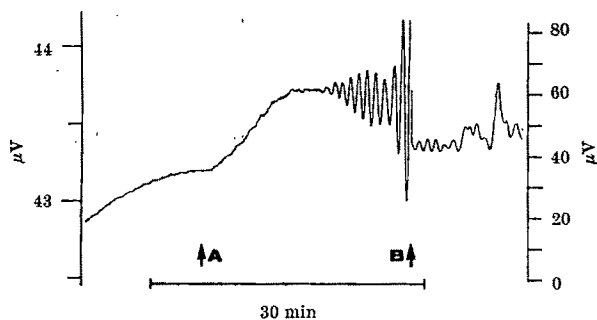


Fig. 2. Section of the temperature record near marginal stability. The quantity plotted against time is the differential output of two copper-Constantan thermocouples placed 0.5 and 1.0 cm above the tank bottom. Heating rate was increased slightly at A. Left-hand voltage scale applies up to B, right-hand scale thereafter.

produce the oscillation in the presence of the particular concentration gradient, and the period of the oscillation. Both Veronis and Sani predict that the critical condition for overstability in this case is very nearly

$$R = \frac{\nu}{K + \nu} R' + 657$$

$$\text{where } R = \frac{g \Delta \rho_i h^3}{\nu K \rho}$$

$$\text{and } R' = \frac{g \Delta \rho_c h^3}{\nu K \rho}$$

Here  $g$  = acceleration due to gravity,  $\rho$  = density,  $\Delta \rho_i$  = density difference produced thermally over the convecting depth,  $\Delta \rho_c$  = density difference caused by the concentration gradient over the convecting depth,  $h$  = convecting depth,  $\nu$  = kinematic viscosity,  $K$  = thermal diffusivity. The value 657 results from the ideal boundary conditions assumed. More realistic boundary conditions would probably raise this value.

Taking 0.7 cm as the convecting depth,  $3 \times 10^{-4} \text{ C}^\circ$  as the expansion coefficient of the solution,  $2 \text{ C}^\circ/\text{cm}$  as the temperature gradient,  $0.01 \text{ cm}^2/\text{sec}$  as the viscosity and  $0.0014 \text{ cm}^2/\text{sec}$  as the thermal diffusivity,  $R' \approx 8,200$  and  $R \approx 9,900$ . The value predicted for  $R$  is about 8,000. Since the critical values of  $R$  predicted by Veronis for the onset of an infinitesimal steady motion and the most favoured finite amplitude steady motion are about 80,000 and 1,200 respectively for this value of  $R'$ , the experimental value clearly agrees best with the value predicted for overstability.

The period predicted by both Veronis and Sani for this case is about 45 sec, which is not markedly different from the observed period of 55 sec.

*Note added in proof.* I am grateful to Dr. D. Nield of the University of Auckland for the interest he has shown in this project. He has made available to me a copy of his recent Ph.D. thesis which contains an analysis of the small-amplitude three-dimensional thermosolutal convection problem for rather general boundary conditions. The evaluation of the critical conditions for overstability in any but the ideal case mentioned above appears to be very tedious, and quantitative results applicable to the present observation are not at present available. However, for ideal boundaries, the values calculated for this case for both  $R$  and the period are the same as those given by the two-dimensional analyses mentioned above.

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## CHEMISTRY

### Adsorption of the Three Hydrogen Isotopes on Charcoal

PREVIOUS studies on hydrogen isotope adsorption on charcoal have been confined to hydrogen and deuterium<sup>1</sup> and show the variation that would be expected from the physical nature of the adsorbent. As part of a general study on isotope effects we have extended this work to include tritium.

The apparatus was of the conventional type for gas adsorption studies, with minimum free gas volumes to ensure maximum accuracy. The charcoal was "granulated activated for gas adsorption" grade supplied by Rubber Industries and Sherman Chemicals, Ltd., and the hydrogen isotopes were 99.95 per cent pure  $\text{H}_2$ , 99.0 per cent  $\text{D}_2$ , 98.4 per cent  $\text{T}_2$ . Protium was the only impurity in the heavier isotopes.

The charcoal samples were thoroughly outgassed *in vacuo* at  $580^\circ \text{C}$  before the start of measurement. The volumes of gas adsorbed,  $V_a$ , were calculated by PVT methods (in standard ml./g) for a series of hydrogen isotope equilibrium pressures,  $P_e$ , at liquid nitrogen temperature. Plots of  $P_e/V_a$  against  $P_e$  for all three isotopes are good straight lines at pressures greater than 100 mm or so, as shown in Fig. 1, indicating that the adsorption follows the Langmuir isotherm<sup>2</sup>, for which

$$P_e/V_a = 1/V_m b + P_e/V_m \quad (1)$$

The weighted means for the slopes,  $s$ , and intercepts,  $i$ , of curves like Fig. 1 are summarized with their standard deviations in Table 1, together with calculated values for  $V_m$ , the volume of gas adsorbed per g of charcoal for monolayer coverage.

The values of  $V_m$  decrease in the sequence  $\text{T}_2 > \text{D}_2 > \text{H}_2$  and are  $\sim 120$  standard ml./g. The parameter  $b$  represents the ratio of the number of molecules which strike and stick to the surface per unit area and pressure to the number of adsorbed molecules which escape per unit area from a saturated surface; the values are identical within the experimental error at  $0.012 \text{ (mm Hg)}^{-1}$ . The isotope effect on adsorption appears to arise principally because of changes in monolayer volume ( $V_m$ ) rather than the adsorption-desorption energetics ( $b$ ). This is unexpected, for  $V_m$  should be a function of molecular size, which is almost identical for the three isotopic molecules, whereas molecular attraction forces and isotopic masses should affect  $b$ , which is given by

$$b = \frac{\alpha e^{q/kT}}{k_0 (2\pi m kT)^{0.5}} \quad (2)$$

where  $\alpha$  is the sticking coefficient,  $q$  the heat of adsorption and  $k_0$  the temperature independent factor for the desorption process (see ref. 2, page 64). The slightly larger heat of adsorption for the heavier isotopes ( $q_{\text{D}} - q_{\text{H}} = 180 \text{ cal/}$

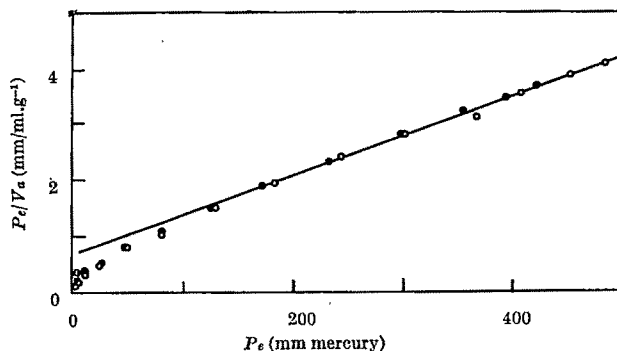


Fig. 1. Langmuir isotherm for tritium at  $77^\circ \text{K}$ . Vertical axis ( $P_e/V_a$ ) in units of mm Hg/standard ml. per g; horizontal axis ( $P_e$ ) in mm Hg. ●, Run 10; ○, run 11.

Table 1. ADSORPTION ISOTHERM AT 77° K

Isotope	$s \times 10^3$	$i$	$b \times 10^3$ (mm Hg) <sup>-1</sup>	$V_m$ std. ml./g
H	9.439 ± 0.07	0.7434 ± 0.0342	1.270 ± 0.059	106.0 ± 0.8
D	8.385 ± 0.019	0.7230 ± 0.0058	1.160 ± 0.010	119.3 ± 0.3
T	7.380 ± 0.043	0.6060 ± 0.0147	1.218 ± 0.030	135.8 ± 0.8

mole, ref. 2) is partly balanced by the mass effect. The  $\alpha$  and  $k_0$  terms contain entropy factors which may vary with isotopic mass though the magnitude of the variation is not calculable.

The isotope effect on  $V_m$  and  $b$  may be explained if more energetic (and therefore less favourable) adsorption sites are available to the heavier molecules because of their higher heats of adsorption. This would not only result in the observed  $V_m$  sequence but would reduce the average heat of adsorption for the heavier isotopes, thus minimizing the effect of the energy term in equation (2).

The isotope effect on adsorption found in this work is comparable with that of 8 per cent found by Van Dingenen<sup>1</sup>.

A high pressure experiment using hydrogen at 1.5° C also conformed with the Langmuir isotherm with  $b = 1.71 \times 10^{-5}$  (mm Hg)<sup>-1</sup> and  $V_m = 35$  standard ml./g. The heat of adsorption for hydrogen on charcoal, calculated from equation (2) with the assumption that  $\alpha$  is independent of temperature, is 1.3 kcal/mole, in excellent agreement with the values of 1.0–1.8 kcal/mole found by Van Dingenen<sup>1</sup>.

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### Step Increase of the Carrier Gas Inlet Pressure in Gas Chromatography

UNTIL now pressure variation has been treated with great care in gas chromatography. Most experiments are carried out in equilibrium conditions, with more or less carefully controlled inlet pressure, and recently interest has been focused on the use of pressure programming<sup>1,2</sup>. In spite of the development of an exponential pressure programmer<sup>3</sup> only slow rates of pressure programming have been used. For theoretical as well as practical reasons, we suggest the use of extremely high programme rates, so that when the injection is made, the inlet pressure is increased almost instantaneously from a value near that of the outlet pressure up to the final value. Clearly, the duration of the programme will be much less than that of the analysis itself. This method has, however, distinct advantages over the more conventional ones.

A rigorous theory of programmed pressure gas chromatography has not yet been published, although some approximate results had been reported<sup>3,4</sup>. These were based on the postulate that the column is always in equilibrium conditions, that is, at a given time  $t$ , the inlet pressure  $p_i$  and outlet velocity  $u_0$  are related by the classical equation

$$u_0 = \frac{k}{2Lp_0} (p_i^2 - p_0^2) \quad (1)$$

This approximation is acceptable only for very low programme rate because there is a time delay between any inlet pressure variation and the time when the outlet flow velocity reaches the value given by equation (1). Furthermore, even at low programme rate the carrier gas mass flow rate is not constant along the column; gas accumulates in the column because at equilibrium the hold-up of carrier gas is proportional to the product of average pressure and corrected air retention volume<sup>5</sup>.

All these phenomena can be accounted for by writing the mass-balance in a differential section of the column and combining this equation with the Darcy's equation which is valid in the flow velocity range useful in gas chromatography<sup>5</sup>. The general equation

$$\frac{\partial p}{\partial t} - \frac{K}{2\eta\epsilon} \frac{\partial^2}{\partial x^2} p^2 = 0 \quad (2)$$

cannot be solved for the limit conditions in which the outlet pressure is constant and the inlet pressure variable. Distribution theory leads us to consider what happens when the inlet pressure is suddenly increased from  $p_0$  to a given value,  $p_i$ . The pressure profile in the column can be calculated at any instant after the pressure step. Numerical calculations have been made by Aronofsky<sup>6</sup>, using reduced values of column length ( $x/L$ ).

pressure ( $p/p_0$ ) and time ( $\frac{Kp_s}{\eta\epsilon L^2} t$ ). Using Darcy's law

$$u = -\frac{K}{\eta} \frac{\partial p}{\partial x} \quad (3)$$

velocity profiles can be calculated from pressure profiles (Fig. 1). The gas velocity profiles change rapidly with time. This variation is caused by the surge of gas in the empty column after the step increase in the inlet pressure and by the dampening action of column pneumatic resistance. A pressure gradient wave moves along the column. This is not a shock wave because in gas chromatographic conditions the process is isothermal and not adiabatic. This wave cannot by itself transport matter, but it causes large gas velocities near the inlet of the column for some time.

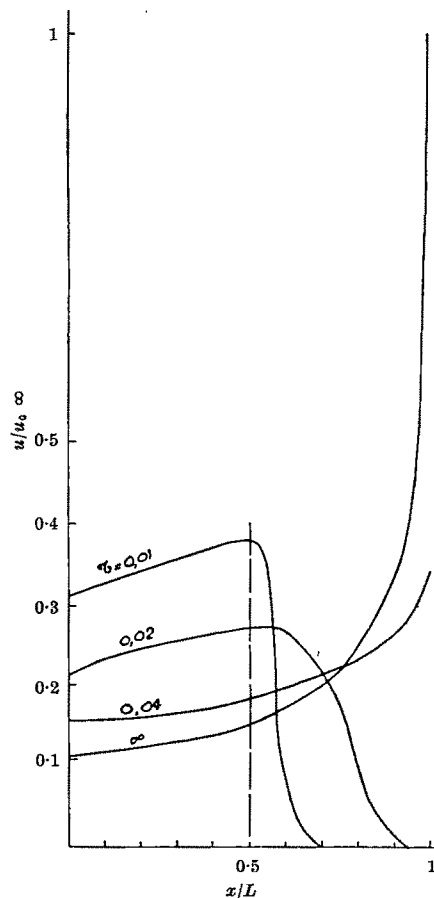


Fig. 1. Carrier gas velocity profiles in the column at various times after an instantaneous increase of the inlet pressure. Variation of the reduced velocity (ratio of the velocity  $u$  at a given time and point to the outlet velocity at infinite time  $u_0$ ) against reduced column length ( $x/L$ ) for different values of the reduced time.



Table 1. COMPARISON OF RESULTS OBTAINED AT EQUILIBRIUM AND WITH STEP INCREASE OF INLET PRESSURE

Compound	$k'$	$p_i = 6$ atm (absolute)					$p_i = 8$ atm (absolute)					$p_i = 11$ atm (absolute)				
		Equilibrium $t_R$ (min)	$t_R$ $W$	Non- equilibrium $t_R$ (min)	$t_R$ $W$	$\Delta t(\%)$ $t_{Req.}$	Equilibrium $t_R$ (min)	$t_R$ $W$	Non- equilibrium $t_R$ (min)	$t_R$ $W$	$\Delta t(\%)$ $t_{Req.}$	Equilibrium $t_R$ (min)	$t_R$ $W$	Non- equilibrium $t_R$ (min)	$t_R$ $W$	$\Delta t(\%)$ $t_{Req.}$
Methane	0	17		10.5		38	12.6		8.0		48					
Isopentane	0.24	21.8	138	15	92	30	15.7	107	9.8	72	37	11.0	100	6.0	53	45
<i>n</i> -Pentane	0.32	22.5	132	16.5	96	27	16.7	113	10.7	70	36	11.6	85	6.6	55	43
<i>n</i> -Hexane	0.61	30.8	131	22.1	82	27	22.8	110	16.5	70	28					
2,4 Dimethyl pentane	1.15						27.0	101	19.8	72	27					
Resolution $t_C/t_n C_s$		8.1		8.0			7.7		5.8			4.7		4.3		

It is remarkable that the profiles have a shape which is opposite to that of the equilibrium profiles obtained at an infinite value of time, since the concavity is in the reverse direction. In conventional slow flow rate programming, on the contrary, the pressure and velocity profiles are not very different from the equilibrium profiles.

In conventional analysis, peaks migrate much more slowly at the beginning of the column than near the outlet. The gas surge in the column which is caused by the pressure step leads to flow rates at the beginning of the column which are much higher than those encountered in equilibrium conditions. The analysis will thus be shortened.

It would be useless, of course, to shorten the analysis time if the resolution was largely decreased. From the pressure and velocity profiles a certain loss of efficiency may be anticipated. Since at the beginning of the column the velocity will be much higher and the pressure only somewhat lower than in equilibrium conditions, the height equivalent to a theoretical plate will probably be increased somewhat.

Experiments were made with an open tube column, 140 m long, 0.25 mm in diameter, at 56° C, using a flame ionization detector. Outlet pressure is atmospheric. Squalane is the stationary phase, with an average film thickness of 0.3  $\mu$ . The same mixture of linear and branched

paraffins was used in all experiments, with values of  $k'$  which vary from 0.25 to 1.15 allowing investigation of the results of this new method with compounds which have a sizable retention. The optimum flow rate for *n*-hexane corresponds to an absolute inlet pressure of about 4 atm.

Fig. 2 shows two of the chromatograms obtained during the experiments reported here. Chromatogram 2a was obtained in equilibrium conditions with a constant inlet pressure of 8 atm. absolute. Chromatogram 2b was obtained by injecting the mixture at a low inlet pressure (1.2 atm. absolute); immediately after injection the inlet pressure was raised to 8 atm. absolute. The retention times are much lower in chromatogram b than in a. The relative time reduction decreases with increasing  $k'$ , as might be predicted, but the resolution seems to be much the same in each case. Table 1 gives retention times in both experiments together with the results obtained at different values of inlet pressure and with data on relative peak thickness and time-based resolution, although we understand from Halasz and Deininger that volume-based resolution would be a more relevant parameter.

In addition to reducing the analysis time with only a small loss in resolution, this method has some other distinct advantages. Very high inlet pressure can be used without any injection problems; injection can be made with a very low differential pressure acting on the septum (0.1 atm. or less), and before increasing the pressure a metal plug can be screwed on to prevent any leak. In addition to the pressure controller only a two-way valve (such as a toggle valve) is needed, which makes experiments very easy to carry out. The detector, however, must accept a fast variation of carrier gas flow rate without any important base line drift. This is not a problem in the case of capillary columns but is a serious one for conventional packed columns.

One or several pressure steps can be made, or a step may be combined with a more conventional pressure programme. It must be emphasized, however, that the largest time reduction is obtained when the pressure variation occurs in only one step. Knowledge of the flow velocity and pressure at any time and point in the column allows peaks, retention times and base width to be calculated. This, however, requires considerable computational work and will be reported later. We thank Jean Merle D'Aubigne and Madame Monique Jacques for their help in performing the experiments.

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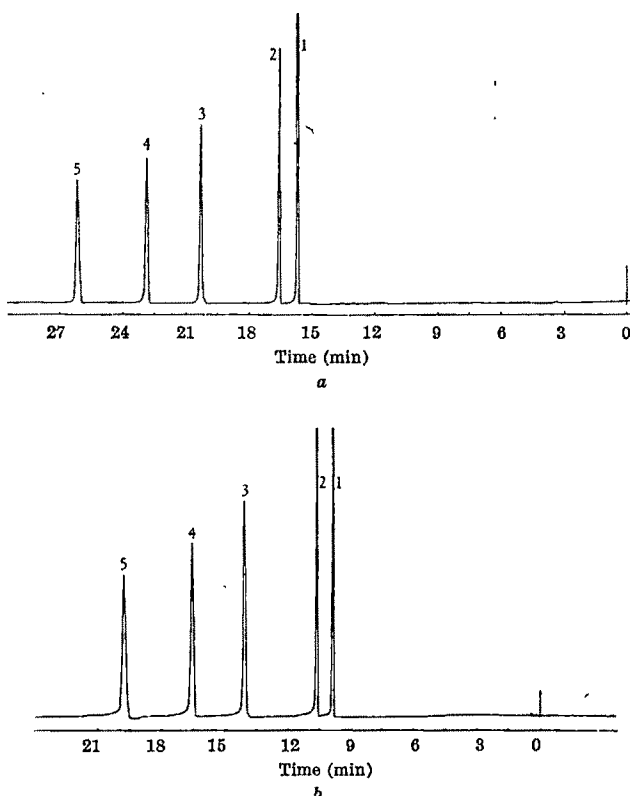


Fig. 2. Comparison of chromatograms obtained in equilibrium and non-equilibrium conditions. Column length 140 m, bore 0.25 mm, 56° C, 1 isopentane-2*n*-pentane-3-2 methyl pentane-4*n*-hexane, 5-2,4 dimethyl pentane. Inlet pressure 8 atm. absolute. Chromatogram a, equilibrium conditions; chromatogram b, instantaneous step increase of the inlet pressure to 8 atm. at the instant of sample injection.

### 5-Benzyl-3-furylmethyl Chrysanthemate: a New Potent Insecticide

FOLLOWING earlier work<sup>1</sup>, we have synthesized more non-ketonic esters of chrysanthemic acid. One new compound, 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate (I), is fifty-five times as toxic to adult female *Musca domestica* L. (house-flies) as the mixed esters of the natural pyrethrins, nearly three times as toxic as parathion and more than five times as toxic as diazinon. The same ester is ten times as toxic to adult *Phaedon cochleariae* Fab. (mustard beetles) as the natural pyrethrins and thirteen times as toxic as parathion. The (±)-*cis-trans*-chrysanthemate (m.p. 43–48°) is the most toxic compound tested so far, of any class of insecticide, to 1–2 day old unfed females of *Anopheles stephensi* and *Aedes aegypti* (results provided by Dr. A. B. Hadaway). This insecticidal activity is greater than has been found before in a compound containing only carbon, hydrogen and oxygen. By contrast, its acute toxicity to mammals seems to be small; for example, the median lethal dose to female rats for the (±)-*cis-trans*-chrysanthemate administered orally is between 600 and 800 mg/kg (personal communication from Dr. J. M. Barnes).

Table 1

Compound	<i>Musca domestica</i> L.* Relative potency†	Synergistic factor‡	<i>Phaedon cochleariae</i> Fab. Relative potency†
5-Benzyl-3-furylmethyl (+)- <i>trans</i> -chrysanthemate	250	0.98	260
5-Benzyl-3-furylmethyl (±)- <i>trans</i> -chrysanthemate	130		150
5-Benzyl-3-furylmethyl (±)- <i>cis-trans</i> -chrysanthemate	100‡	1.4	100¶
5-Benzyl-2-methyl-3-furylmethyl (±)- <i>cis-trans</i> -chrysanthemate	46	1.6	5
5-Benzyl-3-furylmethyl (+)- <i>trans</i> -pyrethrate	25	1.4	120
5-Benzylfurfuryl (±)- <i>cis-trans</i> -chrysanthemate	11	2.1	4.6
Natural pyrethrins	4.6		31
Allethrin			2.2
Parathion	85		18
Diazinon	42		3.7
Dimethoate			1.7
Demeton-methyl			1.4

\* Susceptible strain obtained through the courtesy of Mr. J. C. Wickham, the Cooper Technical Bureau.

† Relative potencies were derived from  $LD_{50}$  values for the compounds and 5-benzyl-3-furylmethyl (±)-*cis-trans*-chrysanthemate (taken as standard and given potency 100) in simultaneous comparisons. Drops (1  $\mu$ l.) of the compounds dissolved in acetone were applied topically from a micrometer syringe (ref. 5) to 3–4 day old adult females (*Musca*) or adult males and females (*Phaedon*).

‡  $LD_{50}$ , 0.016  $\pm$  0.0003  $\mu$ g/female fly.

§ At 1 : 1 compound : piperonyl butoxide ratio.

¶  $LD_{50}$ , 0.0007 per cent w/v.

Previously<sup>1</sup>, high toxicity to insects was found in chrysanthemates in which a planar activating nucleus (cyclopentenolone in the natural pyrethrins and allethrin, benzene in the benzyl esters<sup>1</sup>) held an unsaturated side chain in an appropriate stereochemical relationship to the acid. The best compound in the earlier series was 4-allyl-2,6-dimethylbenzyl (+)-*trans*-chrysanthemate (II).

and mustard beetles, so furfuryl and 3-furylmethyl chrysanthemates were investigated systematically. Methyl groups increased the activity of otherwise unsubstituted compounds, but they diminished toxicity when a benzyl group was present. The change from 5-benzylfurfuryl chrysanthemate to 5-benzyl-3-furylmethyl chrysanthemate dramatically increased the insecticidal activity and even the (±)-*cis-trans*-chrysanthemate (Table 1) was more than twenty times as toxic to house-flies as the natural pyrethrins.

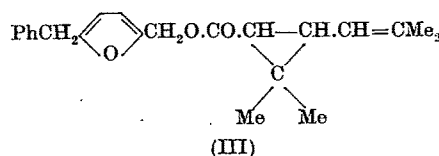
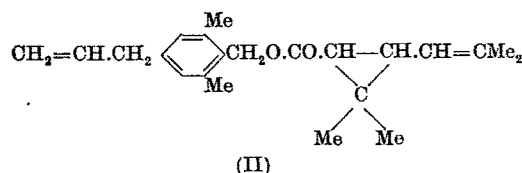
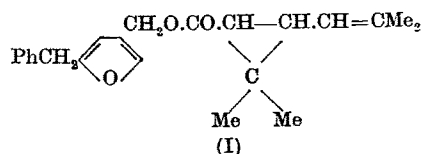
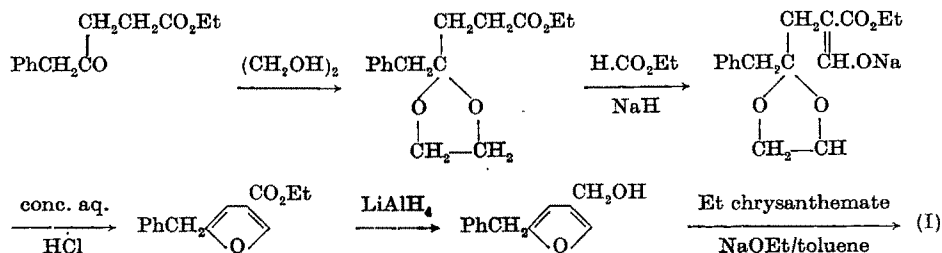


Table 1 compares the activities of some of the compounds synthesized with those of the natural pyrethrins and of some other insecticides. The (–)-*trans*-chrysanthemate and (+) and (–)-*cis* chrysanthemates are less active than the (+)-*trans*-ester, and introducing a 2-methyl group into the 5-benzyl-3-furylmethyl ester decreases activity to both insect species. Although less toxic than the (+)-*trans*-chrysanthemate, 5-benzyl-3-furylmethyl (+)-*trans*-pyrethrate (prepared using the naturally derived crystalline acid chloride<sup>2</sup>) had a somewhat better knockdown power 10 and 15 min after treatment and killed more house-flies after 24 h than the same dose of pyrethrum, when tested by a topical application technique<sup>3</sup>.

Piperonyl butoxide barely synergized the most active esters at a toxicant : synergist ratio of 1 : 1; this may be because the insect cannot readily detoxify the most potent compounds in this series and so activity is not enhanced if the synergist interferes with detoxification mechanisms.

Details of these compounds, of the methods of synthesis, and of the biological tests will be published elsewhere. 5-Benzyl-3-furylmethyl alcohol was conveniently obtained by the following route from  $\delta$ -phenyl laevulic ester<sup>4</sup>.



The furan ring is close in size to a cyclopentenolone, so furfuryl instead of benzyl chrysanthemates was examined. In the furan series, benzyl was an easily introduced and effective side chain. 5-Benzylfurfuryl chrysanthemate (III) showed promising activity against both house-flies

Ester interchange in the presence of sodium ethoxide was a more convenient procedure for making the *cis-trans*-chrysanthemate than use of chrysanthemoil chloride. Spectroscopic and analytical data fully substantiated the structures assigned.

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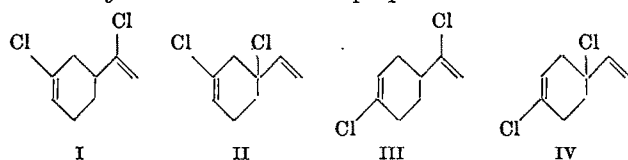
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## A Cyclobutane Derivative from Chloroprene Dimerization

KINETIC studies of the thermal polymerization of chloroprene indicate an unusual mechanism which involves formation of polymer from dimers and not from the chloroprene monomer<sup>1,2</sup>. The polymerization is initiated by traces of impurity in the monomer, and over a considerable range the observed rate and activation energy of polymer formation are identical with those for dimerization, indicating that the initial dimerization is the step which controls the rate in the consecutive reactions leading to polymer<sup>3</sup>. The characterization of the dimers and studies of the dimerization process are clearly prerequisites to understanding the mechanism of the polymerization. This communication is concerned with the initial problem of dimer separation and characterization.

It has been known for some time that chloroprene oligomerizes to form oils of low molecular weight<sup>4</sup>, and that six- and eight-membered cyclic dimers may be isolated from these products<sup>5-8</sup>. Possible Diels-Alder addition compounds are shown below (I-IV), and two isomeric dichlorocyclooctadienes can be proposed.



Brown *et al.*<sup>5</sup> showed that six- and eight-membered ring structures were present in the dimerization products from chloroprene, but did not characterize the individual isomers. Cope *et al.*<sup>7,8</sup> chemically identified the products of the dimerization at 80° as structure (III) and 1:6 dichlorocycloocta-1:5 diene, and structure (IV) was isolated as its dehydrochlorination product 1-chloro-4-vinyl-cyclohexa-1:3 diene. These results were supported by the more recent work of Nazarov *et al.*<sup>9</sup>, who in addition suggested that structure (I) is also present in dimers formed at 20° C and subsequently distilled at 90° C.

In our experiments the temperatures used in the preparation and separation of dimers have not exceeded 41° C. Products have been isolated from mixtures of two kinds, obtained as follows. (a) Chloroprene, which contained 1.0 per cent 1,1-diphenyl-2-picrylhydrazyl as a polymerization inhibitor, was allowed to dimerize at 35° C for 20 days, after which time the unreacted monomer was removed by high-vacuum pumping at -22° C. (b) Chloroprene, inhibited with 0.5 per cent *tert* butyl catechol, was allowed to dimerize at 38° C under gaseous nitric oxide for 20 days. The monomer was removed by distillation under reduced pressure of nitrogen. (We thank the Distillers Co., Ltd., for their co-operation in independently preparing this mixture.)

Fractionation of mixture (b) under reduced nitrogen pressure yielded two fractions, each of which accounted for about 25 per cent of the mixture. The remaining material consisted of residual monomer, high-boiling residues, and an intermediate fraction, shown by gas-liquid chromatography to be a mixture of the two main fractions. Elementary analysis of the two fractions gave results consistent with the empirical formula C<sub>4</sub>H<sub>5</sub>Cl (expected: C=54.25 per cent, H=5.65 per cent, Cl=40.10 per cent; found for fraction 1: C=54.45 per cent, H=5.59 per cent, Cl=39.23 per cent; found for fraction 2: C=53.02 per cent, H=5.38 per cent, Cl=39.70 per cent); and ebulliometric determinations<sup>10</sup> of the molecular weights in benzene solution indicated that the compounds were dimeric (molecular weight of fraction 1: 186 ± 5; molecular weight of fraction 2: 177 ± 5).

The boiling point of fraction 2 (40.0°-40.1° C, <1 mm), and its infra-red and nuclear magnetic resonance spectra, showed it to be a chlorovinyl chlorocyclohexene, as expected.

The infra-red spectrum of fraction 1 (boiling point 25.8-26.5° C, <1 mm) is shown in Fig. 1. The absorption bands at 3,090, 1,642, 1,417, 987 and 927 cm<sup>-1</sup> clearly indicate olefinic unsaturation, and the presence of only one sharp absorption band in the C=C stretching region suggests that the unsaturation is of a single type. Further, apart from the 927 cm<sup>-1</sup> band, the set falls directly within the ranges established for the vinyl group in hydrocarbon structures, and the assignment of the set to this group is supported by the absorption in the 1,855 cm<sup>-1</sup> region. The 927 cm<sup>-1</sup> band lies a little on the high side of the range usually quoted for vinylic out-of-plane C-H vibrations in hydrocarbons (915-905 cm<sup>-1</sup>), but this is not considered to be a serious objection to the interpretation in view of the established presence of chlorine. For the chlorine to have such an influence, it is necessary to assume that it is attached to a carbon α to the vinyl group. A striking feature of the spectrum is the absorption at 2,990 and 2,950 cm<sup>-1</sup>, which contrasts with the behaviour of fraction 2, and is considerably higher in frequency than that expected for C-H stretching vibrations in six-membered rings. It is in fact much more typical of smaller (strained) ring structure, and can be accommodated without any difficulty in the range established for methylene-group vibrations in cyclobutane. The only direct indication of impurity in the material is a band at 1,500 cm<sup>-1</sup>, which could be ascribed to traces of an aromatic component.

In the nuclear magnetic resonance spectrum (Fig. 2), the low-field bands are typical of the type-ABC spin system of a vinyl group not coupled to any other hydrogen nuclei. The upfield band is consistent with two pairs of mutually coupled (A<sub>2</sub>B<sub>2</sub>) cyclic methylenic protons. Integration of the spectrum shows the proton ratio to be three vinylic to two methylenic; that is, two vinyl groups per -CH<sub>2</sub>-CH<sub>2</sub>- fragment.

Taking both infra-red and nuclear magnetic resonance spectral evidence into account, the only feasible structure for fraction 1 is 1,2-dichloro 1,2-divinyl cyclobutane, a compound not hitherto suspected as a principal product of chloroprene dimerization.

The mixture obtained by procedure (a) was separated by thin-layer chromatography on a preparative scale.

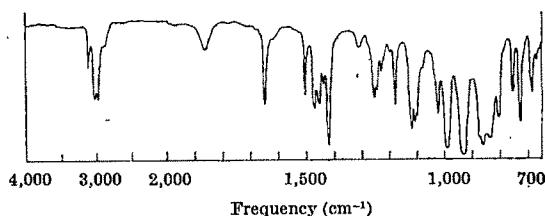


Fig. 1. Infra-red spectrum of fraction 1.

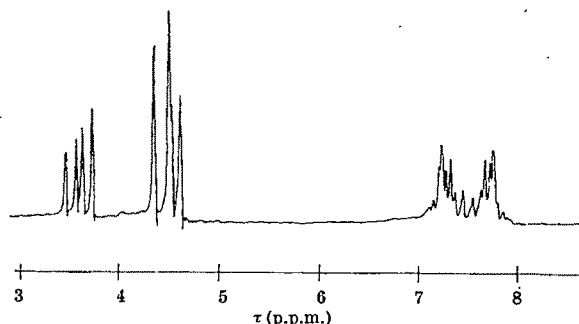
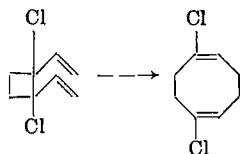


Fig. 2. Nuclear magnetic resonance spectrum of fraction 1. Chemical shifts measured with respect to tetramethyl silane,  $\tau=10$ .

This was done at room temperature using *n*-hexane as eluent on silica gel 'HF254 (Merck)' plates. (The absorbent was coated to a thickness of 1.5–2 mm, and was then activated for 3 h at 120° C. A 2 mm band of 50 mg of the mixed dimers was coated on to the plate at 40° C. The separated bands were detected by two methods: (i) irradiation with 254 mμ ultra-violet light, and (ii) the development of an edge-strip with a 2 per cent solution of perchloric acid in methanol. The bands were scraped out and then soxhlet-extracted with ether.) Two bands were observed, and approximately the same weight of product was isolated from each. The products were spectroscopically characterized as identical with fractions 1 and 2 from preparation (b).

Thus 1,2-dichloro 1,2-divinylcyclobutane is one of the principal products of chloroprene dimerization at relatively low temperature. It is possible that the failure of other workers to isolate this compound may involve the higher temperatures previously used in the preparation or isolation of the dimers. At such temperatures the dichlorodivinyl cyclobutane might undergo a Cope rearrangement to form 1,6-dichloro 1,5-cyclooctadiene<sup>11</sup>. In the present work, a peak corresponding to this compound has been observed in high temperature gas chromatograms of dimer samples which have been shown spectroscopically to contain no 1,6-dichloro 1,5-cyclooctadiene before injection. This provides indirect evidence for the rearrangement.



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## IMMUNOLOGY

### Suppression of Antibody Production by Phytohaemagglutinin

THE *in vitro* effect of phytohaemagglutinin (PHA) on small lymphocytes from the peripheral blood is now well known<sup>1,2</sup>, and a limited number of studies of the effect of this substance *in vivo* have been made in man<sup>3,4</sup> and laboratory rodents<sup>5-7</sup>. Calne and his colleagues<sup>8</sup> reported the use of PHA to augment the immunosuppressive effect of 'Imuran'. We have studied the effect of PHA on the immune response of rats to foreign red blood cells, and the preliminary results of our experiments are reported here.

The animals used in these experiments were young Wistar albino rats of the same inbred strain weighing about 200–220 g. The PHA used was obtained from Burroughs Wellcome. The animals used were divided into several groups and were treated as follows:

**Group 1.** Three doses of 0.5 ml. PHA were injected intraperitoneally at 24 h intervals, followed 24 h later by 1 ml. of washed 1 per cent chicken erythrocyte suspension also injected intraperitoneally.

**Group 2.** These animals were given PHA as described but were not immunized with the erythrocytes.

**Group 3.** The animals in this group were not treated with PHA but were given an immunizing injection of chicken erythrocytes as in Group 1.

**Group 4.** These rats were immunized with erythrocytes as described but also received one injection of 0.5 ml. PHA 6 h later.

All animals were bled at the start of the experiment (day 0), immediately before immunization (day 3) and on the second, fourth and sixth days after immunization (that is, days 5, 7 and 9). The treated rats in Groups 1 and 2 were also bled daily during the administration of the PHA.

Complement dependent haemolytic antibodies were detected by adding 2 per cent washed chicken erythrocytes to doubling dilutions of test sera in the presence of excess complement. The serum-erythrocyte-complement mixtures were incubated at 37° C for 60 min and then examined for haemolysis. 50 per cent lysis was taken as the end point of antibody activity.

Results of a typical experiment are summarized in Table 1; other experiments have given similar results.

Table 1. HAEMOLYSIN PRODUCTION IN RATS WITH AND WITHOUT PHA TREATMENT

Day	Group 1	Group 2	Group 3	Group 4
0	—	—	—	—
1	—	—	—	—
2	—	—	—	—
3*	2	2	2	—
5	—	—	—	—
7	—	—	16; 128; 64;	32; 8; 128;
			64; 64	64; 256
9	2; 4; —	32; 8	128; 64; 128;	256; 1,024; 256;
			128; 64	512; 1,024

Antibody production is expressed as the reciprocal of the highest dilution giving 50 per cent lysis of chicken erythrocytes.

\* Immunization after the sample was taken.

Haemolytic antibody was found in low titre in a number of Group 1 animals before immunization. No activity was found on either the second or fourth day after immunization in Group 1 animals, but low titre antibody was found in most 2 days later.

In Group 2 animals also, little haemolytic antibody activity was found with the exception of low titre in one animal on the seventh and ninth days of the experiment.

Naturally occurring haemolytic antibody was not found in pre-immunization samples from Group 3 rats. No antibody was detected until the fourth day after immunization, when considerable titres were obtained in all animals, persisting until at least the sixth day after immunization.

As in Group 3, no haemolytic antibody was detectable in Group 4 until the fourth day after immunization.

Fairly high titres were recorded in most animals on the fourth day, and on day six the titres were higher than those found in any other group in this experimental series.

Similar clear-cut results of antibody production were found using a haemagglutination technique, although in our hands it appeared to be less sensitive than the test using complement.

From these results it is readily apparent that parenteral administration of PHA before immunization with chicken erythrocytes considerably suppresses humoral antibody formation. On the other hand, PHA given 6 h after the antigen is not followed by immune suppression but seems, from our limited data, to enhance the production of antibody. Experiments are being undertaken to determine whether or not similar results are obtained when rats are given more vigorous post-immunization treatment with PHA. There is some evidence from these results and previous experiments carried out by us that the administration of PHA can elicit the production of naturally occurring antibody. This point is being investigated further.

Other experiments which we have carried out in which tetanus toxoid was given before immunization showed no suppressive effect on anti-chick erythrocyte antibody production. This suggests that PHA did not act by a mechanism involving antigenic competition.

In view of the fact that PHA transforms small lymphocytes of peripheral blood and thoracic duct to large blast-like cells *in vitro* it seems not unreasonable to suppose that a similar transformation of immunologically competent cells occurs *in vivo* with the "siphoning off" of such cells so that they are not available to react with the immunizing antigen. A possible site for such a transformation may be the germinal centres of the lymphoid tissues. It has been shown that the spleens of rats receiving intraperitoneal injections of PHA have larger numbers of germinal centres which are also larger in size than in control rats<sup>7</sup>.

Thus PHA may become of value as an immunosuppressive drug. It is apparently harmless in man in doses so far used<sup>3,4</sup>. It may give rise to untoward side effects in animals if used in high doses<sup>5</sup>. We noticed that a number of rats treated with PHA tended to be less active than those left untreated and sometimes assumed a hunched posture. This apparent lack of toxicity is in contrast to the marked side effects which may be brought about by other immunosuppressive agents such as X-irradiation and cytotoxic drugs. It may therefore be of some value in the field of organ transplantation. Our preliminary observations of the enhancement of antibody production brought about by PHA administered after the antigen suggest that it would be ineffective, or possibly deleterious, if used to treat auto-immune diseases such as haemolytic anaemia. Its use in such a context might result in an enhancement of the disease process. The effect of PHA as an immunosuppressive drug in transplantation immunity has yet to be critically assessed.

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## Immune Response to Substituted Arsanil-azo-Human Gamma Globulin Conjugates in Mice Tolerant to Human Gamma Globulin

I HAVE recently investigated the immune response to a variety of azo-human gamma globulin derivatives in mice tolerant to the carrier protein, human gamma globulin (HGG)<sup>1</sup>. Judged by the elimination of trace-labelled HGG, it seemed that a single injection of azo-protein in Freund incomplete adjuvant had only a marginal effect on tolerance. Two exceptions were noticed, however, in experiments where mice tolerant to HGG were challenged with either double substituted azo-HGG or with *o*-sulphanil-azo-HGG. On the other hand, compared with controls challenged with HGG, two injections of azo-HGG, 4-5 weeks apart, usually caused a significant increase in the number of responsive mice. Gel-diffusion techniques revealed that the injection of azo-proteins did not regularly cause a full return to responsiveness to HGG in mice neonatally rendered tolerant to this protein. The specificity of the antibody formed was found to be directed against antigenic determinants comprising the haptenic group and part of the protein carrier. The altered HGG preparations used had 2.6-13.6 azo groups per molecule of HGG and cross-reacted to 80-100 per cent with native HGG. The following experiments were devised to investigate the effect of azo-HGG with more haptenic groups attached and cross-reacting to a lower extent than the preparations previously used.

Diazonium salt of *p*-amino benzenearsonic (arsanilic) acid (*p*-A) was coupled with HGG as described earlier<sup>1</sup>, except that the molar ratios of diazotized arsanilic acid to HGG were greater by factors of 2 and 4. Furthermore, conjugates were prepared at pH 7.5 and pH 9.5, respectively, by simultaneous addition of 0.2 normal sodium hydroxide. (Other than the pH conditions of preparation these conjugates were identical.) After completion of the reaction the mixtures were kept for 12 h at 0° C and were then extensively dialysed against distilled water for several days and lyophilized. The analytical data are shown in Table 1. For the sake of comparison, a preparation similar to the *p*-A-HGG used in the previous investigation is presented in the first column. It can be seen that when the amount of diazonium salt added to the reaction mixture was increased by a factor of 2 and 4, the number of azo groups determined in the conjugates increased by a factor of about 1.5-3, whereas the content of arsenic rose by a factor of about 2-4. The ratio of arsenic groups to azo groups was found to be 2.87-4.4, which indicates that a substantial amount of arsanilic acid had been coupled to the protein by linkages other than —N=N—. The pH of the reaction mixture did not influence the total amount of azo groups, but in all conjugates prepared at pH 9.5 the amount of arsenic was higher than in those made at pH 7.5. The extent of cross-reaction determined by quantitative precipitation using a rabbit anti-HGG serum<sup>1</sup> is shown in the eighth column. Unfortunately, preparations *B* and *C* were insoluble; however, data on the degree of substitution found in previous experiments and obtained with preparations *D* and *E* suggest that cross-reaction of *B* and *C* with native HGG must be intermediate—probably between 80 and 100 per cent.

Tolerance was induced in neonatal *C57BL* mice by injecting 20 mg HGG within 24 h of birth<sup>2</sup>. The techniques used in the immunization, the challenge procedure, and the tests for tolerance were the same as those described earlier. Briefly, mice were injected at various intervals after birth with 0.1 mg HGG or *p*-A-HGG in 0.05 ml. of incomplete Freund adjuvant, distributed among three foot-pads<sup>3</sup>. Antibody formation was determined by following the elimination of trace-labelled antigen<sup>4</sup> and the tolerance was evaluated graphically according to the pattern of elimination<sup>2,5</sup>.

The results obtained after a single injection of *p*-A-HGG at an age of 7-9 weeks are shown in the last column.



Table 1. ANALYSIS AND DEGREE OF CROSS-REACTION OF VARIOUS *p*-A-HGG PREPARATIONS AND THE EFFECT OF ONE INJECTION AT THE AGE OF 7-9 WEEKS ON TOLERANCE TO HGG

Preparation	Diazotized arsanilic acid added (moles)	pH	-N=N-/HGG	As/HGG	Arsenic (per cent)	As/-N=N-	Cross-reaction with native HGG (per cent)	Responding mice (per cent)†
A	115	7.5	10.1	29.0	1.36	2.87	98	55
B	230	7.5	14.2	51.0	2.39	3.50	ND*	60
C	230	9.5	15.5	67.0	3.14	4.32	ND*	7
D	460	7.5	27.4	85.8	4.02	3.13	37	42
E	460	9.5	27-30	120.0	5.62	4.0-4.4	37	14

\* Not done: B and C were insoluble.

† Challenged with *p*-A-HGG. Twenty-five per cent of the control group, challenged with HGG, showed a low degree of responsiveness. The average size of the groups was seventeen mice.

At this time, 25 per cent of the controls, challenged with HGG, already showed a low degree of responsiveness, as judged by a somewhat faster elimination rate of the tracer as compared with a second control which had neither been in contact with HGG after birth nor had been injected with HGG in adjuvant (elimination control). On the other hand, 55, 60 and 42 per cent of the mice challenged with *p*-A-HGG, A, B and D, respectively, showed a low degree of responsiveness. The responsiveness, however, was only partial. In no instance did any mouse reveal an immune elimination of the tracer as did mice which had been injected after birth with *p*-A-HGG or HGG in adjuvant (immunization control) instead of soluble HGG. In contrast to these findings are the results obtained with the preparations C and E. In both groups, fewer animals than in the controls were responsive. This may reflect certain chemical properties (that is, high arsenic content) inherent in these conjugates prepared at pH 9.5.

The degree of cross-reaction of azo-protein conjugates with native HGG did not seem to correlate with their ability to "break" immunological unresponsiveness to the carrier protein<sup>1</sup>. This view is clearly demonstrated by the present findings. Preparations A and D had similar effects on the tolerance to HGG, although they cross-reacted to 98 and 37 per cent, respectively. The conjugates D and E, which cross-reacted to the same extent, clearly acted differently. It is concluded that it is not the degree of 'foreignness' to the tolerogen but rather the chemical nature of a hapten-protein conjugate which is of prime importance for the termination of tolerance to the carrier protein.

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### Identification of an Antigenic Determinant [of Collagen treated with Hydroxylamine]

THE treatment of collagen with hydroxylamine, which splits ester-like bonds, produces subunits carrying newly formed aspartic acid hydroxamates<sup>1,2</sup>. Their alcohol counterparts are not yet fully identified. A series of investigations on the immunogenicity and specificity of collagen<sup>3,4</sup> showed that collagen treated with hydroxylamine (HC) is an antigen which induces the formation of two types of immune sera: type 1 containing only antibodies against the area of hydroxylamine treatment (HC-antibodies); type 2 containing additionally antibodies against the general collagen structure. HC therefore acts when used for immunization like a conjugated protein. Investigation of haemagglutination inhibition showed that early sera were predominantly of type 2. Hyperimmune sera, however, were mostly of type 1. Inhibition experiments proved that sera with only HC-antibodies (type 1 immune sera) can be inhibited by HC, but are not inhibited by collagen preparations such as parent gelatine. Antisera which possess both anti-

bodies (type 2 immune sera) are inhibited extensively by HC and to a lesser degree by parent gelatine. Additionally, inhibition experiments were carried out with peptides obtained from HC, acid-soluble collagen and insoluble collagen by treatment with trypsin or collagenase<sup>5</sup>. Only peptides obtained from HC inhibited HC-antibodies and these peptides when used in sufficient amounts caused a complete inhibition of potent antisera.

The experiments described here were designed to obtain further information about the antigenic determinants of HC. Antisera were obtained in rabbits by immunization with HC from calf joint collagen (No. 49, 51, 82, 186), with HC from rabbit skin collagen (No. 56) and with parent gelatine from calf skin collagen (No. 181). The preparation of HC used for coating red cells and used as inhibitor was obtained from calf skin collagen. Haemagglutination and haemagglutination-inhibition were carried out as previously described<sup>3,4</sup>. Further inhibitors were amino-acid hydroxamates and amino-acids.

Two serological systems were selected for the inhibition experiments. HC served in both as coating antigen on red cells. These red cells were either agglutinated by type 1 immune sera, containing only HC-antibodies, or by anti-parent gelatine sera which served as controls. The anti-parent gelatine antibodies react only with the collagen structure of HC and do not detect the specificities caused by hydroxylamine treatment.

Table 1. INHIBITORY ACTIVITY OF HC, AMINO-ACID HYDROXAMATES AND AMINO-ACIDS IN PASSIVE HAEMAGGLUTINATION OF ANTI-HC AND ANTI-PARENT GELATINE SERA WITH HC-COATED CELLS

Inhibitor	Concentration (μg/0.05 ml.)	Reciprocal titre of serological systems HC/anti-HC	HC/anti-parent gelatine
Saline	—	128	64
Hydroxylamine-treated collagen	0.0025	<2	<2
DL-Aspartic acid hydroxamate	0.25	<2	64
" " "	0.025	2	64
" " "	0.0025	16	64
α-Alanine hydroxamate	0.25	32	64
β-Alanine hydroxamate	0.25	64	64
Glycine hydroxamate	0.25	32	64
Aspartic acid	0.25	64	64
Asparagine	0.25	64	64
Glycine	0.25	128	64

Table 1 shows the results of inhibition experiments; 0.05 ml. of HC-preparation, amino-acid hydroxamates and amino-acids in concentrations as indicated were added to 0.05 ml. each of geometrically progressing dilutions of antiserum. Red cells coated with HC were later added. Titre controls were carried out by using 0.05 ml. of saline instead of inhibitor. The extent of titre decrease indicated the inhibiting activity. Investigations in the HC/anti-HC system showed that HC was a strong inhibitor. DL-Aspartic acid hydroxamate also showed significant inhibition which depended on the concentration used. Hydroxamates of the other amino-acids and the parent amino-acids caused little or no inhibition. In investigations in the HC/anti-parent gelatine system (control) HC inhibited because of its collagen property. DL-Aspartic acid hydroxamate as well as the other compounds were inactive.

The minimum inhibiting doses of HC and different amino-acid hydroxamates were determined by the technique of Springer and Williamson<sup>6</sup>. Five different type 1 immune sera (HC-antibodies) were diluted to a titre of 1:8 (8 agglutinating units). Equal volumes of serum and

inhibitor in different concentrations were incubated for 10 min at 23° C before addition of red cells coated with HC. The minimum amounts of inhibitor which prevented the antisera from agglutinating the cells are recorded in Table 2.

Table 2. MINIMUM AMOUNTS (MG/ML.) OF COLLAGEN TREATED WITH HYDROXYLAMINE AND AMINO-ACID HYDROXAMATES GIVING COMPLETE HAEMAGGLUTINATION-INHIBITION OF EIGHT MINIMUM AGGLUTINATING DOSES OF ANTI-HC SERUM

Inhibitor	Anti-HC sera used				
	No. 49	No. 51	No. 56	No. 82	No. 186
Hydroxylamine-treated collagen	0.0012	0.0012	0.0012	0.0025	0.0012
DL-Aspartic acid hydroxamate	0.04	0.08	0.08	0.16	0.02
$\alpha$ -Alanine hydroxamate	>5	>5	5	>5	5
$\beta$ -Alanine hydroxamate	>5	>5	>5	>5	>5
Glycine hydroxamate	>5	>5	5	>5	>5

$\alpha$ - and  $\beta$ -Alanine hydroxamate and glycine hydroxamate showed little or no activity at the highest concentration used. Between the minimum inhibiting amounts of HC and DL-aspartic acid hydroxamate a weight ratio of 1:16 to 1:66 was observed. For determination of the molar ratio, the number of hydroxamate groups in the HC molecule (6 moles/1,000 moles of amino-acids<sup>2,7</sup>) has to be considered. The calculation according to the respective molecular weights leads to a factor of approximately one hundred. The molar ratio between HC and DL-aspartic acid hydroxamate is therefore in the order of 1:2,000 to 1:6,000. Similar ratios were observed in typical azo-hapten systems<sup>8</sup>. The high ratio makes it likely that the combining site of HC-antibodies is directed against an area larger than that of aspartic acid hydroxamate alone. Probably one or more adjacent amino-acids are also involved.

The investigations performed showed that aspartic acid hydroxamate has a specific activity in the HC/anti-HC system in contrast to all other amino-acid hydroxamates and amino-acids tested. Aspartic acid hydroxamate can be considered at least as one of the antigenic determinants of collagen treated with hydroxylamine.

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### Immunological Tolerance induced by Cyclophosphamide assayed by Plaque Spleen Cell Method

SINCE the demonstration in the rabbit of specific immunological tolerance when 6-mercaptopurine was administered together with the antigen bovine serum albumin<sup>1</sup>, numerous examples of tolerance induced by immunosuppressive drugs have been demonstrated<sup>2</sup>. This phenomenon of drug-induced tolerance is a particularly favourable system for the analysis of the fundamental and still incompletely understood problem of specific immunological unreactivity, but so far it has received little attention in this connexion.

The present communication is our first report on the analysis of immunological tolerance induced with the alkylating agent cyclophosphamide<sup>3</sup>. We have chosen the antigen sheep cells because the newly described technique of Jerne<sup>4,5</sup> allows the determination of the cellular kinetics of developing tolerance. In this system we have observed that the cells of the unstimulated mouse spleen which produce 19S sheep cell haemolysin disappear after

injection of cyclophosphamide only when antigen (sheep cells) is administered concurrently, that is in the same condition of antigen administration which is necessary for the induction of tolerance.

A satisfactory system employing sheep cells as the antigenic stimulus for the evaluation of immunosuppressive drugs in mice has been described<sup>6</sup>. We have obtained complete and lasting (longer than 2 months) specific immunological tolerance after a single intravenous injection of 0.8 ml. of a 30 per cent sheep cell suspension ( $6 \times 10^8$  cells) when four intraperitoneal injections of cyclophosphamide (each dose 85 mg/kg) are given daily beginning on the day of antigen administration. Complete tolerance is regularly obtained in CBA mice without significant mortality and can be maintained by weekly intraperitoneal injections of sheep cells. Analysis of this system by means of Jerne's technique for determining the number of anti-sheep cell antibody-producing cells in mouse spleen is presented in Fig. 1. In these experiments each point represents the arithmetic mean of six individual mouse spleens, each of which was plated on a single 100 mm  $\times$  15 mm disposable 'Falcon' Petri dish. Each spleen was separately sieved with a 'Cyto-sieve', and the cells were counted and then concentrated by centrifugation into 0.2 ml. of Gey's balanced salt solution. Agarose solution (0.5 ml., 2.5 per cent) was added to 1.5 ml. of Gey's heated to 47° C, and to this were added the spleen cell suspension and 0.1 ml. of 20 per cent sheep cells, both at 37° C. After mixing, this cell suspension was poured into the Petri dish which contained a sublayer of 10 ml. of 1.2 per cent agarose. When the agarose had gelled, the dish was incubated at 37° C for 2 h, complement was added (1 ml. of a 1:10 dilution of lyophilized guinea-pig serum), and the plate was incubated for an additional hour. The haemolytic plaques surrounding each antibody-producing

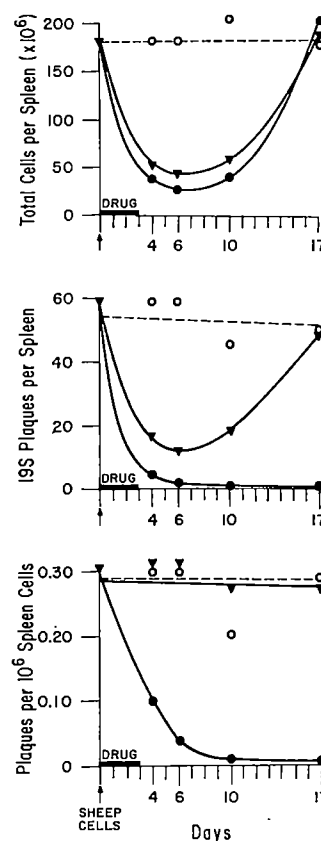


Fig. 1. The response of total spleen cells and 19S haemolysin-forming spleen cells to cyclophosphamide and cyclophosphamide plus sheep cell administration. —○—○, Control; —▼—▼, cyclophosphamide; —●—●, cyclophosphamide plus sheep cells.

cell were counted with the aid of a bacteria colony counter. Other details are similar to those of the original publication<sup>4,5</sup>.

The upper part of Fig. 1 shows the total spleen cell count in control animals, and at various times after the administration of *cyclophosphamide* both with and without antigen (sheep cells). In the middle part of Fig. 1 the total number of haemolytic plaques (antibody-forming cells) are graphed, and in the lower portion the number of plaques/million spleen cells is presented. The total spleen cell count drops sharply after the injections of *cyclophosphamide*, reaching a minimum on about the seventh experimental day and returning rapidly to normal by the seventeenth day. Of most interest is the effect of these regimens on the number of haemolysin-producing cells (19S plaques) of the spleen. Thus, the untreated CBA mouse spleen contains an average of forty to sixty cells producing antibodies to sheep cells. After administration of *cyclophosphamide* alone the fall and subsequent return of haemolysin-producing cells closely parallel these alterations of total spleen cell count. This parallel is illustrated in the lower part of Fig. 1 where it is seen that the number of plaques/million spleen cells remains constant throughout the period of observation. When sheep cells are administered at the time of the first dose of the drug the results are strikingly different; there is now a selective disappearance of the anti-sheep cell antibody-producing spleen cells which is complete by the tenth experimental day. On the seventeenth day there is an average of one plaque in two spleens (0,0,0,0,3,0 in the spleens examined) in these animals. Furthermore, as the spleen of the animal treated with sheep cells and *cyclophosphamide* is repopulated, there is no restoration of haemolysin-producing cells. The lower portion of the figure calls attention to the precipitous and permanent fall of 19S plaques in these antigen-treated animals when expressed for each million spleen cells.

It seems likely that the antigen-dependent disappearance of haemolysin-producing cells observed during tolerance induced by *cyclophosphamide* is related to the specific unresponsiveness produced. (Administration of ox cells or rabbit cells together with *cyclophosphamide* produced neither tolerance to sheep cells nor the disappearance of sheep cell haemolysin-producing plaques.) The present work, therefore, supports a clonal selection theory of antibody synthesis<sup>7-9</sup> and the additional supposition that the 19S cells observed in the unstimulated spleen are related to the cells which respond to the sheep cell antigen. A falling quotient of 19S haemolysin-producing cells to total spleen cells may prove to be a very early indication of developing tolerance.

It must be recognized that the experimental evidence connecting the 19S haemolysin-producing cell of the unstimulated spleen with that cell observed after antigen stimulation is circumstantial. Experiments which used a technique of *in vivo* cloning<sup>10</sup> suggest that the number of cells responsive to sheep cells in the normal mouse spleen is about 5,000 rather than 50-100 as indicated by the Jerne technique<sup>4</sup>. A plausible solution to this dilemma is that the Jerne technique detects a cell which is related to, or derived from, or is a selected part of the antigen-responsive clone rather than the clone itself. The present experiments are none the less additional evidence relating the 19S cell of the resting spleen to the antigen-responsive clone.

Two explanations have been put forward for drug-induced immunological tolerance<sup>2,11,12</sup>. The first proposes that these compounds act in conjunction with antigen to turn off the immune mechanism by blocking the transcription of information somewhere within the receptor or effector lymphoid cell; essentially, a ribonucleic acid mechanism is implied. Alternatively, it has been proposed that antigen renders these cells selectively susceptible to the cytotoxic effects of the immunosuppressive drugs, essentially a deoxyribonucleic acid mechanism.

Our experiments do not distinguish with certainty which of the two possible mechanisms is responsible for the antigen-dependent, drug-induced disappearance of 19S plaques. It seems plausible, however, that the metabolic alterations preparatory to cell division render the antigen-stimulated, haemolysin-producing cells particularly susceptible to *cyclophosphamide*. In view of the massive cell destruction which occurs throughout the spleen simultaneously and the known cytotoxic effects of *cyclophosphamide*<sup>13</sup>, enhanced destruction of these stimulated cells is an attractive explanation of the immunosuppressive and tolerance production of the drug. There is convincing evidence that *cyclophosphamide*, along with other alkylating agents, has its primary effect on deoxyribonucleic acid<sup>14</sup>, replication of which is necessary for mitosis.

*Cyclophosphamide* (administered as a single dose) however, will induce complete tolerance to sheep cells when given up to three days before the injection of antigen. Thus, the somewhat unlikely assumption must be made that enhanced susceptibility to antigen-stimulated cell death persists for 72 h after administration of *cyclophosphamide*.

The present experiments do not exclude the possibility that 19S-producing cells, rather than being destroyed, are converted to cells which no longer produce 19S antibody. We have examined directly the possibility of conversion to 7S-producers by employing the techniques of Dresser and Wortis<sup>15</sup> and Sterzl and Riha<sup>16</sup> which utilize a rabbit anti-mouse  $\gamma$ -globulin serum to detect 7S antibody-forming cells. No 7S haemolysin-producing cells were detected 5 days after treatment with *cyclophosphamide* and sheep cells. (The plates were incubated for an additional hour with 2 ml. of a 1:50 dilution of rabbit antiserum before complement addition<sup>15</sup>). It remains possible, however, that 19S cells have been converted to cells which do not produce antibody. This alternative does not reduce the likelihood that the antigen-dependent 19S plaque disappearance is closely related to immunological tolerance induced by the drug.

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### Synergistic Action of Antibodies: Detection of Circulating Antileucocyte Antibodies after Skin Transplantation

LEUCOCYTE isoantibodies, which appear after skin homo-transplantation in rabbits and rats, cannot usually be detected by simple agglutination. This report describes a

method for demonstrating these isoantibodies, which is based on the synergistic action of two antibody systems<sup>1,2</sup>, and which is also suitable for leucocyte typing. In this method donor leucocytes are agglutinated by the joint action of two different antibodies, each used in a subagglutinating titre.

Local wild strain rabbits and albino rats were used. Full thickness skin homografts, 2 cm × 1 cm, were performed in rabbits on the inner surfaces of both ears, without the use of sutures<sup>3</sup>. In rats, one full thickness transplant, 3 cm × 2 cm, was sutured on the back, and covered by a gauze pressure bandage for 5 days. The grafts were inspected daily and the progress of rejection was noted. Blood was collected from graft recipients by cardiac puncture, both at the time of rejection and later. Sera were inactivated, and stored at -20° C until testing.

Anti-rabbit and anti-rat-leucocyte sera were prepared by immunization of hens with washed suspensions of rabbit and rat leucocytes respectively, and these were injected intravenously and subcutaneously with complete Freund's adjuvant. The sera were absorbed with washed erythrocytes, free of leucocytes. Any weak antiglobulin activity was neutralized by the addition of normal rabbit or rat serum. The sera were titrated with rat and rabbit leucocytes, respectively, and dilutions giving a very weak positive reaction (a few leucocyte clumps of two to four cells) were used. Leucocytes were separated from the donor of the skin graft by differential sedimentation, as described previously<sup>4</sup>. A suspension of about 30,000-60,000 cells/cm<sup>2</sup> was prepared.

The test was performed in siliconized glassware by addition of one drop of the leucocyte suspension to 0.2 ml. of the recipient's serum, in a series of double dilutions. The tubes were incubated for 30 min at room temperature. After centrifugation at 3,000 r.p.m. for 15 sec the serum was removed and 0.2 ml. of the serum containing hen anti-rabbit or hen anti-rat antibodies was added. Results were read microscopically after further incubation for 30 min at room temperature, and centrifugation at 3,000 r.p.m. for 15 sec.

Additional tests were carried out with leucocytes of animals chosen at random and the graft recipient's own leucocytes as well as normal sera were used instead of the graft recipient's sera. The test was performed repeatedly on twenty-one rabbits and twenty-six rats. The same pattern of results was observed both in homografted rabbits and rats, although titres were slightly lower in rats. Typical results are given in Table 1.

Very weakly positive, or negative, results were obtained whenever the heteroantibodies were incubated with the donor's white blood cells first and the isoantibodies were added later. Positive results were also found with leucocytes of some random animals, as shown in Table 2.

The test based on the synergistic action of subagglutinating quantities of iso- and hetero-antileucocyte antibodies

was found to be a simple and reliable serological method for the detection of post-homograft humoral antibodies.

The antibodies formed during skin rejection and which react with donor's leucocytes are probably antibodies elicited by histocompatibility antigens. The cross-reactivity observed between the donor and random leucocytes suggests the presence of at least one histocompatibility antigen common to the skin donor and the random animal. This method is at present being used for the detection of common histocompatibility antigens and, in consequence, for tissue typing.

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### Species Specific and Cross-protective Functional Antigens of the Tapeworm Embryo

EVIDENCE<sup>1</sup> has been reported which supports the hypothesis that at least two immune responses may occur to larval taeniid tapeworms in the intermediate host, and that both responses can be elicited by a single parenteral injection of homologous viable eggs or activated embryos. The initial response appears to prevent the establishment of the challenge infection at the site of election, possibly by preventing the invading embryo from penetrating the intestinal barrier. The later response occurs at the site of election against the growing metacystode.

Further data<sup>2</sup> showed that part of the functional antigen complex is shared by the embryos of two species of the same genus parasitizing the same host. These two species are *Taenia hydatigena* and *T. ovis*. The evidence for a shared functional antigen between these two sheep metacystodes and the rabbit metacystode *T. pisiformis* was less certain.

The question was raised whether or not embryos of different genera also share part of this functional antigenic complex. To determine this, a trial, involving four groups each of twenty sheep free of worms (*Strongyloides* spp. excepted), has been carried out. Half the lambs in each group were vaccinated by the intramuscular route with the viable eggs and half with the activated embryos of *Echinococcus granulosus*, *T. hydatigena*, *T. ovis* and *T. pisiformis*. These lambs together with ten control animals were challenged 94 days later with 2,500 eggs of *E. granulosus*. About 30 months later, these animals were autopsied.

The activated embryos antigen complex for each species was prepared by immersing eggs expressed from gravid proglottids in Silverman hatching solution<sup>3</sup>. The viable egg antigen consisted of untreated eggs. The dose rate of antigen was 1,000-50,000 eggs or embryos for each species.

Table 1 summarizes the results. The range of hydatid cysts established and surviving is given for each treatment. The number of cysts was by no means normally distributed, thus the transformation  $y = \log(1 + x)$  has been used to "normalize" the distribution, so that significance tests would be valid. These data show that when sheep were vaccinated with the eggs of each of the sheep metacystodes, only the eggs of the homologous species induced significant protection against hydatid

Table 1

Recipient's serum, rabbit	Agglutination titre with donor's leucocytes
Before grafting	—
10 days after grafting	—
12 days after grafting	1/8
19 days after grafting	1/32
29 days after grafting	1/32
9 days after second set	1/64
15 days after second set	1/128
25 days after second set	1/128

In our rabbits first set skin homografts survived for 7-12 days; second set grafts survived for 4-6 days.

Table 2

Dilution of recipient's serum	Leucocytes of skin donor	Leucocytes of random rabbit	Leucocytes of additional 6 random rabbits
1/2	+	+	—
1/4	++	++	—
1/8	++	++	—
1/16	+	±	—
1/32	+	—	—
1/64	±	—	—

Negative results were always obtained when the graft recipient's own leucocytes or normal sera were used.

Table 1. PROTECTIVE RESPONSE TO *Echinococcus granulosus* IN SHEEP INDUCED BY VACCINATION WITH THE EGGS AND ACTIVATED EMBRYOS OF HOMOLOGOUS AND HETEROLOGOUS SPECIES OF TAENIIDAE

Source of antigen	Species	No. of sheep*	Viable and dead hydatid cysts			Viable hydatid cysts			Resistance indices ‡	
			Mean †	Standard deviation †	Range	Mean †	Standard deviation †	Range	Establishment of cysts	Survival of cysts
Activated embryos	<i>E. granulosus</i>	9	0.6	0.4	0-22	<0.1	0.1	0-1	91.2§	96.0§
	<i>T. hydatigena</i>	8	1.3	0.7	2-165	0.3	0.4	0-8	31.3	39.0§
	<i>T. ovis</i>	9	1.4	0.6	0-177	0.1	0.2	0-3	38.4	97.4§
	<i>T. pisiformis</i>	10	1.7	0.6	0-112	1.0	0.8	0-98	0.0	19.3
Viable eggs	<i>E. granulosus</i>	8	0.7	0.4	0-15	0.0	0.0	0	91.9§	100.0§
	<i>T. hydatigena</i>	8	1.8	0.3	18-199	1.1	0.8	0-109	0.0	6.9
	<i>T. ovis</i>	7	1.7	0.2	21-103	1.4	0.8	5-67	19.8	0.0
	<i>T. pisiformis</i>	8	1.7	0.6	1-191	1.2	0.6	1-99	0.0	0.0
Controls		9	1.8	0.3	19-154	1.2	0.6	1-100	—	—

\* Surviving for 30 months.

† Mean and standard deviation obtained from transformed figures  $y = \log(1+x)$ .

‡ Resistance indices calculated from the following:

$$\text{Resistance to the establishment of } E. \text{ granulosus is } 1 - \left[ \frac{Nt + Vt}{Nc + Vc} \right] \times 100$$

$$\text{Resistance to the survival of } E. \text{ granulosus is } \left[ \frac{NtVc - NcVt}{Vc(Nt + Vt)} \right] \times 100$$

§ Significant at 1 per cent level.

$Nt$  is the number of necrotic cysts per animal within each test group;  $Nc$  is the number of necrotic cysts per animal within each control group;  $Vt$  is the number of viable cysts per animal within each test group;  $Vc$  is the number of viable cysts per animal within each control group.

cysts. When sheep were immunized with the activated embryos, however, resistance was also induced to the challenge infection by the embryos of some of the heterologous species.

This evidence suggests that there are at least two functional antigen complexes. One is species specific, and is present in the eggs of the homologous species; the other is present in the activated embryos but not the eggs of the heterologous species. The former exerts an effect against both the establishment and the survival of the challenge organism, but the latter induces resistance only to the growing larval form at the site of election.

Although it is possible that the appropriate immunogen is not produced in sufficient amounts in the abnormal host, the previously recorded<sup>2</sup> and present evidence for a shared antigen between the sheep metacestodes and the rabbit metacestode *T. pisiformis* suggests that not all species within the same or different genera have a common functional antigen.

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## MICROBIOLOGY

### Isotope Effects on the Morphology of Fully Deuterated Bacteriophage $T_2$

CONSIDERABLE changes in the isotopic composition of living organisms may result in gross changes in the structure of cells and organelles<sup>1</sup>. The morphological changes attendant on isotopic substitution have a special interest, because the isotopically altered organisms are viable and biologically competent; and, as a consequence, correlations between structure and function become possible. Thus, for example, the chloroplasts of fully deuterated green algae<sup>2</sup> are observed under the electron microscope to be markedly different from those of the prototype organism although the deuterated algae are fully capable of photosynthesis. Because deuterated bacteriophage have been found useful in genetic<sup>3</sup> and biochemical<sup>4</sup> investigations, we have now fully examined deuterated phage by electron microscopy to determine the morphological consequences of deuteration on a virus.

*Escherichia coli* B (A.T.C.C. No. 11303) and bacteriophage *E. coli*  $T_2$  (A.T.C.C. No. 11303- $T_2$ ) were used. The bacteria were grown in aerated broth and, while the cul-

tures were in their logarithmic growth phase, they were inoculated with about 0.2 ml. of phage suspension. Aeration was continued for 24 h. Then the infective phage in the culture were assayed according to the double layer technique of Adams<sup>5</sup>. The Petri dishes containing twenty or fewer plaques were used for donor plaques. Phage from a single donor plaque were inoculated into two parallel *E. coli* cultures, one containing deuterated broth<sup>6</sup>, the other broth of normal isotopic composition. Incubation of the ordinary cultures was continued for 24 h, while the deuterated cultures were incubated for 7 days. Then the ordinary cultures were refrigerated for 1 week, while the deuterated cultures were refrigerated for 2-4 weeks. The longer the cultures were held within these periods, the greater the number of phage particles that could be collected. Concentration of the phage was achieved by centrifuging the cultures at 5,000g for 15 min, followed by centrifugation at 35,000-170,000g for 1-4 h. The higher forces and longer periods were used to concentrate the deuterated phage.

The samples were prepared for electron microscopy by negative staining with sodium or potassium phosphotungstate<sup>7</sup> in water and deuterium oxide at pH's (or pD's) in the range of 6-8. Just before staining, each sample was assayed for virulence, and only infective samples were examined electron microscopically.

Table 1. APPROXIMATE DIMENSIONS OF ORDINARY AND DEUTERATED BACTERIOPHAGE  $T_2$  (m $\mu$ )

	Head length	Head width	Tail length*	Tail width
Ordinary	9.9	7.1	10.2	1.6
Deuterated	11.0	8.9	11.4	2.64

\* Indicates distance from head to, but not including, the tail fibres. The neck above the sheath and the plate are included.

Deuterated  $T_2$  bacteriophage are larger than phage grown under normal conditions (Table 1). Besides the size difference, there is a difference in shape. The deuterated phage appear to have more bulbous heads than their normal counterparts. The sides of normal phage heads seem to be approximately flat planes, whereas those of deuterated phage seem to bulge. The tail fibres of deuterated phage are shorter than those of their normal counterparts. Frequently, in deuterated phage, these fibres are not seen at all or are anomalous (Fig. 1). High resolution electron micrographs suggest that there is a difference in the structure, or at least in the arrangement, of the subunits (capsomeres) of the phage tail. Micrographs taken at moderately high resolution show that two kinds of striations can be seen in the tails of bacteriophages. One variety of striation suggests a shallow pitched helix. The other type of striation exhibits a far steeper pitched helix. The second kind of striation is observed in both deuterated and normal forms. (It was not investigated carefully and we shall only suggest that it is the wrapped-up tail



fibres.) When the wrapped-up tail fibres are not present, the normal phage tail shows the shallow pitched striations; on the other hand, the deuterated phage tail has a lumpy appearance. At highest resolutions, the tail of normal phage appears to contain, and to consist largely of, spheroid bodies arranged in linear order. Fig. 1 shows some of these bodies arranged at nearly a right angle to the long axis of the tail in the hydrogen phage, but some other micrographs indicate that there is a helical arrangement of the sub-units. Deuterated virus also show tail sub-units, but linear arrangement is seldom observed. Instead, the capsomeres, which appear to be somewhat larger than those in ordinary virus, seem to be irregularly distributed.

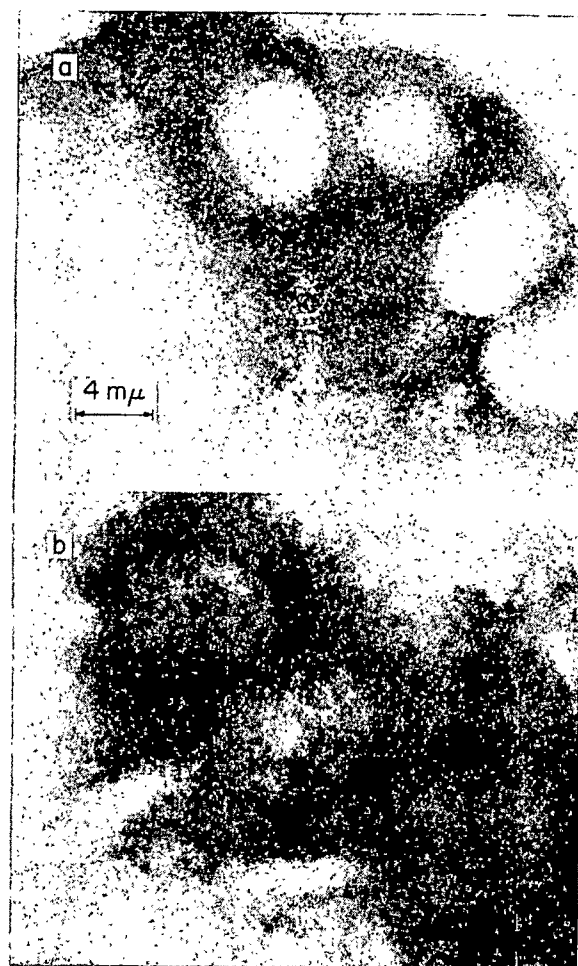


Fig. 1. *E. coli* T<sub>2</sub> bacteriophage grown under normal (a) and deuterated (b) conditions.

That mere immersion in deuterium oxide is not sufficient to produce the observed differences is indicated by the fact that viruses maintained the morphology typical of their culture type regardless of whether staining solutions were made up in water or deuterium oxide. For the present, we are inclined to the view that the peculiarities result from the size and location of the protein sub-units. The disordered arrangement of these sub-units in the deuterated virus could account not only for the extraordinary morphology of the phage but also for the reduced infectivity observed in these and in partially deuterated phage<sup>8</sup>. Such disordered protein sub-units might also contribute to the reduced morphological order seen in all deuterated micro-organisms that have so far been described.

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### Viruses of the Lymphogranuloma- Psittacosis Group isolated from Opossums: Opossum Virus B

EARLIER I reported<sup>1</sup> the isolation from opossums of two distinct new disease agents belonging to the lymphogranuloma-psittacosis group (PLGV), designated them opossum viruses A and B, and described studies with opossum virus A (OVA). This communication reports investigations with opossum virus B (OVB), by the same procedures used with OVA.

OVB was isolated from a brown masked opossum (*Metachirus nudicaudatus*)<sup>2</sup>. This opossum was killed when it was found to be in respiratory distress twenty days after its arrival at the Carlos Finlay Institute in Bogota, Colombia, from the place near Villavicencio where it had been trapped<sup>3</sup>. Autopsy revealed pulmonary consolidation.

A suspension of lung tissue from the opossum was injected intracerebrally, intraperitoneally and intranasally into mice. Those inoculated by the intracerebral and intraperitoneal routes remained well, but those receiving the agent intranasally developed signs of respiratory illness in 2-6 days, depending on the OVB concentration in the inoculum, and died. Elementary bodies characteristic of the PLGV group of viruses were demonstrated in impression smears of lung tissue stained according to Macchiavello's method. Yolk sac suspensions from chicken embryos infected with an inoculum from the mouse lungs contained not only elementary bodies but also a toxin with a titre of 320/0.5 ml. in mice which had been inoculated intravenously. Mice inoculated intranasally with yolk sac material developed pneumonitis; while those inoculated intracerebrally or intraperitoneally, although they had no signs of illness, had elementary bodies in their brains or spleens. Such brain or spleen tissue proved infective to mice when inoculated intranasally, but it was not possible to establish serial passage in mice with these suspensions by the intracerebral or intraperitoneal route. Virus was not found in lungs of mice injected intracerebrally or intraperitoneally, nor in brains or spleens of mice injected intranasally.

Three grey masked opossums (*Metachirops*) and one woolly opossum (*Caluromys laniger*) were injected intranasally with a suspension of infected mouse lung. Four or five days later the animals presented the same respiratory symptomatology as the opossum (*Metachirus nudicaudatus*) from which the agent was originally isolated. Virus was recovered from lung suspensions of all four, and pathological study demonstrated pneumonitis. The

Table 1. INFECTIVE AGENTS OF THE LYMPHOGRANULOMA-PSITTACOSIS GROUP: PATHOGENICITY FOR MICE AND GUINEA-PIGS AND SULPHONAMIDE SUSCEPTIBILITY

Agent	i.n.	Pathogenicity*		Guinea-pigs i.c.	i.p.	Susceptible
		Mice i.c.	i.p.			
Psittacosis	+	+	+	±		Yes
Ornithosis	+	+	±			No
Lymphogranuloma	+	+	±			Yes
Feline pneumonitis	+	+	±			No
Louisiana pneumonitis	+	+	+		+	No
Meningopneumonitis	+	+	±	±	±	No
Mouse pneumonitis	+	+	±			Yes
Opossum A	+	+	+	+	+	No
Opossum B	+	+	+	+	+	No

\* i.n., Intranasally inoculated; i.c., intracerebrally inoculated; i.p., intraperitoneally inoculated; +, generally fatal; ±, occasionally fatal; -, no effect.

virus was not pathogenic for guinea-pigs injected intracerebrally or intraperitoneally.

Table 1 presents a comparison of OBV and other members of the PLGV group with respect to pathogenicity for mice and guinea-pigs, and susceptibility to sulphonamides<sup>1</sup>. Investigations of possible cross-immunity were also undertaken. An immune relationship was demonstrated between OVA and OVB. Mice immunized intraperitoneally with OBV resisted intracerebral challenge with 1,000 mouse  $LD_{50}$  of OVA. In a similar test with meningopneumonitis virus (which is, like OVB, not susceptible to sulphonamides) OVB conferred no protection. It was not possible to obtain a clear-cut result on immunity by challenging mice immunized with OVB with the homologous virus intranasally. The same situation had been found with mice immunized and similarly challenged with OVA.

Neutralization tests were used to determine the relationship of OVB to mouse pneumonitis virus which behaves similarly in white mice (Table 1). The yolk sac toxin produced by the culture of OVB in the chicken embryo was neutralized by homologous immune serum from roosters injected intraperitoneally with the virus; but was not neutralized by immune serum prepared in the same manner with mouse pneumonitis virus. This lack of neutralization, together with the non-susceptibility of OVB to the sulphonamides, established a difference between OVB and mouse pneumonitis virus.

The fact that mice immunized with OVB resisted challenge with OVA raised the question that the two viruses might be identical. A difference was established, however, in that the B virus was not pathogenic for white mice injected intracerebrally, even with rich yolk sac culture.

These investigations were begun at the Carlos Finlay Institute, Bogota, Colombia, when it was jointly supported by the Ministry of Health of the Republic of Colombia and the International Health Division of the Rockefeller Foundation, and were terminated at the Research Division, American Cyanamid Company, Pearl River, New York.

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### Formation and Identification of the Pigment Pterorhodin in a Mutant of *Fusarium vasinfectum* Atk.

WE are investigating the pathogenicity of *Fusarium vasinfectum* Atk. in the cotton plant *Gossypium arboreum* and the present communication deals with studies of two mutants of *F. vasinfectum* obtained by nitrous acid treatment of their conidia. The synthesis of pterorhodin pigment by a mutant of *F. vasinfectum* is also reported.

The nitrous acid treatment was carried out on normal conidia obtained from 10 day old cultures in Czapek's agar medium. A suspension containing  $5 \times 10^8$  conidia/ml. of 0.1 molar acetate buffer was prepared according to the method of Siddiqi<sup>1</sup>. A pair of nutritionally deficient mutants, one being a partial requirer of nicotinic acid and the other requiring *para* amino benzoic acid (PABA), obtained during one such treatment was further investigated. The pathogenicity of these two mutants was compared with the parent strain by different methods. It was interesting to observe that the partial nicotinic acid requirer formed a bluish violet pigment in the Czapek's agar medium supplemented with niacin. A typical pigment producing mycelia is given in Fig. 1.

It was of interest to isolate and identify the pigment, which was insoluble in common solvents like benzene, ether, chloroform, alcohol, and also in dilute acids, either in cold or on heating. It dissolved in concentrated sulphuric acid, however, to give a red solution which on dilution gave a crystalline precipitate. The pigment was slightly soluble in alkali, but it slowly became hydrolysed on keeping for some time. The absorption spectra were taken in a recording spectrophotometer and also read in a colorimeter, and the absorption maxima were found to be at 508 m $\mu$  and 535 m $\mu$ .

On an examination of the many naturally occurring pigments, we found that the *Fusarium* pigment resembled pterorhodin. A pure synthetic sample of pterorhodin was compared with the *Fusarium* pigment. The absorption spectra observed in both cases were found to be identical and the *Fusarium* pigment possessed all the chemical properties of pterorhodin<sup>2-4</sup>.

Pathogenicity tests showed that the pigment forming partial nicotinic acid requirer was found to be doubly toxic when compared with the parent strain, whereas the PABA requiring mutant was non-toxic (Fig. 2).

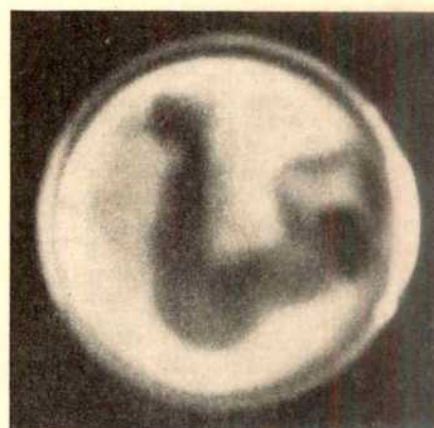


Fig. 1. The formation of bluish violet pigment by the partial nicotinic acid requiring mutant of *Fusarium vasinfectum*.



Fig. 2. Comparison of the toxicity of the different strains of *F. vasinfectum*. C, Control; P, PABA requiring mutant; W, wild strain; N, partial nicotinic acid requiring mutant.



The fusaric acid concentrations in the culture medium, as determined by the cup-plate method and the serial dilution method, were also more than twice that of the parent strain of *F. vasinfectum*. The pigment which required PABA formed little fusaric acid<sup>6</sup>.

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### Esterase Zymogram Method for classifying Mycobacteria

THE biochemical tests usually used in classifying mycobacteria are based mainly on quantitative differences in enzyme activities, with the exception of the niacin test. It would be important to examine the qualitative differences in enzyme activities such as substrate specificity and multiplicity of molecular forms. Esterases are thought to be the most suitable materials for this purpose because of their wide distribution in mycobacteria and the ease with which their zymogram can be obtained by electrophoresis. Cann *et al.*<sup>1</sup> examined the esterase patterns of some groups of rapid growers and suggested that this technique could be applied to the classification of mycobacteria. The present communication describes the species (or group) specificities of esterase zymograms obtained from numbers of mycobacterial strains using an advanced technique of thin layer electrophoresis.

The mycobacterial strains used were seven strains of *M. hominis*; two of *M. bovis*; eight of *M. kansasii*; seven of *M. avium*; six of *M. fortuitum*; twelve of *M. smegmatis*; eleven of *M. phlei*; and twenty-eight non-photochromogens (of which fifteen were received from Dr. E. H. Runyon and thirteen were isolated in Japan). The strains were checked by the usual methods of classification, such as the niacin test, the nicotinamidase test, the urease test, the formamidase test, Bönicke's amidase series test, the arylsulphatase test, the heat-stable catalase, esterase<sup>2</sup> and acid phosphatase<sup>3</sup> tests, and Gordon, Smith and Mihm's *M. fortuitum* tests.

0.8–1.5 g of wet cells grown on 1 per cent Ogawa egg medium were suspended in 1–1.5 ml. of water and sonicated for 20 min with a 10 kc/s, 200 W sonic generator. The sonicate was centrifuged at 17,000 r.p.m. for 20 min and the supernatant was used as the crude enzyme preparation to be analysed.

Agar thin layer electrophoresis was carried out using the method of Ogita<sup>4</sup>. The conditions used in this experiment were as follows. The supporting gel consisted of 0.7 per cent agar and 0.8 per cent polyvinylpyrrolidone in potassium phosphate buffer, pH 6.8,  $\Delta/2 = 0.025$ . The gel plate measured 165 × 120 × 0.9 mm.  $\Delta/2$  for the buffer in the electrode vessels was 0.05. A potential of 180–200 V was applied at both ends of the agar gel layer and a current of 26–28 m.amp passed; the duration of the electrophoresis was 60–90 min. The enzyme activity was detected using a  $\beta$ -naphthylacetate-naphthanyl-diazoblu B system.

The results are summarized in Fig. 1.

Three distinct bands were observed in *M. hominis* and *M. bovis*, and the two species could not be distinguished from each other by the present esterase zymogram

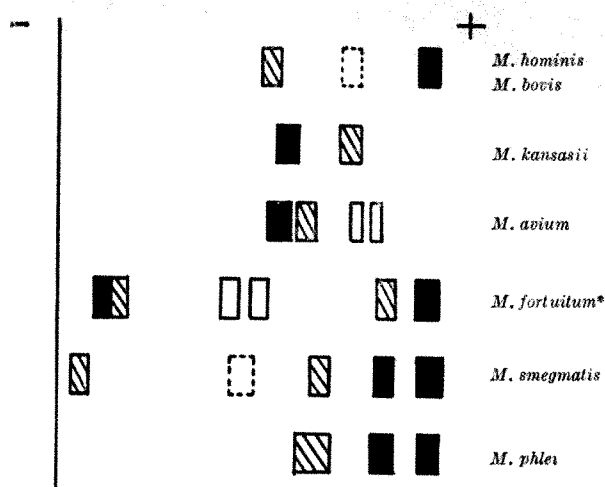


Fig. 1. Esterase zymogram of mycobacteria. Intensity of reaction  $\blacksquare$  >  $\square$  >  $\square$ .  $\square$  shows the existence of some variations in position. \* Some bands were slightly shifted or missing in several strains of *M. fortuitum*.

method. In two strains of *M. hominis*, a slight shift in the position of a band was observed. *M. kansasii* gave two bands and *M. avium* two principal bands and two weaker bands. *M. fortuitum* was found to have, in general, a specific pattern consisting of five or six bands. *M. smegmatis* typically gave a pattern consisting of five bands, but some variations in one of these bands were observed in several strains. Three characteristic bands were found in *M. phlei*. *M. runyonii* seemed to show different bands from *M. fortuitum*. The patterns of non-photochromogens resembled that of *M. avium* to some extent and they could be subdivided into at least three groups according to the position of the strongest band.

In accordance with the results of Cann *et al.* in some species of rapid-growers, the esterase patterns of mycobacteria seemed to be characteristic for each species, although some variations were observed. The esterase zymogram method using the  $\beta$ -naphthylacetate-naphthanyl diazoblu B system can therefore be used as a new method of classification for mycobacteria.

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### *Penicillium sclerotigenum*: a New Source of Griseofulvin

*Penicillium sclerotigenum* Yamamoto<sup>1</sup> (CMI, 68,616) has been shown to produce griseofulvin. The fungus causes tuber rots of the Chinese yam, *Dioscorea batatas*. It was grown in Roux bottles in a medium containing 75 g 'Dextrosol', 2.9 g potassium nitrate, 5.0 g potassium dihydro-orthophosphate, 1.0 g magnesium sulphate heptahydrate, 1.0 g sodium chloride, 0.5 g 'Difco' yeast extract, and 1 ml. of minor element concentrate (containing 0.1 g ferrous sulphate heptahydrate, 0.015 g copper sulphate pentahydrate, 0.05 g zinc sulphate heptahydrate, 0.01 g manganese sulphate heptahydrate and 0.01 g sodium molybdenate, in 100 ml.) made up to 1 l. with distilled water. The mycelium and broth were extracted with chloroform after 26 days growth.

A bioassay of the culture filtrate using *Botrytis allii* as the test organism showed the very characteristic effects of griseofulvin<sup>2</sup>. The presence of this antibiotic was confirmed by its isolation in low yields from both extracts as colourless needles, identity with authentic material being established by mixed melting point, and by comparison of ultra-violet and infra-red spectra and of  $R_F$  on thin layers. Isolation from mycelial extracts was hampered by the co-occurrence with griseofulvin of a colourless non-aromatic compound of closely allied polarity which was identified as ergosterol peroxide<sup>3</sup>.

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## PHYSIOLOGY

### Inhibition of Release of Acetylcholine by Strychnine and its Implications regarding Transmission by the Olivo-cochlear Bundle

In previous experiments, we have investigated the effects of various cholinergic blocking agents and other drugs on the release of acetylcholine (ACh) from the Ringer-Locke perfused superior cervical ganglion of the cat after injection of carbachol (0.5–1.0  $\mu$ g) into the perfusion solution or supramaximal electrical stimulation (10 c/s for 3 min) of the preganglionic trunk (ref. 1 and our work in preparation). Hexamethonium ( $10^{-4}$  molar), which does not block the release of ACh by preganglionic stimulation<sup>2,3</sup>, markedly reduced release of ACh induced by carbachol. Similarly, atropine sulphate (10–33  $\mu$ g) consistently, and  $3 \times 10^{-5}$  molar diallyl bisnortoxiferine or  $3 \times 10^{-4}$  molar dihydro- $\beta$ -erythroidine occasionally, reduced the amount of ACh released by carbachol, but not by preganglionic stimulation;  $2 \times 10^{-4}$  molar mecamylamine did not affect release of ACh with either type of stimulus. Procaine, at 50 or 10  $\mu$ g/ml., reduced release of ACh which had been induced by either preganglionic stimulation or carbachol, but at 1  $\mu$ g/ml. it was effective only against carbachol-induced release of ACh. Of all the drugs tested, only strychnine blocked or reduced selectively the release of ACh induced by preganglionic stimulation, an effect that was statistically significant ( $P < 0.05$ ) for the dosage range of 100–1.0  $\mu$ g/ml.; carbachol-induced release of ACh was reduced markedly at a concentration of 100  $\mu$ g/ml., but inconsistently or not at all at concentrations of 31.6  $\mu$ g/ml. or lower<sup>4</sup>. Strychnine in concentrations of  $10^{-6}$ – $10^{-5}$  g/ml. has been shown to reduce the output of ACh by the ileum of guinea-pig after coaxial stimulation or treatment with various drugs<sup>4</sup>. The inhibition by strychnine of the release of ACh by nerve impulse suggests a possible explanation for the apparent inconsistency between histochemical and pharmacological findings at the terminals of the olivo-cochlear bundle of Rasmussen.

The axonal terminals of the olivo-cochlear bundle, which exert a centrifugal inhibitory effect on the passage of impulses over the afferent fibres of cranial nerve VIII during auditory stimulation<sup>5</sup>, have been shown histochemically to stain distinctly for acetylcholinesterase<sup>6–8</sup>. This suggests that the olivo-cochlear bundle is cholinergic<sup>10,11</sup>. Pharmacological investigations, however, have not supported this implication. It was shown originally that strychnine, in doses as low as 0.1 mg/kg, given

intravenously, or applied topically (1/400) to the round window, or higher doses of the related drug, brucine, suppresses the neural ( $N_1$ ) inhibitory effect of stimulation of the olivo-cochlear bundle mentioned previously<sup>12</sup>. Strychnine and brucine likewise block another effect of stimulation of the olivo-cochlear bundle, the potentiation of cochlear microphonics (CM)<sup>13</sup>, which probably results from hyperpolarization of the hair cells<sup>14</sup>. Strychnine has been shown to produce blockade at peripheral sites of cholinergic transmission, but its potency in this respect is relatively low<sup>15,16</sup>. On the other hand, classical cholinergic blocking agents including D-tubocurarine, dihydro- $\beta$ -erythroidine, and atropine produce no detectable modification of either of the foregoing effects of stimulation of the olivo-cochlear bundle<sup>14,17,18</sup>. While the systemic administration of physostigmine or neostigmine<sup>19</sup> or the iontophoretic application of acetylcholine (ACh) to the vicinity of the cochlear hair cells<sup>17</sup> caused depression of the  $N_1$  response to auditory stimulation, in the latter experiments the CM response was simultaneously depressed, presumably through depolarization of the hair cells; both these actions of ACh, unlike those of stimulation of the olivo-cochlear bundle, were blocked by D-tubocurarine<sup>17,18</sup>. Thus, it seems unlikely that ACh is the neurohumoral transmitter of the olivo-cochlear bundle, despite its pharmacological actions in that vicinity and the high activity of AChE of the terminals.

ACh has been suggested to play an intermediate part in the release of the actual transmitter at the terminals of certain non-cholinergic neurones, including the neurosecretory fibres of the neurohypophysis<sup>20,21</sup>, adrenergic fibres<sup>22</sup>, and certain primary afferent fibres<sup>23</sup>. With the exception of adrenergic fibres of the cat all such fibres stain for AChE (refs. 24 and 25). This concept has been incorporated into a general hypothesis in which it was suggested that at the terminals of cholinergic fibres as well the ACh released initially by the nerve impulse may act at the same terminals to produce a brief ( $< 1.0$  msec) prolongation of depolarization and, thus, amplify the quantity of ACh released<sup>26,27</sup>. The hypothetical presynaptic cholinoreceptors involved at these various sites would not necessarily be expected to be pharmacologically identical with those at the corresponding postsynaptic sites. Thus, the action of strychnine in blocking the release of ACh from the cholinergic fibres of the superior cervical ganglion could be a result of blockade of pharmacologically distinctive presynaptic cholinoreceptive sites. Similarly, its blocking action at the terminals of the fibres of the olivo-cochlear bundle stained with AChE could be caused, at least in part, by blockade of the release by ACh of an inhibitory, or hyperpolarizing, transmitter which in turn acts at the auditory afferent terminals and hair cells. Certain features common to strychnine-sensitive inhibitory receptors and various cholinoreceptors have been pointed out<sup>28</sup>. The term "S" (strychnine-sensitive) cholinoreceptors might be assigned to the hypothetical presynaptic receptors in distinction to the well established M (muscarinic) and N (nicotinic) postsynaptic cholinoreceptors.

Our proposal does not necessarily preclude the possibility of another one<sup>14</sup>, that the prevention by strychnine of the effects of stimulation of the olivo-cochlear bundle is caused by the postsynaptic blockade of an inhibitory transmitter, as has been assumed to occur at the terminals of the Renshaw cells on the anterior horn cells<sup>29</sup>. Nevertheless, the evidence obtained at both these sites can be explained equally well in terms of a presynaptic block by strychnine of release of transmitter<sup>30</sup>. The incorporation of a step in the latter alternative mediated by ACh would then account for the presence of AChE at the terminals of the olivo-cochlear bundle. In addition, the assumption of a predominantly presynaptic site of action of strychnine would eliminate blockade by strychnine as an essential qualification for the identification of a putative inhibitory transmitter at the central nervous system<sup>31</sup>.

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### Functional Behaviour of Pial and Cortical Arteries in Conditions of Increased Metabolic Demand from the Cerebral Cortex

ANY marked increase in the metabolic demands of the cerebral cortex, caused, for example, by increased activity, is accompanied by an intensification of cortical circulation<sup>1-3</sup>. This occurs independently of the systemic arterial pressure which remains unaltered. The width of the major arteries of the brain (internal carotids and vertebrals) does not change in conditions of increased cortical activity<sup>4</sup> and even diminishes after a temporary deficiency of blood supply to the brain<sup>5,6</sup>. These arteries therefore do not, in the above conditions, contribute to an increase in cerebral circulation. It was therefore necessary to learn more about the functional behaviour of both pial and cortical arteries, as the possible vascular mechanisms responsible for increase of blood supply to the cerebral cortex when its metabolic demands are heightened.

The experiments were carried out with adult rabbits, either unanaesthetized (only novocain was locally administered in the course of surgical manipulations), or under light urethane anaesthesia. The changes in diameter of pial arteries were recorded by taking serial photomicrographs of them through a trephine opening in the skull and then using an ocular micrometer to measure the diameter of various vessels on the separate frames of the films (taken successively at fixed time intervals). Investigation of cortical blood vessels was carried out on microscope slides after rapid fixation of supravital tissue. For this purpose the cerebral vessels were, whenever the experiment necessitated it, perfused with formaldehyde solution (12 per cent, with saline, both dissolved in 96°

alcohol). In unstained slides of the cortex (30 $\mu$  thick and cut along the radial arteries) all structural elements of the vascular walls could be observed because of differential refraction. The external and internal diameters were measured along the cortical arteries at intervals of 20 $\mu$ .

The local increase in cortical activity recorded (on an electroencephalogram) was induced by application of a 0.5 per cent solution of strychnine nitrate. The dilatation of pial arteries overlying the corresponding areas of the cortex is caused not by the direct effect of strychnine on vascular walls but by the influence of the cerebral cortex<sup>4</sup>. The functional behaviour of pial and cortical arteries after 1-2 min of treatment with strychnine is shown in Fig. 1. A complete stoppage of cerebral circulation for a period of 30-60 sec was produced by exsanguination from the abdominal aorta and by a subsequent pumping of blood back into the arterial system. Fig. 2 shows how pial and cortical arteries behave when the cerebral cortex is in the post-ischaemic state.

Thus the increase of metabolic demands of the cerebral cortex (caused by increased activity or a temporary stoppage of cerebral circulation) is accompanied by a dilatation of pial arteries, especially of those of smaller calibre, whereas the lumina of cortical arteries even undergo contraction. The implication is that it is the pial arteries that are to be regarded as the vascular mechanism responsible for the increase of blood supply to the cerebral cortex in the circumstances described above. Situated as they are over the surface of the brain in the porous tissue of the pia mater they are able to change their width within a very wide range. The cortical arteries, on the other hand, are in quite different conditions, for there is no free space surrounding them<sup>7,8</sup>, and their dilatation would exert pressure on the tissue elements of the cerebral cortex. Thus there is the essential significance of the fact that their external diameter does not increase even when the cerebral circulation is intensified<sup>9</sup>. The data presented here show that the internal diameter of the cortical arteries even tends to diminish. The problem of whether this contributes to increased resistance to blood flow in the cortical area concerned cannot be solved yet. The Fåhræus-Lindquist phenomenon<sup>10,11</sup>, however, may suggest that the fluidity of blood in arteries of such small calibre rises as the lumina contract. Only when this contraction of cortical arterioles (especially of very small ones) reaches a certain point (for example, in a cortical focus of convulsive activity of some 10 or 15 min duration<sup>12</sup>) can it become a cause of deficiency of blood supply<sup>3</sup>.

These results agree with previous findings<sup>13</sup>. Using an electroplethysmographic method it was shown that in man inhalation of carbon dioxide results in an increase of blood

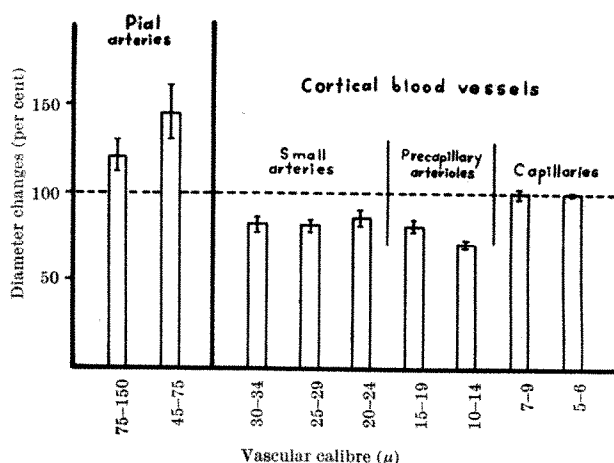


Fig. 1. Changes in diameter (in percentage of control values) of cerebral blood vessels under conditions of increased cortical activity (in rabbits). The data represent mean ( $M$ ) and standard deviations ( $\sigma$ ).



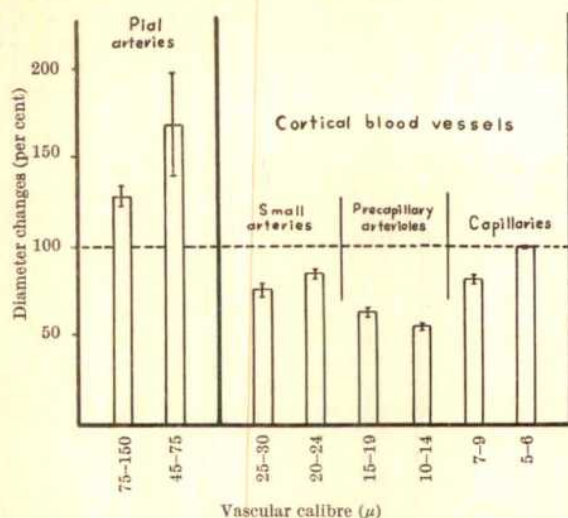


Fig. 2. Changes in diameter (in percentage of control values) in post-ischaemic state of the cerebral cortex (in rabbits). The data represent mean ( $M$ ) and standard deviations ( $\sigma$ ).

volume only in the surface vessels of the brain; within the brain it seems to decrease.

Consequently, in conditions of increased metabolic demand from the cortex of the brain, the pattern of the functional behaviour of various parts of the cerebral arteries is not identical. Furthermore, changes in the diameters of their lumina may even proceed in opposite directions. The pial arteries seem to play the main part in the increase of the blood supply to the cerebral cortex in these conditions.

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### Inhibition of Uterine Contractions *in vivo* in the Unanaesthetized Rabbit

WE have found that a non-steroidal oestrogen antagonist<sup>1</sup> will inhibit spontaneous (4  $\mu$ g/ml.) and electrically induced (20  $\mu$ g/ml.) contractions of the oestrous rat uterus *in vitro*<sup>1,2</sup>. Similar results were obtained *in vitro* with electrically stimulated uteri (about 30  $\mu$ g/ml.) removed from rabbits immediately after coitus. This report

concerns the effect of CN-55,945-27\* on uterine contractility when infused intravenously into unanaesthetized rabbits *post partum*.

Uterine contractions were recorded by means of transducer units similar to those previously described<sup>3</sup>. Such units (Fig. 1B) were calibrated (Fig. 1A) and then sutured on to the serosal surface of the myometrium overlying placental sites (Fig. 1C) in New Zealand white rabbits on the twentieth day of pregnancy. The transducer leads were run subcutaneously from a mid-ventral incision in the abdomen to an external connector which was anchored by the skin at the back of the neck (Fig. 1E). A permanent cannula was sutured into the jugular vein and exteriorized adjacent to the electrical connector (Fig. 1D). The recordings were made on an Offner (Type RS) dynograph.

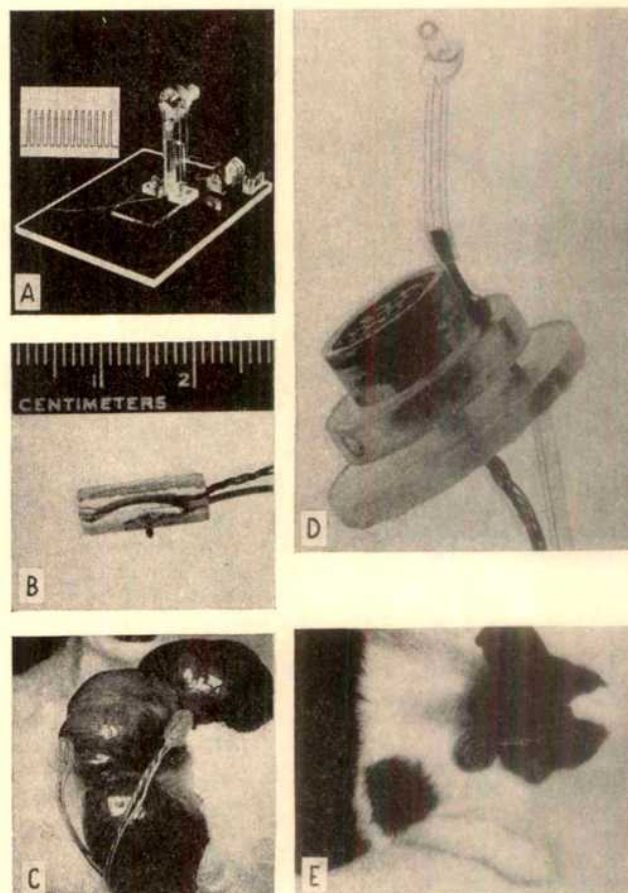


Fig. 1. A, Calibration apparatus in which known weights are intermittently lowered on to one end of the transducer unit by means of a small battery operated motor; B, combination electrode-transducer unit (see ref. 3); C, transducer units on pregnant rabbit uterus (unit on right overlies placental site); D, acrylic plug holding electrical (female) connector and jugular cannula outlet; E, rabbit (Dutch-Belted strain) showing acrylic plug healed into skin of neck region.

CN-55,945-27 was dissolved in 0.6 per cent saline and infused intravenously (30 mg/kg/h). Fig. 2 is a record typical of those observed repeatedly in eleven animals on days 2-7 *post partum*. All the animals were allowed to keep and raise their young. Lines A and B (Fig. 2) show a control pattern obtained during infusion of 0.6 per cent saline. Infusion of the compound over a period of 2 h, as shown in lines C, D and E (Fig. 2), resulted in a progressive decrease in both the frequency and amplitude of uterine contractions in all the animals studied. When the infusion of CN-55,945-27 ended, uterine activity returned towards normal as shown in lines F and G (Fig. 2).

\* (1-[2-(p-[a-(p-methoxyphenyl)- $\beta$ -nitrostyryl]phenoxy)ethyl]pyrrolidine, monohydrate).

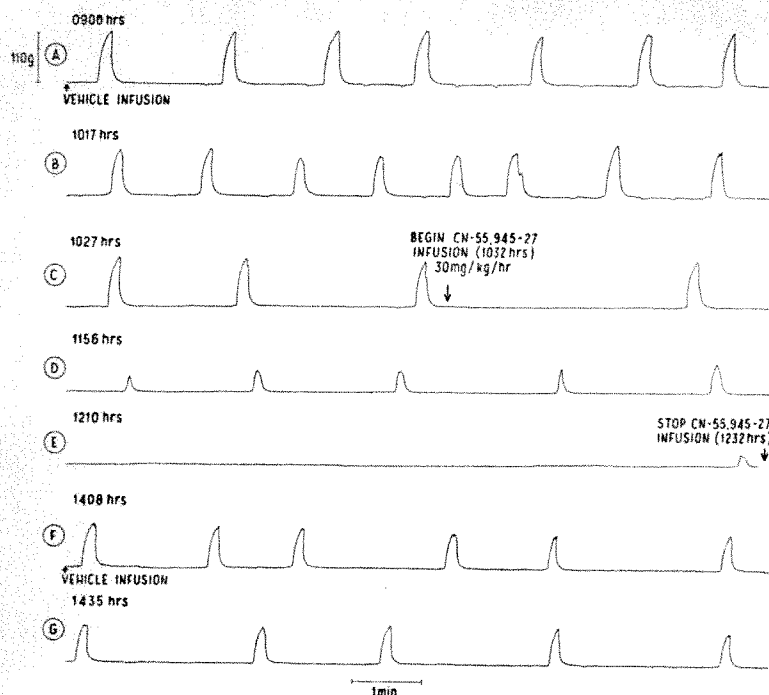


Fig. 2. Effect of intravenous infusion of CN-55,945-27 on uterine contractions *in vivo* in the unanaesthetized post partum rabbit. g, Tension in grams.

A quantitative analysis of the data presented in Fig. 2 is plotted in Fig. 3. These data were obtained by a method which we call linear displacement analysis. An inexpensive device, which is normally used to determine distances on maps, was used to trace the total record from which the recordings presented in Fig. 2 were taken. That is, the number of "miles" was traced during each interval of 15 min for the total period of infusion. Next, the horizontal length ("miles") of a 15 min period was subtracted from the total length of each of these tracings. This calculation allows the percentage decrease in uterine activity to be expressed on a 100 per cent basis. Finally, we calculated the percentage change in "linear displacement" of each interval during drug infusion from the mean of eight similar 15 min tracings during the control period. Although the meaning of this type of analysis is uncertain, we have found the method to be simple, rapid and cheap and to represent accurately the effect of various hormones and synthetic

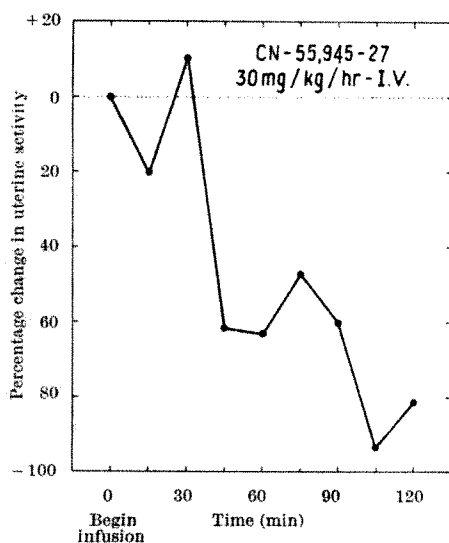


Fig. 3. Quantitative analysis of data presented in Fig. 2 by linear displacement method (see text).

agents on uterine contractility. Previously we attempted to make use of the planimeter method<sup>4</sup> to analyse this type of data and found it very laborious. The data presented in Figs. 2 and 3 are quite typical of those obtained during forty-seven different experiments in eleven rabbits. Higher doses (for example, 60 mg/kg/h) cause a qualitatively similar but more rapid effect. The initial stimulatory effect of the compound on uterine contractions (Fig. 3) was observed in the majority of our experiments.

It should be noted that the transducer units used in this experiment are essentially uni-directional and that they were placed on the longitudinal axis of the uterus. Our results are therefore limited to the longitudinal layer of muscle and may not reflect the activity of the circular muscle. It is interesting, however, that this oestrogen antagonist can inhibit uterine motility both *in vitro* and *in vivo*. Thus, CN-55,945-27 may have clinical potential in situations in which it would be desirable to inhibit uterine activity rapidly.

We thank Mr. Ralph Hartnagel for preparing the photographs shown in Fig. 1, and Dr. Horace A. DeWald for providing the CN-55,945-27.

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### Effect of Hemicholinium on the Number of Synaptic Vesicles

SYNAPTIC vesicles contain a bound fraction of acetylcholine<sup>1,2</sup>. Vesicles were identified by the quantity of acetylcholine, which was 400 molecules<sup>3</sup> or twenty to a hundred times as much<sup>4,5</sup>. Hemicholinium, on the other hand, inhibits the cellular mechanism which synthesizes acetylcholine<sup>6</sup>. Thus the question arises as to whether the number of synaptic vesicles shows any alterations after treatment with hemicholinium.

Eight albino rats (160–180 g body weight) were injected intraperitoneally with  $\alpha, \alpha$ -dimethylethanolamino-4,4'-biacetophenone (HC-3) in a dose of 10 mg/kg. Three of the animals died spontaneously 23–35 min after the injection; the rest were killed 40 min after the injection. Eight untreated rats were used as controls. The caudate nucleus and the superficial layer (stratum zonale) of the parietal cortex were subjected to routine electron microscopic investigations, using Millonig's osmic acid solution. Blocks were embedded in 'Araldite'; thin sections were prepared on an ultratome and photographed on a table electron microscope.

The numbers of synaptic vesicles were determined in a large number (more than a thousand) of axonal profiles and the surface areas of the individual profiles were estimated by means of a planimetric procedure. Axon profiles, exhibiting membrane thickenings, were designated "synaptic" profiles, whereas those without membrane



specification were regarded as "transient" profiles. The numbers of synaptic vesicles in each square micron were then calculated both for synaptic and transient axons. The results are summarized in Fig. 1.

The numbers of synaptic vesicles are higher in the axons of the parietal cortex than in the caudate nucleus. The difference is significant. Treatment with HC-3 did not induce any significant alteration in the synaptic vesicles within the caudate nucleus, whereas the decrease in the parietal cortex is highly significant. Even in the cortex, however, HC-3 did not destroy synaptic vesicles; their number decreased only to that in the caudate.

Hebb<sup>7</sup> recently reported a 90 per cent decrease of acetylcholine content in the caudate nucleus after local treatment with HC-3. A marked decrease of acetylcholine in the pons and medulla has been found after systemic administration of HC-3<sup>8</sup>, and an increased central latency (probably caused by decreased acetylcholine content) in the spike responses of Renshaw cells affected by HC-3 has been observed<sup>9</sup>.

It seems very unlikely that vesicles contained acetylcholine in their cavities when it is realized that: (a) acetylcholine is synthesized by a cytoplasmic, non-mitochondrial enzyme (choline acetylase) which may be either soluble<sup>10-12</sup> or associated with some structural components (neurotubuli or vesicles), according to species differences<sup>11,13</sup>; (b) synaptic vesicles are derived from mitochondrial membranes<sup>14</sup>; (c) treatment with HC-3 markedly reduces synthesis of acetylcholine; (d) treatment with HC-3 does not abolish the formation of synaptic vesicles; (e) acetylcholinesterase is located in surface membranes<sup>10-13</sup>. The hypothesis that acetylcholine is adsorbed and attached to the external surfaces of synaptic vesicles seems more plausible. Accordingly, the function of vesicles was only to transport acetylcholine molecules to their site of action (Fig. 2). Binding of acetylcholine to the external surface of a vesicle ( $\sim 500,000 \text{ \AA}^2$ ) could easily account for the high figures recently obtained for the quantum of acetylcholine<sup>4</sup> which are inconsistent with the extreme concentrations (up to 10 molar) required for an intravesicular localization. Also the pre-transmitter (modulator) function of acetylcholine in most of the

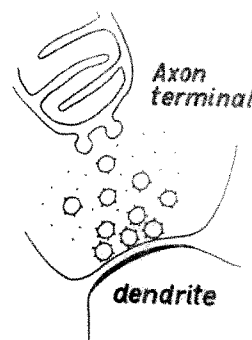


Fig. 2.

central synapses suggested on the basis of histochemical and electron microscopic investigations<sup>15</sup> is consistent with the location of acetylcholine at the external surfaces of synaptic vesicles.

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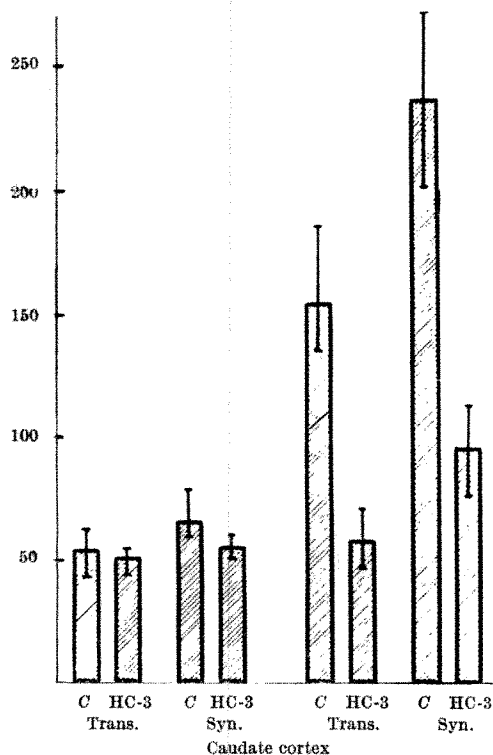


Fig. 1.

### Effects of Vasodilators on the Isolated Taenia Coli of the Guinea-pig

DESPITE a long history of the clinical use of nitroglycerine as a powerful coronary vasodilator, very little is known about the basic mechanism by which this agent relaxes vascular smooth muscles. As a first step to search into the underlying mechanism of this relaxant action, the effect of this compound on the electrical activity of the isolated guinea-pig taenia coli was examined using the sucrose-gap technique, and this was compared with the actions of several other representative vasodilators, such as papaverine, theophylline and adenosine.

Guinea-pigs were stunned by a blow on the head, the abdomen opened and the colon was exposed. A 30 mm *in situ* length of taenia was dissected free from the underlying tissue and mounted in a sucrose-gap apparatus as described by Burnstock and Straub<sup>1</sup>, so that it lay in a horizontal insulating tube containing isotonic sucrose solution, while each end was suspended in a vertical tube through which Tyrode solution flowed. The temperature of the Tyrode solution flowing through the one vertical tube was kept below 15° C, to keep the muscle inactive, thus maintaining a stable potential against which the potential change of the preparation in the other vertical tube, kept at body temperature, could be compared.

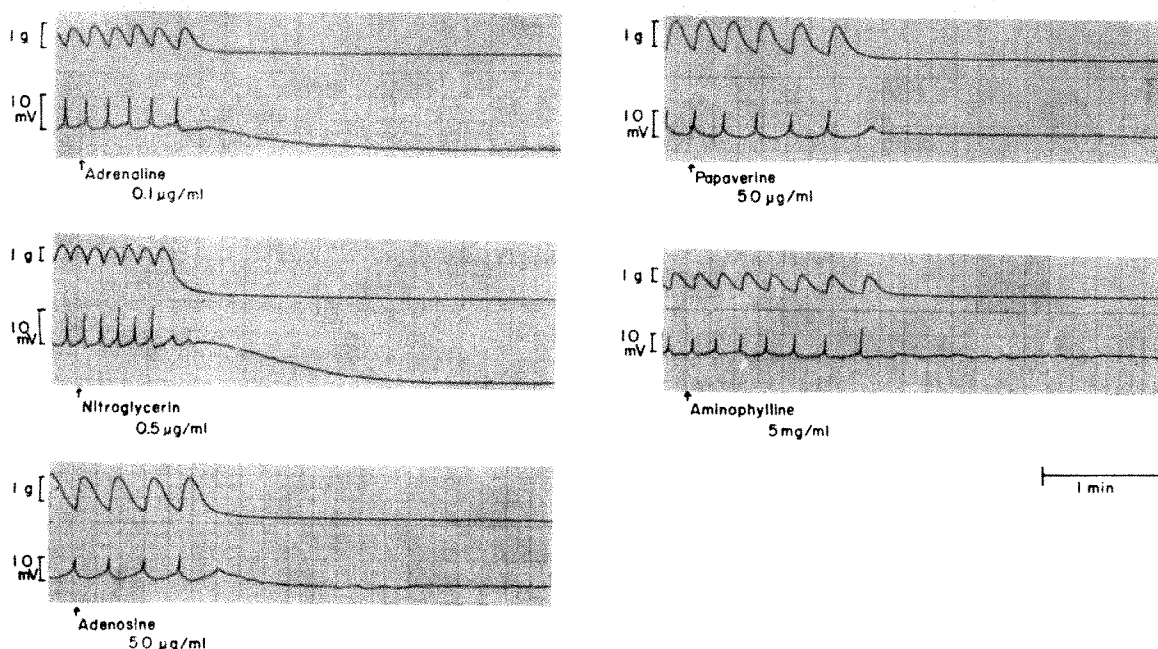


Fig. 1. Effects of vasodilators on the electrical activity of the isolated guinea-pig's taenia coli. Sucrose-gap method. Contractile tension was also recorded isometrically using a strain-gauge transducer. Although no remarkable hyperpolarization is discernible in this figure with papaverine and theophylline ethylenediamine, these compounds produced hyperpolarization in some other preparations not shown here.

Arrangements were made, using a strain-gauge transducer, to record isometric contractions of the preparation simultaneously with electrical activity. The initial tension of the muscle was adjusted to 0.5–1.0 g.

The composition of the Tyrode solution used was as follows: sodium chloride, 154; potassium chloride, 5.6; calcium chloride, 2.2; magnesium chloride, 2.1; sodium bicarbonate, 8.0; dextrose, 5.5 (mmoles). This solution was saturated with a gas mixture of 95 per cent oxygen + 5 per cent carbon dioxide.

Compounds used were: nitroglycerine, papaverine hydrochloride, theophylline ethylenediamine, adenosine and adrenaline bitartrate.

As shown in Fig. 1, all the five compounds tested induced qualitatively similar changes in the membrane potential, that is, an inhibition of spike discharge and a hyperpolarization, at the same time as they produced a relaxation of the preparation. The threshold doses for these actions were found to be  $10^{-8}$ – $10^{-9}$  for nitroglycerine,  $10^{-8}$ – $10^{-7}$  for adenosine,  $10^{-6}$  for papaverine and  $10^{-3}$  (g/ml.) for theophylline ethylenediamine. These changes are the same as those reported for adrenaline by many investigators and it seems that all these compounds could produce relaxation of this preparation through their action on the membrane activity. Further studies in our laboratory, however, revealed certain differences concerning the mechanism of the observed effects of these compounds on the membrane activity. Although the relaxant effect of papaverine and theophylline ethylenediamine persisted after complete depolarization of the membrane by isotonic potassium sulphate, the action of nitroglycerine and adenosine was abolished in the depolarized preparation, just as the effect of adrenaline was (Fig. 2). There is now considerable evidence to suggest that the tonic part of the potassium contracture in smooth muscles is maintained by the inward movement of calcium from the extracellular space into the muscle fibres. Presumably papaverine and theophylline ethylenediamine inhibited this inward movement of calcium, thereby abolishing the sustained potassium contracture. This mechanism may probably be operating, when these compounds relax the normal polarized taenia coli, in

co-operation with their stabilizing action on the membrane.

In contrast, nitroglycerine, adenosine and adrenaline, produce a change only in the polarized membrane, most probably an increase in affinity towards calcium, as is postulated by some research workers<sup>2</sup>, which results in a cessation of spike activity and a hyperpolarization, which in turn lead to a relaxation. Although it was reported earlier<sup>3</sup> that the relaxant action of adrenaline could be elicited even in the depolarized uterine muscle, the recent report by Axelsson *et al.*<sup>4</sup> demonstrated that the relaxing effect in the depolarized taenia coli could not be ascribed to adrenaline itself, but rather to some pre-

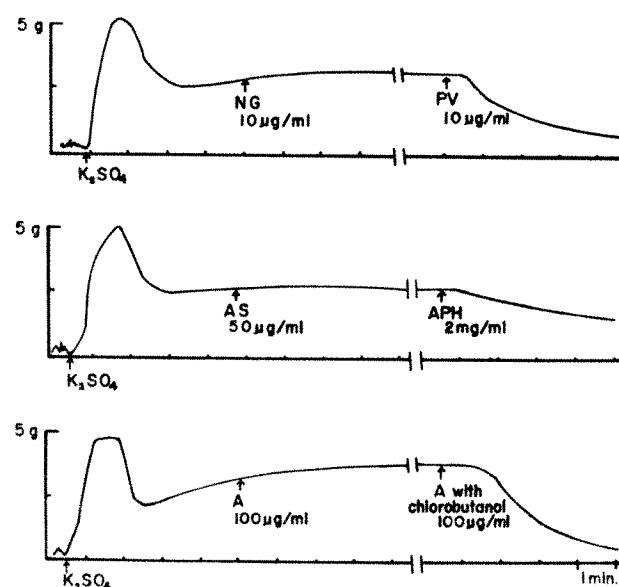


Fig. 2. Effects of vasodilators on the completely depolarized taenia coli. NG, Nitroglycerine; PV, papaverine; AS, adenosine; APH, theophylline ethylenediamine; A, adrenaline bitartrate; and A with chlorobutanol, that is, a commercial sample of adrenaline hydrochloride containing chlorobutanol as a preservative.

servatives, such as chlorobutanol, commonly contained in the commercially available adrenaline samples. Our experiments with a freshly prepared aqueous solution of adrenaline confirmed their results.

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## RADIOBIOLOGY

### Localization of Radioiodinated Endotoxin in Organs of Mice and Rabbits: Effect of Thorotrast, Trypan Blue, Endotoxin and Carbon administered intravenously

THE splenic uptake of intravenously introduced sheep erythrocyte stroma in mice is increased if certain finely divided agents (carbon, trypan blue, saccharated iron oxide, thorotrast, polystyrene latex) are injected by the same route simultaneously or before the administration of the stroma<sup>1</sup>. It seemed of interest to investigate whether the distribution of endotoxin in organs was altered in similar conditions.

Crude endotoxin ("68 per cent ethanol-precipitated aqueous ether extract"<sup>2</sup>), from *E. coli*, strain McElroy, was labelled with iodine-131 by a method similar to that of Greenwood *et al.*<sup>3</sup>; it was precipitated immediately with two volumes of chilled ethanol and the precipitate was washed with 68 per cent ethanol. The yield from 5 mg of crude endotoxin was dissolved in 10–15 ml. of saline; rabbits were given 1 ml., and mice 0.5 ml., intravenously. A sample was incubated at 37° C for 24 h and aliquots were treated with two volumes of ethanol; 86 per cent to 90 per cent of the radioactivity remained precipitable. The addition of two volumes of ethanol did not precipitate the iodine-131 which was not bound to endotoxin.

Iodine-131 was determined with Ekco equipment; whole mouse organs and aliquots of homogenized rabbit

organs were counted. To estimate iodine-131 in organs of rabbits given both thorotrast and labelled endotoxin, two counts were made with 8 days between them and the difference was multiplied by a factor of 1.99, the mean reduction in radioactivity in the organs of control rabbits given tagged endotoxin only.

The effect of trypan blue (Hopkin and Williams) and of thorotrast (Fellows 'Testagar') on the splenic and hepatic uptake of labelled endotoxin was examined in rabbits (Table 1), and the effect of thorotrast and of endotoxin was tested in mice (Table 2). Injections were given intravenously; trypan blue and thorotrast were administered 2 h, and the unlabelled endotoxin 1 day, before the labelled endotoxin. An hour after the injection of the labelled endotoxin, a rabbit given thorotrast and one of the controls died. Two hours after the injection, two rabbits appeared moribund; one of these had been given thorotrast and the other was a control. Two hours after the injection of the labelled endotoxin, the mice and the surviving rabbits were killed.

The uptake of iodine-131 in the spleens and livers of rabbits given trypan blue or thorotrast was consistently higher than in the controls, irrespective of sex and in spite of the considerable variations in body weight. The proportionate increase in uptake was greater in the spleens than in the livers. In mice given thorotrast, similar results were obtained; spleen counts in mice which had been pre-treated with endotoxin were twice as high as in the controls while the counts in the livers were only slightly larger.

In a further experiment, labelled endotoxin was injected intravenously into mice given Indian ink (0.5 ml., one in five dilution in saline of Günther Wagner 'Pelikan' ink C 11/1431a, containing approximately 10 mg carbon/dose) by the same route 2 h previously, and into controls. Groups of three treated and three untreated animals were killed 15 min, 1 h and 5.5 h after injection of the endotoxin and a group of untreated mice after 20 h; the iodine-131 was counted in the spleens, livers, neck tissues including the thyroid glands, and the hearts, lungs and kidneys (Table 3).

In the treated animals, the total iodine-131 found in the organs was larger after 15 min than in the controls; the splenic counts were undiminished at 5.5 h, although they had declined steeply in the controls. Counts in the livers, hearts, lungs and kidneys decreased in all groups; the fall was slower in the mice given carbon. In the neck tissues, counts were low early, then rose, more rapidly in the untreated than the treated mice. Certain forms of inorganic iodine are known to be taken up by the thyroid of the rat after intraperitoneal injection<sup>4</sup> and the ratios of iodide in serum: thyroid in mice and rats appear to be similar<sup>5</sup>. Rising iodine-131 counts in the neck were therefore probably a result of uptake by the thyroid gland of inorganic radioiodine released from the labelled endotoxin. In the treated mice, the delay in uptake of iodine-131 in the thyroid gland was probably caused by its slower release in the organs; thus, the metabolism of the iodinated endotoxin appeared changed.

An experiment of the same design, in which the treated mice received 0.5 ml. of endotoxin diluted one in fifty in saline 1 day before the labelled endotoxin, gave similar, but less marked, results.

The generalized Shwartzman reaction, elicited classically in rabbits by two intravenous injections of appropriate doses of endotoxin given a day apart, is characterized by

Table 1. SPLENIC AND HEPATIC UPTAKE OF LABELLED ENDOTOXIN IN RABBITS; EFFECT OF PRETREATMENT WITH TRYPAN BLUE OR THOROTRAST

Weight and sex of rabbit	Preliminary conditioning	Iodine-131 counts/sec/organ			
		Spleen		Liver	
910 g, female	Trypan blue 1 per cent 9 ml.	140		5,530	
1,470 g, female	" " " 15 ml.	98	206	4,850	5120
2,590 g, male	" " " 26 ml.	380		4,980	
1,020 g, ** female	None (control)	64		3,140	
1,580 g, female	" " "	46	61	2,640	2830
1,610 g, female	" " "	72		2,720	
2,990 g, male	Thorotrast 8.7 ml.	240		4,180	
3,340 g, * female	" 9.9 ml.	380	290	7,550	5580
4,850 g, * female	" 14.5 ml.	250		5,020	
3,510 g, * female	None (control)	26		2,020	
3,650 g, female	" " "	96	55	2,920	2450
4,029 g, male	" " "	44		2,420	

Injections were intravenous. Endotoxin was given 2 h after trypan blue or thorotrast and surviving rabbits were killed 2 h later.

Thorotrast (1 ml.) gave 375 counts/sec with the equipment set for counting iodine-131. The counts given represent iodine-131 only; for the method of estimation of the proportion of counts from the thorotrast and iodine-131, respectively, see text.

\* Died 1 h after injection of labelled endotoxin.

\*\* Moribund 2 h after injection of labelled endotoxin.

Table 2. SPLENIC AND HEPATIC UPTAKE OF LABELLED ENDOTOXIN IN MICE; EFFECT OF PRETREATMENT WITH THOROTRAST OR ENDOTOXIN

Preliminary conditioning	Interval	Between injection of conditioning agent and injection of labelled endotoxin	Between injection of labelled endotoxin and killing of mice	No. of mice in group	Mean iodine-131 counts/sec	
					Spleen	Liver
Thorotrast (0.5 ml., in 10)	2 h		2 h	5	48 (a)	880 (c)
None (controls)				5	28 (b)	470 (d)
Endotoxin (0.5 ml., in 10)	1 day		2 h	9	37 (e)	238 (g)
None (controls)				9	17 (f)	202 (h)

All injections were intravenous. Significance of differences between means: (a) vs (b):  $0.01 < P < 0.02$ ; (c) vs (d), (e) vs (f) and (g) vs (h):  $P < 0.01$ .



Table 3. FATE OF LABELLED ENDOTOXIN IN MICE AFTER INTRAVENOUS INJECTION: EFFECT OF PRETREATMENT WITH INDIAN INK

Preliminary conditioning	Interval between endotoxin administration and killing of animals	Spleen	Mean iodine-131 counts/sec (three mice)					Iodine-131 accounted for (per cent of that injected)	
			Liver	Heart, lungs, kidneys	Neck tissues, including thyroid gland				
Indian ink		720	7,490	1,180	58				
None (controls)	15 min	380	5,920	690	37	66			50
Indian ink	1 h	960	4,880	2,030	460	48			
None (controls)	5-5 h	830	3,300	510	590	37			24
Indian ink		82	1,000	360	1,480				20
None (controls)	20 h	22	530	170	4,940				40

Indian ink 0.5 ml, diluted 1 in 5 in saline (10 mg of carbon/dose) was injected intravenously. Labelled endotoxin was injected 2 h later into all animals.

capillary thrombi, those in the renal glomeruli leading to cortical necrosis<sup>6</sup>. Thorotrast, trypan blue<sup>7</sup>, Indian ink and saccharated iron oxide<sup>8</sup> have been used successfully in place of the first dose of endotoxin; it has been suggested that these substances block the reticulo-endothelial system, and thus prevent the removal of the endotoxin from the circulation<sup>8</sup>. In pregnant rats, however, the activity of the reticulo-endothelial system was found to be increased, yet susceptibility to the deposition of thrombi in the renal glomerular capillaries was greater<sup>9</sup>; in mice which were given thorotrast intravenously, labelled endotoxin injected by the same route after certain intervals of time was deposited in the liver in quantities greater than in the controls<sup>10</sup>. Endotoxin was injected intravenously into rabbits and 1 day later endotoxin labelled with chromium-51 was injected into these animals and into untreated controls<sup>11</sup>. In the treated rabbits, the splenic uptake of chromium-51 was larger and the hepatic uptake was somewhat smaller.

The experiments reported here suggest that the more severe effects of endotoxin in rabbits previously treated with agents such as carbon, thorotrast and trypan blue<sup>8</sup> were caused not by reduced uptake of endotoxin by reticulo-endothelial organs but, rather, by increased uptake and delay in the disposal of the endotoxin lodged in the liver and spleen; this delay could itself have contributed to the presence of larger amounts of endotoxin in the spleens and livers of treated animals. The findings may be relevant to the mechanism of the generalized Schwartzman reaction, since the preliminary injection of endotoxin also led to increased uptake in the spleen and liver of a further dose of endotoxin given on the following day, and to delay in the clearance of endotoxin injected in the second dose.

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## CYTOLOGY

### Internal Asynchrony in Late Replicating X Chromosomes

DURING investigation of patients with Klinefelter's syndrome, autoradiography of chromosome preparations was carried out in a number of cases. The main object of this

investigation was to determine the number and nature of late replicating chromosomes, and the results agree with the assumption that one middle group chromosome replicates late in the S phase in individuals with forty-seven (XXY) and forty-six (XX) chromosomes, two in those with forty-eight (XXXY) chromosomes and three in those with forty-nine (XXXXY) chromosomes. In addition, it was found that in many instances the late replicating chromosomes had a peculiar internal labelling pattern.

Internal asynchrony has been recorded in late replicating X chromosomes<sup>1</sup>, but the consistent pattern described later was not seen<sup>1</sup>. In lightly labelled cells the centromeric region was found to be relatively free of label<sup>2</sup>.

Three types of chromosomes could be distinguished during the present investigation. One was labelled uniformly on the whole length of the chromosome. Another showed a distinct "gap" in the labelling over the centromeric region (Fig. 1). Finally, some chromosomes showed an intermediary pattern.

The chromosome investigations were carried out in most instances on blood cells<sup>3</sup>, in one case on fibroblasts<sup>4</sup>. Tritiated thymidine was added to the suspension 5-6 h before collection of the cells giving a final concentration of 1 µc./ml. 'Colcemid' was added 3 h later. The slides were covered with liquid emulsion (Ilford K 5). Cells which were well spread and showed an intermediate amount of labelling were photographed. The silver grains were then removed<sup>5</sup>, the cells were restained with Giemsa, and compared with the photographic pictures. A total of fourteen individuals was investigated. The results are listed in Table 1.

Table 1

Diagnosis	Karyotype	No. of patients	No. of X chromosomes investigated	No. of these showing definite "gap"
Klinefelter's syndrome skin cultures	47 (XXY)	1	14	3 (21.4 per cent)
Klinefelter's syndrome blood cultures	47 (XXY)	7	105	51 (48.6 per cent)
" "	48 (XXXXY)	1	56	32 (57.1 per cent)
" "	49 (XXXXXY)	1	69	31 (44.9 per cent)
" "	46 (XX)	1	22	12 (54.5 per cent)
Normal females blood cultures	46 (XX)	3	54	30 (55.6 per cent)
Total		14	320	159 (49.7 per cent)

Approximately half of the observed X chromosomes showed a definite "gap" in the labelling over the centromeric region. Only three presumed X chromosomes showed a similar gap outside this region, in all instances on the long arms. No connexion with secondary constrictions could be demonstrated. These only occurred in 4 per cent of the late replicating X chromosomes. A less distinct labelling pattern was seen in 18 per cent of the late replicating X chromosomes. The rest was diffusely labelled on the entire length of the chromosome. The skin cells showed rather few (3/14) characteristically labelled chromosomes. This may be the result of a different rate of synthesis in the fibroblast cultures. The behaviour of the late replicating X chromosomes in the poly-X Klinefelter's is of special interest. In the subjects with forty-eight (XXXY) chromosomes, twenty-six cells were investigated. In seven of these both labelled X chromosomes showed a definite gap, in nine only one chromosome, and in ten none. In the patient with forty-

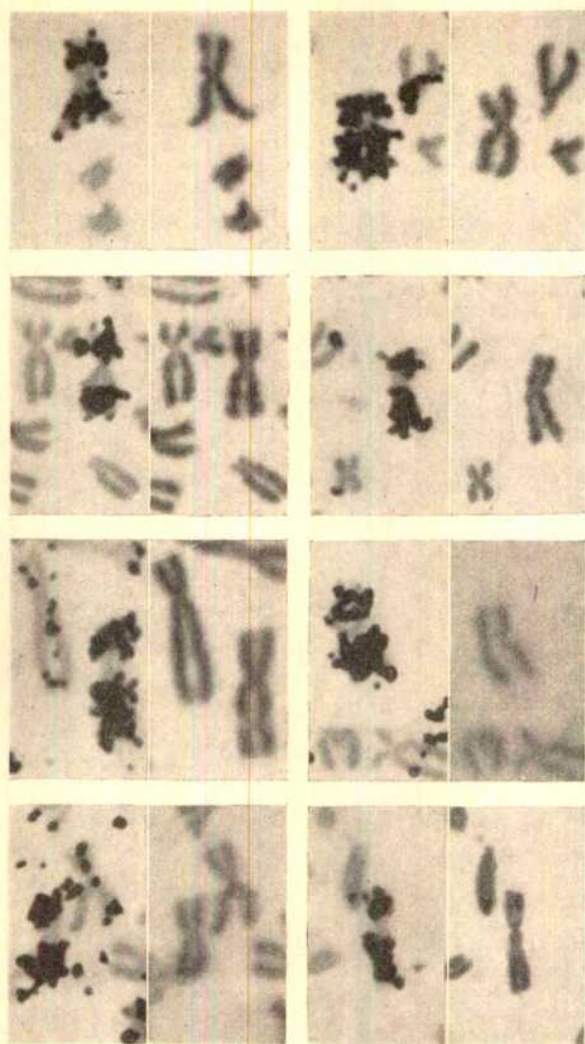


Fig. 1. Eight late replicating X chromosomes showing a gap in the pattern of labelling. The chromosomes are shown covered with silver grains, and after these have been removed and the chromosomes re-stained. ( $\times$  c. 1,000.)

nine (XXXXY) chromosomes, twenty-three cells were distributed as follows. Four cells showed the pattern in three chromosomes, five in two, nine in one, and five showed no chromosomes with a distinct "gap" in the centromeric region.

The distribution between the cells showing two, one or none in patients with forty-eight (XXXY) chromosomes and three, two, one or none in those with forty-nine (XXXXY) chromosomes is not significantly different from the distribution that would be expected if the labelling pattern occurred at random with a frequency of 0.5. The X chromosomes in these cases may not be exactly parallel in their time sequence of replication.

The significance of the internal asynchrony of the X chromosome is not clear. Only future research will show whether the phenomenon has anything to do with the assumption that not all parts of the late replicating X chromosome are genetically inactive, for example, the  $Xg^+$  locus<sup>6</sup>.

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## BIOCHEMISTRY

### Lactate Dehydrogenase Isoenzymes in Chicken Tissues

LACTATE dehydrogenase isoenzymes from a variety of animal sources have been reported to differ in a number of their properties, including substrate affinities<sup>1,2</sup>, utilization of coenzyme analogues<sup>3</sup>, substrate specificity<sup>4</sup>, thermal stability<sup>5</sup> and sensitivity to inhibitors<sup>6,7</sup>. A regular gradation in these properties has been found which appears to correlate with the difference in electrophoretic mobility of each isoenzyme fraction. This difference is thought<sup>8,9</sup> to be determined by their A and B sub-unit composition. According to these workers,  $LD_1$  and  $LD_5$  are "pure types" comprising four B and four A sub-units, respectively, and  $LD_{2-4}$  are intermediate hybrid types. Cahn *et al.*<sup>9</sup> report the presence of five lactate dehydrogenase isoenzymes in young chicken tissues, the heart muscle isoenzyme being a tetramer of four B sub-units and the breast muscle isoenzyme a tetramer of four A sub-units. We could not confirm the existence of five bands of activity in adult tissues, and this communication reports studies on isoenzymes derived from adult chicken tissues which showed different properties but similar electrophoretic mobility.

Adult chickens were killed by breaking their necks, the tissues excised within 10–15 min and stored at  $-18^\circ\text{C}$  until required, except for two birds, the tissues of which were used immediately. Extracts were prepared in ice cold 0.067 molar Sørensen phosphate buffer, pH 7.4, using a Potter–Elvehjem homogenizer. After centrifuging at 6,000g for 20 min at  $4^\circ\text{C}$ , the supernatants were removed for enzyme analysis. Cellulose acetate electrophoresis was carried out according to Kohn<sup>10</sup> and the isoenzymes located by a tetrazolium staining technique<sup>11</sup>. For isoenzyme studies, the strips were cut into 3 mm sections and eluted with phosphate buffer. Polyacrylamide gel electrophoresis was carried out in a vertical apparatus<sup>12</sup> at pH 9.2. Starch-block electrophoresis in barbitone buffer (I 0.1; pH 8.6) was used as a quantitative procedure, the isoenzymes being eluted from 1 cm strips as described by Plummer *et al.*<sup>13</sup>. Dehydrogenase activities were determined spectrophotometrically at pH 7.4 using final concentrations of 0.7 mmolar pyruvate<sup>14</sup> or 3.3 mmolar 2-oxobutyrate<sup>4</sup> and 0.35  $\mu\text{moles}$   $\text{NADH}_2$ . In the inhibition experiments, potassium oxalate or urea to give final concentrations of 0.2 mmolar and 2 molar, respectively, were incorporated into the reaction mixture (3.0 ml.). Enzyme-inhibitor mixtures were allowed to stand for 30 min at  $25^\circ\text{C}$  before determining the activity at 340 m $\mu$  in a spectrophotometer.

The isoenzyme patterns of chicken heart and breast muscle are shown in Fig. 1 together with that of a human haemolysate obtained by starch-block electrophoresis. The single band from each of the chicken tissues had the same mobility and coincided with that of human  $LD_4$ , but the activity of the heart isoenzyme with 2-oxobutyrate as substrate was much greater than that of the breast isoenzyme. Electrophoresis on cellulose acetate at pH 8.6 and on polyacrylamide gel at pH 9.2 of extracts of all the principal chicken organs from five different birds showed one chief band of activity with a mobility very similar to that of human  $LD_4$ . In one hen and one cock bird, in the kidney and liver extracts only, traces of two other isoenzyme bands, with approximately the mobility of human  $LD_1$  and  $LD_2$  were detected.

Michaelis constants for pyruvate and 2-oxobutyrate of the partially purified heart and breast isoenzyme fractions were determined using Lineweaver–Burk plots, and the results, together with those for the crude tissue extracts and those for human  $LD_1$  and  $LD_5$ , are shown in Table 1. The heart isoenzyme has an affinity for pyruvate four and a half times greater than that of the breast isoenzyme, and an affinity for 2-oxobutyrate seven times greater.



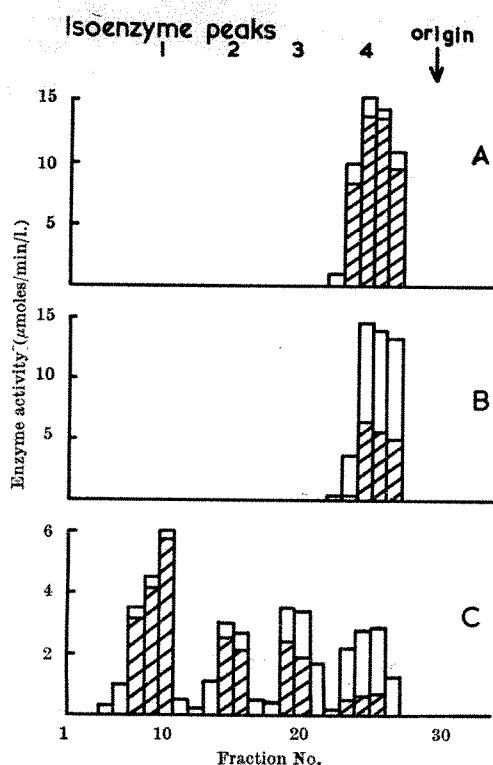


Fig. 1. Lactate dehydrogenase isoenzymes of chicken heart (A), chicken breast (B), and human haemolysate (C), obtained by starch-block electrophoresis in barbitone buffer, pH 8.6. Substrates: 0.7 mmolar pyruvate (—) and 3.3 mmolar 2-oxobutyrate (///).

The  $K_m$  values of the heart isoenzyme are very similar to those of  $LD_1$ , while the values for breast for both substrates are slightly lower than for  $LD_5$ . The other tissues had intermediate values, which were, however, closer to those for heart. This difference in substrate affinities is also reflected in the different ratios of activity with 2-oxobutyrate to those with pyruvate. Heart isoenzyme had the highest activity ratio and breast the lowest with the other lying between the two.

Oxalate has been shown to inhibit selectively human  $LD_1$  while having little effect on  $LD_5$  (ref. 15), and was found to differentiate similarly between chicken heart and breast isoenzymes (Table 1) although the chicken tissues were less affected than the human. In the same way 2 molar urea, which preferentially inhibits  $LD_5$  (ref. 16), inhibited the chicken breast isoenzyme much more than the heart isoenzyme (Table 1), although both were less sensitive to urea than human  $LD_1$  and  $LD_5$ .

Table 1. SOME PROPERTIES OF CHICKEN AND HUMAN TISSUE LACTATE DEHYDROGENASE ISOENZYMES

Source of tissue	$K_m$ (mmoles)	Ratio of dehydrogenase activities: 2-oxo-butyrate/pyruvate	% Inhibition produced by 0.2 mmolar oxalate (pyruvate)	% Inhibition produced by 2 molar urea (2-oxo-butyrate)
Chicken:				
heart	0.095	0.98	0.85	71
kidney	0.110	1.28	0.73	67
ova	0.110	1.45	0.78	63
red cells	0.120	1.43	0.80	58
liver	0.130	1.28	0.71	61
spleen	0.097	1.33	0.73	59
testis	0.150	1.50	0.77	63
leg muscle	0.200	1.26	0.61	45
breast muscle	0.340	6.30	0.37	24
Chicken:				
heart isoenzyme	0.084	0.90	0.89	67
breast isoenzyme	0.390	6.40	0.40	25
Human:				
$LD_1$	0.080	0.84	0.95	70
$LD_5$	0.830	10.00	0.31	32

All results are the mean of duplicate determinations on three birds. Human  $LD_1$  was obtained from heart muscle, and  $LD_5$  from skeletal muscle.

This finding of one band of activity with the same mobility in different tissues is anomalous. It suggests either that the difference in electrophoretic mobility is so small as to be undetectable by what are usually very effective techniques, or that the intermediate hybrid isoenzymes are very unstable, or that they are present in the young bird and disappear in the adult.

These results do, however, support the idea that chicken heart and breast muscle isoenzymes constitute extreme types of lactate dehydrogenase and that although they have the same or nearly identical electrophoretic mobility, they have different properties strongly suggestive of different sub-unit composition resembling B and A sub-units, respectively.

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### Uptake of 5-Iodo-deoxyuridine by a Single Colony of Developing Embryos of *Arbacia punctulata*

PREVIOUS investigations have shown that 5-iodo-deoxyuridine (IUDR), an analogue of thymidine, is incorporated into DNA and within certain limitations can be used as an indicator of DNA synthesis (refs. 1-4, and work of Kreuger and others in preparation). Details of incorporation and elimination of iodo-deoxyuridine in various cells of mice may be found in a review<sup>4</sup>. According to the authors IUDR is a "convenient, sensitive, and specific tracer of DNA metabolism *in vivo*". Incorporation of IUDR labelled with iodine-131 has been used as an indication of the rate of DNA synthesis at different stages of the developing sea urchin embryo<sup>5</sup>. The facility of detecting the  $\gamma$ -radiation of iodine-131 made it seem possible to use iodine-131 labelled IUDR to follow DNA synthesis in the same group of living cells without fixation, extraction, or digestion of the cells. This communication pursues the use of repetitive exposure to labelled IUDR of a single colony of sea urchin embryos as a method for the investigation of developmental changes in the rate of DNA synthesis in the living cell.

Eggs and embryos of the sea urchin, *Arbacia punctulata*, were used. Each experiment was confined to the eggs obtained from a single female. The eggs were collected, washed, fertilized, and suspended in 500 ml. of sea water at the prevailing temperature of sea water (19° C–20° C)<sup>5</sup>. After fertilization, 20 ml. of the embryo suspension was taken to be used as the continuous culture (that single colony of embryos which was to be exposed to

repeated "pulses" of labelled IUDR). After incubation in labelled IUDR (121  $\mu\text{Ci/l.}$ ) for 30 min, the colony of embryos was washed four times in sea water, counted in a well-type  $\gamma$ -ray spectrometer, and maintained in 20 ml. of sea water until the next pulse. From the original embryo suspension, periodic 20 ml. aliquots were removed, incubated in labelled IUDR for 30 min, washed four times in sea water, and counted. The labelled IUDR was prepared and diluted as described previously<sup>5</sup>. Concentrations of labelled IUDR are given in Table 1.

Table 1. UPTAKE OF LABELLED IUDR BY EMBRYOS

Time after fertilization (h)	Individual groups of embryos		Single colony of embryos	
	Aliquots (c.p.m.)	Aliquots summed (c.p.m.)	Cumulative pulses (c.p.m.)	Uptake/pulse (c.p.m.)
3.5	5,590	5,590	5,216	5,216
4.5	4,526	10,116	10,821	5,105
5.5	5,910	16,026	16,083	5,762
9.0	9,842	25,868	22,941	6,858
12.0	8,059	33,927	29,849	6,908
14.0	7,975	41,902	32,246	2,397

In experiments in which a single colony of embryos was repeatedly incubated with labelled IUDR at progressive stages of development (those exposed to pulses of the radioactive compound) the embryos took up increasing amounts of labelled IUDR with each pulse. Table 1 presents the amount of radioactivity remaining in the embryos after each pulse, after they have been washed in sea water to remove all activity that can diffuse from the embryos. Thus, the c.p.m. represent the sum of two fractions of radioactivity, the acid-soluble fraction, which contains the precursor pool of intermediary DNA metabolites (which apparently is very small), and the acid-insoluble fraction containing DNA<sup>5</sup>. The last column in Table 1 shows the amount of uptake consequent to each pulse of labelled IUDR.

Simultaneously with the administration of each pulse to the continuous colony, an aliquot of embryos from the original suspension was taken and treated similarly. These control experiments were carried out to test the possible effects of repeated administration of labelled IUDR and repeated washing. In Table 1, the amount of uptake at each developmental stage is shown and the sums of these individual measurements are compared with the values obtained in the pulse experiment.

Preliminary investigation of the stability of labelled IUDR in sea water indicates that the molecule undergoes changes when it remains in solution for as long as 24 h (20° C). Very little labelled IUDR is incorporated by the embryos from such solution. This effect on labelled IUDR could be caused by bacterial action.

In these experiments involving the use of consecutive pulses of labelled IUDR with a single colony of developing embryos, it is anticipated that the amount of uptake of labelled IUDR will be cumulative and be related to the rate of DNA synthesis of the embryos. Table 1 shows such a cumulative response. Embryo aliquots incubated simultaneously with the administration of each pulse quantitatively demonstrate the amount of uptake which occurs at a given time after fertilization. The sum of these individual aliquots is approximately equivalent to the cumulative amounts in the single colony. By 9 h, however, the single colony shows decreased uptake as compared with the individual aliquots. This may be an effect of the accumulation of labelled IUDR in the embryos of the single colony. It may also be caused by experimental error or loss of cells as a result of repeated washing.

The primary pathway of degradation of IUDR in mammalian systems is by way of iodouracil which is then de-iodinated<sup>6</sup>. Degradation of IUDR in sea water or in the sea urchin embryo has not been investigated. It was, however, observed that solutions of labelled IUDR, if not used within a week of synthesis, were markedly less effective in experiments with the embryos. Preliminary work shows that the stability of labelled IUDR changes if it is allowed to remain diluted with sea water for as long as

24 h. This, however, is not considered to be significant in investigations concerned with developmental stages to gastrulation (15–19 h after fertilization) if fresh solutions of labelled IUDR are made shortly before fertilization.

In a previous publication<sup>5</sup>, it was shown that the rate of incorporation of IUDR by sea urchin embryos increases up to about 16 h after fertilization. At this time it was found that the rate of incorporation begins to decrease and ultimately reaches a very low level. It may be that this decrease of the rate of incorporation, which was most evident during the second day of development, was caused in part by a change in the IUDR molecule, as a result of bacterial action.

The present experiments show that successive changes in the rate of uptake of labelled IUDR can be detected at different stages of development in the same group of living embryos. It is anticipated that these changes reflect primarily changes in the rate of DNA synthesis. Other factors, however, such as developmental changes in the facility with which the IUDR moves across the cell membrane, may be reflected in the results.

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### Hyperglycaemia induced by Insulin B Chain in Dietary Diabetes in Rats

WE have recently shown that the intraperitoneal injection of reduced insulin B chain complexed with an equal amount of crystalline bovine serum albumin produces a significant hyperglycaemia in normal rats<sup>1</sup>. In accordance with the theory proposed by Ensink, Mahler and Vallance-Owen<sup>2</sup> we assume that this effect is due to the inhibition of endogenous insulin activity by the complex of reduced insulin B chain and albumin.

The possibility of demonstrating the effect of the B chain in normal animals seems to make it more likely that this mechanism operates in diabetes, as, if increased insulin splitting is a factor in diabetes, there would be relatively less intact insulin and more B chain present. The obvious next step in the investigation of this phenomenon was to investigate the action of B chain in animals in a diabetes-like state. Alloxanized or depancreatized animals present well known variability and maintenance problems, and it was decided initially to try animals on a high fat, high protein diet. Such diets produce a condition analogous to "starvation diabetes", which has been quite thoroughly

Table 1. EFFECT OF REDUCED INSULIN B CHAIN-ALBUMIN COMPLEX ON THE BLOOD GLUCOSE OF RATS ON NORMAL AND DIABETOGENIC DIETS

No. of rats	Diet	Injection*	Blood glucose, mg/100 ml., mean $\pm$ standard error			
			Control	30 min	60 min	90 min
17	Normal	1 mg albumin	71 $\pm$ 1.8	75 $\pm$ 1.8	76 $\pm$ 1.1	80 $\pm$ 1.8
20	Normal	1 mg B chain + 1 mg albumin	68 $\pm$ 1.5	83 $\pm$ 2.0	89 $\pm$ 2.0	82 $\pm$ 1.7
		Significance, <i>P</i> value	> 0.2	< 0.02	< 0.001	> 0.4
8	Diabetogenic	1 mg albumin	80 $\pm$ 1.4	89 $\pm$ 3.0	85 $\pm$ 2.8	90 $\pm$ 4.1
9	Diabetogenic	1 mg B chain + 1 mg albumin	76 $\pm$ 1.5	113 $\pm$ 3.7	131 $\pm$ 4.3	108 $\pm$ 4.8
		Significance, <i>P</i> value	< 0.1	< 0.001	< 0.001	0.02

\* All injections were in 1 ml. of 0.0062 molar cysteine - 0.02 molar *tris* buffer and two of each were given, one at time zero and one at 31 min.

investigated since it was first described by Claude Bernard in 1859<sup>3</sup>. A number of metabolic changes resembling those which occur in human diabetes have been observed in these conditions, among them abnormally high and prolonged glucose tolerance curves, a reduced blood glucose response to insulin, a reduced glucose uptake by muscle tissue, an increase in muscle phosphorylase *a*, and a decrease in the concentration of insulin in the pancreas<sup>4,5</sup>.

The mechanisms involved in these effects are not yet clear, but the work of Randle *et al.*<sup>6</sup> and other investigators suggests that the higher rate of fatty acid metabolism in the absence of dietary carbohydrate decreases the availability of endogenous insulin.

In previous metabolic investigations, we have maintained weanling rats on the high fat, high protein diet used here, and although abnormal glucose tolerance curves were observed throughout, the animals remained in reasonably good health for up to 4 months<sup>7</sup>.

Male Sprague-Dawley rats weighing 170-200 g were fed *ad libitum* for 7 days on a diet of the following composition: 315 g high nitrogen casein; 620 g hydrogenated vegetable oil; 1 g salt mixture<sup>8</sup>; 2 g choline chloride; and 1 g vitamin mixture<sup>9</sup>. Other animals in the same weight range were fed a standard laboratory chow pellet diet. *S*-sulpho B chain was prepared from crystalline bovine insulin, reduced with reducing solution in a hot water bath, and combined with crystalline bovine serum albumin as previously described<sup>1</sup>. Only *S*-sulpho B chain prepared according to the procedure of Dixon and Wardlaw<sup>9</sup> was used in the experiments reported here. *S*-sulpho B chain prepared by the Swan procedure<sup>10</sup>, which had shown some insulin inhibitory activity in the mitochondrial assay, was ineffective *in vivo* under the conditions used.

After an 18 h fast, the animals were injected intraperitoneally with 1 ml. of 0.0062 molar cysteine-0.02 molar *tris*(hydroxymethyl)aminomethane solution, pH 8.6-8.9, containing either 1 mg of reduced B chain plus 1 mg of albumin or 1 mg of albumin alone. A second injection of either the same amount of reduced B chain and albumin or albumin alone was given after the 30 min blood sample had been taken. Blood samples (0.1 ml.) were taken from the heart under light ether anaesthesia before the first injection and 30, 60 and 90 min thereafter. Blood glucose levels were determined by 'Technicon AutoAnalyzer' method N-9.

The results are shown in Table 1. The hyperglycaemia previously observed in normal rats<sup>1</sup> was again seen in this experiment. In the animals fed on chow, the increases in blood sugar over the control values at 30, 60 and 90 min for the group treated with reduced B chain-albumin were 22, 31 and 21 per cent respectively, in comparison with the increases of 6, 7 and 13 per cent respectively for the group treated with albumin at the same intervals of time. The Student *t* test shows a significant difference at 30 min and a highly significant difference at 60 min between the two groups. The difference at 90 min is not significant, and probably reflects an offsetting of the reduced B chain inhibition by an increased secretion of insulin.

In the animals on the high fat, high protein diet the increases in blood glucose in the group given reduced B chain-albumin, calculated from the control values at 30, 60 and 90 min, were 49, 72 and 42 per cent respectively, in comparison with increases of 11, 6 and 12.5 per cent respectively for the group treated with albumin. The differences

between the reduced B chain-albumin and the albumin groups were highly significant at 30 and 60 min and significant at 90 min.

An explanation for these results other than inhibition of insulin activity by B chain would be that the preparation injected produced hyperglycaemia through a non-specific effect such as adrenal stimulation. Such an effect is unlikely, however, because the injection of B chain alone, *S*-sulpho B chain with albumin, or B chain-albumin from which the reducing solution had been dialysed did not produce hyperglycaemia.

The results suggest that the B chain does not have the configuration or chemical properties required for an inhibitory effect unless it is actively maintained in the reduced state and complexed with an agent such as albumin. When these conditions are met, and when a sufficiently large excess is given to provide for oxidation and other modes of attrition which can occur after injection, the anti-insulin effect can readily be demonstrated.

The enhanced inhibitory effect of insulin B chain in the diabetes-like state induced by the high fat, high protein diet seems to add weight to the hypothesis that this mechanism is important in true diabetes.

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### Histochemical Localization of Intestinal Disaccharidases: Application to Peroral Biopsy Specimens

THIS communication describes a technique for localizing intestinal disaccharidases developed from that of Dahlqvist and Brun<sup>1</sup>. Intestinal biopsies were obtained by a multi-purpose suction biopsy tube<sup>2</sup> from four normal children and five patients with sucrose and isomaltose "intolerance". Intestinal specimens from rats and guinea-pigs have also been investigated by the same procedure. The biopsies and animal tissues were rapidly frozen and stored at -25°. Sections from 8 $\mu$  were cut in a cryostat, mounted on coverslips and fixed in cold acetone for 15 min.

The chromogenic reagent (I) was prepared by a solution in phosphate buffer (pH 6.5; 0.1 molar) of the following reagents: 5 mg/ml. gelatine; 0.05 mg/ml. phenazine methosulphate; 0.33 mg/ml. tetranitroblue-tetrazolium (TNBT); and 0.2 mg/ml. glucose oxidase.



The substrate solutions (II) were made up with 100 mg of saccharose, lactose or palatinose per ml. of distilled water. When maltose and trehalose were used as substrates, the concentrations were 25 mg and 50 mg per ml. respectively.

The mounted sections were placed on the flat lid of a tissue culture vessel and 0.05 ml. of a concentrated solution of glucose oxidase (4 mg/ml. phosphate buffer pH 6.5, 0.1 molar) were deposited on each. They were then covered with a piece of thin rapid filter paper, previously cut to the shape of the lid and moistened with 0.2 ml. of the chromogenic reagent (I). The vessel containing 1 ml. of chromogenic reagent (I) and 0.1 ml. (0.2 ml. for lactose) of the appropriate substrate solution (II) was closed by the cover to which the coverslip and filter paper remained attached. The vessel was then sealed with silicone grease, shaken vigorously and turned upside down.

Incubation for 3–6 h at 37° generally gave a suitable brown stain. Thereafter, the sections were washed thoroughly in hot water (45°) and mounted in 'Apathy' syrup.

Control sections were incubated in a staining solution from which the substrate had been either omitted or replaced by melibiose, a non-hydrolysable disaccharide.

Some sections were treated before incubation with sulphhydryl inhibitors such as *p*-chloromercuribenzoate (PCMB) in order to inhibit the non-specific staining of sulphhydryl compounds. These sections were dipped for 15 min in phosphate buffer at pH 7.4 containing 0.0025 molar PCMB and then washed in phosphate buffer at pH 6.5.

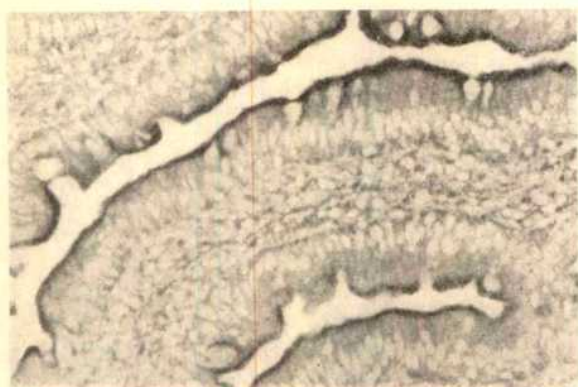


Fig. 1. Maltase activity in normal duodenal biopsy. ( $\times c. 165$ .)

Our modifications to the original procedure of Dahlqvist and Brun were devised to reduce diffusion of glucose as far as possible. This was achieved by increasing the concentration of glucose oxidase and by using a filter paper which maintains a high glucose oxidase concentration close to the site of enzyme activity. Otherwise, the filter paper moistened with gelatine solution (I) retains a superficial precipitate which results from the unavoidable diffusion of a small amount of glucose. Finally, the use of TNBT instead of nitroBT gives an improved staining picture because the precipitate of TNBT formazan is finer and far more localized<sup>3</sup>.

The results of this histochemical procedure are similar in human normal duodenal mucosa, as well as in guinea-pig mucosa, whether maltose, saccharose or palatinose is used as the substrate. The reaction, however, is stronger with maltose. The precipitate is accurately localized in the brush border of the villous epithelial cells whereas the staining is absent from or weak in the cytoplasm (Fig. 1). There is no stain in the crypts. Precipitate is found in some cells of the lamina propria. In the muscularis mucosae and the submucosal vessels, a faint staining is in all likelihood non-specific since it does not occur after treatment by PCMB.



Fig. 2. Normal trehalase activity. ( $\times c. 65$ .)

The activity of trehalase is also intense in the brush border, but the reaction is rather localized near the tip of the villi (Fig. 2). With lactose as the substrate, a stain is obtained not only in the brush border but also in the apical cytoplasm of the villous epithelial cells (Fig. 3).

Control sections of human and guinea-pig material incubated without substrate give consistent negative results, whereas rat intestinal sections incubated in the same manner develop a stain throughout the cytoplasm of the epithelial cells. This non-specific staining is blocked by previous treatment with PCMB.

Sections from tissue without disaccharidase activities always failed to react significantly. This result excludes any interference of disaccharidases which may contaminate the glucose oxidase preparations in the staining process<sup>1</sup>.

Intestinal biopsies of patients with congenital sucrose and isomaltose "intolerance", have normal trehalase and lactase activities, whereas they react weakly with maltose and not at all with saccharose and palatinose. These results are in close agreement with the quantitative enzyme determinations in tissue homogenate<sup>4</sup>.

Our improved staining method can thus be applied to the demonstration and localization of disaccharidase activities in both normal and pathological intestinal biopsies. This technique shows particularly that the disaccharides splitting enzymes are primarily if not

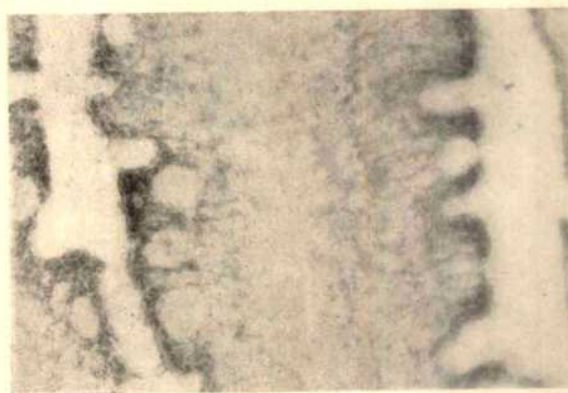


Fig. 3. Normal lactase activity. ( $\times c. 265$ .)

exclusively localized in the brush border in accord with the biochemical findings<sup>5,6</sup>.

Detailed reports will be published elsewhere.

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### Composition of Membranous Tissues obtained from Tendon and Skin

THE presence of a sheath surrounding the collagen fibrils of skin and tendon was first shown histologically by Kaye<sup>1</sup> in 1925, and its restrictive effects on swelling and eventual rupture were demonstrated by Jordan Lloyd and Marriott<sup>2</sup>. Recently, Kwon, Mason and Rigby<sup>3</sup> have again directed attention to the presence of this sheath in tail tendon and have pointed out its probable effect on hydrothermal shrinkage as well as on swelling.

A plentiful supply of kangaroo tail tendons has made it possible to isolate sufficient of the sheath to carry out an amino-acid analysis and limited histological and electron microscope examination. The tendons were removed from the tails in Australia, washed several times in 5 per cent sodium chloride, then in water, and dehydrated in acetone for transport. After resoaking in water, the tendons were swollen in 0.1 molar acetic acid, and by splitting the sheath longitudinally down one side it was possible to remove it from the bulk of the tendon fibres.

The membrane stained with acidic and basic dyes in a similar manner to collagen and there were indications of a very fine fibrillar structure. Numerous cells could be seen, but there was no evidence of the presence of elastin. A few larger collagen fibrils were visible on the surface but were not included in the ground structure of the membrane; these presumably arise from the tendon proper. Direct and microscopical observations both indicated that these extraneous fibrils represented less than 10 per cent of the total material. Electron micrographs were typical of connective tissue systems, showing a few fibrils with characteristic collagen banding, together with a generalized matrix or continuum<sup>4</sup>.

A sample of sheath (30 mg) was hydrolysed with 5 ml. 6 normal hydrochloric acid at 105° C for 18 h. The amino-acid composition was determined using an automatic analyser and the elution procedure described by Hannig<sup>5</sup>. Hydroxyproline was determined directly by the method of Stegemann<sup>6</sup> modified for use with the 'Technicon' autoanalyser.

The amino-acid composition of the sheath is clearly different from that of the tendon as a whole. The hydroxyproline content indicates the presence of about 65 per cent collagen, more than can be accounted for by contamination with collagen fibrils from the tendon, together with about 35 per cent of a non-collagenous protein. Some indication of the composition of this non-collagenous fraction can be obtained by subtracting the contribution due to the collagen. The results so obtained expressed in terms of residues per 1,000 total residues are given in Table 1. The protein shows certain resemblances to other non-collagenous protein fractions obtained from collagenous tissues, for example, the protein extracted from skin by dilute alkali<sup>7</sup> and the protein of the chondroitin sulphate complex of cartilage<sup>8</sup>. It differs in containing an appre-

Table 1. COMPOSITION OF THE TENDON SHEATH AND OF THE MEMBRANE UNDERLYING THE SKIN

	Amino-acid nitrogen as per cent total nitrogen			Residues per 1,000 residues corrected for collagen content*		
	Tendon	Tendon sheath	Membrane from flesh surface of sheepskin	Tendon sheath	Membrane from skin	Alkali soluble protein
Hydroxyproline	8.02	5.35	6.91	0	0	0
Aspartic acid	3.57	4.84	4.34	138	124	121
Threonine	1.51	2.45	2.11	62	85	53
Serine	2.85	3.31	2.81	85	57	68
Glutamic acid	5.76	6.27	5.94	133	93	112
Proline	9.54	7.26	8.18	14	0	53
Glycine	24.51	17.68	23.30	20	20	84
Alanine	8.55	7.20	8.30	72	77	68
Cystine	0.85	0.57	0.67	33	33	0
Valine	2.01	2.01	2.61	55	123	68
Methionine	0.58	0.65	0.63	22	28	8
iso-Leucine	0.70	1.36	1.37	40	53	56
Leucine	2.02	2.89	2.89	81	124	104
Tyrosine	0.34	0.79	0.65	35	42	38
Phenylalanine	1.27	1.32	1.48	37	61	33
Histidine	1.01	1.84	1.02	20	1	24
Lysine	3.86	5.52	5.05	76	70	53
Hydroxylysine	1.04	0.56	0.56	17	0	0
Arginine	14.72	14.52	13.63	60	10	49
Ammonia	3.64	5.23	4.54	188	148	109
Total	95.85	91.42	96.99			

\* Corrected on the basis that the hydroxyproline nitrogen in collagen represents 8.22 per cent of the total nitrogen and that all hydroxyproline arises from collagen<sup>10</sup>.

ciable amount of cystine, which no doubt accounts for its greater insolubility compared with collagen. The rather low recovery of nitrogen as amino-acids and the low nitrogen content—15–16 per cent on dry weight—suggest the presence of mucoid material. It seems probable, therefore, that the tendon sheath, like renal reticulin<sup>9</sup>, is composed of very fine collagen fibrils, but in this case embedded in a matrix of a non-collagenous type protein, probably a mucoprotein, rather than in predominantly lipid material.

A membranous tissue can also be peeled off from the underside of many types of skin. Some of this material removed from skin treated with alkali was also examined. It appears to consist of collagen together with about 16 per cent of another protein. The composition of this protein is also similar to that of the tendon sheath, but differs in the apparent absence of proline and in its high contents of leucine and valine. In spite of the alkali treatment, it still contained between 0.8 and 1 per cent of hexosamine on a dry weight basis.

It would seem, therefore, that there is a variety of membranous structures in collagenous tissues which consist predominantly of very fine collagen fibrils embedded in a matrix containing non-collagenous protein, mucoid and in some cases lipid material. The function of such sheaths is presumably to regulate penetration, swelling, and generally to maintain strength and stability. Certainly *in vitro* the presence of such sheaths plays an important part in such processes.

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## PATHOLOGY

### Inhibition of Tumour Formation with Adenovirus 12 Tumour Cells in Hamsters Immunized with Various Adenoviruses

TUMOUR production by adenovirus type 12 in newborn hamsters inoculated subcutaneously has been confirmed in several laboratories<sup>1,2</sup>. Eddy and co-workers<sup>3</sup> found inhibition of this tumour production in hamsters repeatedly inoculated with live adenovirus type 12 during the incubation period of tumour formation. More recently, Peries *et al.*<sup>4</sup> demonstrated inhibition of the formation of adenovirus 12 induced tumours in hamsters inoculated subcutaneously with a live heterotypic virus, adenovirus type 5. No inhibition occurred, however, when killed adenovirus type 5 was used. The mechanism of tumour inhibition in both sets of experiments was not established.

We have studied tumour formation in Syrian hamsters (*Mesocricetus auratus*) inoculated with tissue culture cells (*H212* line) derived in this laboratory from a hamster tumour induced by adenovirus type 12. This communication describes the inhibition of *H212* cell-induced tumours in hamsters immunized with either homotypic or heterotypic adenoviruses.

The *H212* cell line was established from a primary transplant of a hamster tumour developing 65 days after subcutaneous inoculation of the "Huie" strain of adenovirus type 12 cultivated in human embryo kidney cells. These cells have been grown *in vitro* for 2 years through ninety-nine serial subcultures and their cultivation and cellular characteristics will be reported elsewhere. *H212* cells resemble hamster cells transformed by adenovirus type 12 described by other workers<sup>5</sup> in morphology, freedom from infective adenovirus and the continuous production of virus-specific antigens<sup>6,7</sup>. These cells induce progressively growing tumours at the site of inoculation in hamsters. In contrast to adenovirus 12 induced tumours, which only develop after the inoculation of newborn animals, *H212* cells induce tumours in a proportion of hamsters aged 3 months or more.

The adenovirus strains\* used for the preparation of antigens for immunization experiments were grown in either human embryo kidney (*HEK*) or HeLa cell cultures maintained in Eagle's basal medium with 2 per cent inactivated calf serum. Infected cell cultures at a late stage of virus cytopathic effect were frozen and thawed three times and the fluids were centrifuged at 800*g* for 20 min to remove cell debris before storage at  $-20^{\circ}\text{C}$ . In some experiments inactivated adenovirus antigens were prepared by treating the fluids with formalin using the method of Hilleman *et al.*<sup>8</sup>. The live antigens were tested for tumour-inducing capacity by subcutaneous inoculation into hamsters at the same age as those used in the immunization experiments. No tumours developed in animals inoculated with the live virus antigens only.

Hamsters from a closed colony of random bred animals were used in the immunization experiments. Batches of animals of the same age were immunized by four subcutaneous injections of 0.2 ml. of the various antigens at 10, 14, 21 and 28 days of age. As a control to antigens containing virus, some batches of animals were not immunized while others received virus-free cultures of *HEK* or HeLa cells.

That the virus antigens had induced the formation of antibody was shown by tests for specific homologous neutralization using sera collected from animals at the time of challenge. At 33–35 days of age the mothers of immunized or control animals were removed and their litters randomized before challenge with *H212* cells. Groups of randomized animals were inoculated subcutaneously with a range of serial ten-fold dilutions of cells (between  $3 \times 10^3$  and  $3 \times 10^6$  cells per hamster) and the hamsters observed for tumour formation for the following 180 days.

Table 1 shows the proportions of hamsters developing tumours in the groups immunized with adenovirus and the control groups. In five groups of hamsters which were not immunized, or received virus-free *HEK* or HeLa cells

\* U.S.A. prototype strains kindly supplied by Dr. M. S. Pereira, Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London.

Table 1. EFFECT OF IMMUNIZATION WITH LIVE OR INACTIVATED ADENOVIRUS ANTIGENS ON TUMOUR INDUCTION IN HAMSTERS BY *H212* CELLS

	No. of expt.	Immunization procedure	Original infectivity titre of virus inoculum (TCID <sub>50</sub> /ml.)	No. of <i>H212</i> * cells inoculated per hamster				Hamsters developing tumours	
				$3 \times 10^3$	$3 \times 10^4$	$3 \times 10^5$	$3 \times 10^6$	Proportion	per cent
Inactivated (formolized) antigens	1	Adenovirus 12 ( <i>HEK</i> )	$10^{4.2}$	2†	4	0	0	6	25
		Controls, non-immunized	—	8	8	8	8	24	
	2	Adenovirus 5 (HeLa)	$10^{6.5}$	6	5	3	1	15	58
		Controls, non-immunized	—	8	7	7	8	26	
	3	Formolized HeLa cells (virus-free)	—	0	1	1	0	2	7‡
		Controls, non-immunized	—	8	8	8	4	28	
				9	4	3	1	17	50
				10	9	10	5	34	
Non-activated antigens	4	Adenovirus 12 ( <i>HEK</i> )	$10^{3.3}$	1	0	—	—	1	10
		Adenovirus 1 (HeLa)	$10^{5.6}$	5	5	—	—	10	
		Adenovirus 2 ( <i>HEK</i> )	$10^{4.7}$	2	0	1	—	3	18
		Adenovirus 2 ( <i>HEK</i> )	$10^{4.7}$	5	8	8	—	17	
		Adenovirus 5 ( <i>HEK</i> )	$10^{6.8}$	0	0	—	—	0	0
		Adenovirus 5 ( <i>HEK</i> )	$10^{6.8}$	4	4	—	—	8	
		Adenovirus 5 (HeLa)	$10^{6.5}$	0	0	—	—	0	0
		Adenovirus 5 (HeLa)	$10^{6.5}$	3	3	—	—	6	
		Adenovirus 7 ( <i>HEK</i> )	$10^{4.2}$	1	3	—	—	4	36
		Adenovirus 7 ( <i>HEK</i> )	$10^{4.2}$	6	5	—	—	11	
		Adenovirus 7 (HeLa)	$10^{4.7}$	0	0	—	—	0	0‡
		Adenovirus 7 (HeLa)	$10^{4.7}$	5	5	—	—	10	
		Controls <i>HEK</i> cell antigen (virus free)	—	0	0	0	—	0	0‡
		Controls <i>HEK</i> cell antigen (virus free)	—	4	8	3	—	13	
		Controls HeLa cell antigen (virus free)	—	3	2	—	—	5	42
		Controls HeLa cell antigen (virus free)	—	6	6	—	—	12	
		Controls, non-immunized	—	7	2	4	—	13	40
		Controls, non-immunized	—	11	10	11	—	32	
				20	7	2	—	29	44
				20	20	5	—	45	

\* *H212* cells from the thirtieth to the fifty-fifth tissue culture generations used in the experiments.

† No. of hamsters developing tumours  
No. inoculated with *H212* cells

‡ Experiments with significant tumour inhibition at the 5 per cent probability level ( $P < 0.05$ ).

(experiments 1, 2 and 4), the proportion of animals developing tumours was between 41 and 58 per cent. In experiment 3, however, a lower incidence of tumours was formed. In all experiments a lower proportion of hamsters immunized with adenovirus developed tumours compared with that in the control groups. This trend of tumour inhibition was significant (Yates's corrected  $\chi^2$  test) in the groups of hamsters immunized with inactivated adenovirus type 5 ( $P < 0.001$ ), live adenovirus type 2 grown in HEK cells ( $P < 0.05$ ) and live adenovirus type 7 grown in HEK or HeLa cells ( $P = < 0.01$ ). Tumour inhibition after immunization with heterotypic adenoviruses was unexpected and, for adenoviruses types 2, 5 and 7, was greater than that in animals immunized with homotypic adenovirus type 12.

For adenovirus types 5 and 7, live antigens prepared in HEK and HeLa cells were compared (experiment 4). With adenovirus type 7 a similar degree of inhibition of tumour formation was noted for antigens prepared in both types of culture, while adenovirus type 5 grown in HEK cells produced a greater degree of inhibition than antigen grown in HeLa cells. Tumour inhibition appeared to depend on the presence of adenovirus antigen, since in control animals inoculated with virus-free HEK or HeLa cells there was no significant inhibition of tumour formation.

These results are of interest in relation to the demonstration by Huebner *et al.*<sup>9</sup> of nonvirion "neoantigens" in hamster tumours induced by adenovirus type 12. Such antigens are shared by adenovirus types 12, 18 and 31, but cross-reacting "neoantigens" were not demonstrated for adenovirus types 1, 2, 5 and 7. Further work is in progress to determine whether the tumour inhibition described here is associated with soluble group specific adenovirus antigens, antigens resembling "neoantigens" or adenovirus particle antigens. In addition it is of interest to determine whether immunization with heterotypic adenoviruses can inhibit tumour formation in hamsters inoculated with adenovirus type 12 when newborn.

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### Urinary Phenols in Melanoma

RECENT observations<sup>1</sup> on the urinary metabolites of 3:4-dihydroxyphenylalanine (DOPA) have prompted us to examine the excretion of phenolic acids in five patients with malignant melanoma, three of whom had hepatic metastases and melanuria and two only locally recurrent cutaneous and lymphatic lesions; all were on uncontrolled

diets. Phenolic acids were examined chromatographically<sup>2</sup> in both hydrolysed and unhydrolysed urines in all cases.

No abnormality was found in the two patients with clinically localized disease. In addition to a few unidentified spots, the following phenolic acids were found to be excreted in abnormally high quantities by all three subjects with generalized melanoma: 4-hydroxy-3-methoxyphenyllactic<sup>1</sup> (VLA), 4-hydroxy-3-methoxyphenylpyruvic<sup>1</sup> (VPA), 4-hydroxy-3-methoxyphenylacetic (HVA), *p*-hydroxyphenyllactic (*p*-HPLA), *p*-hydroxyphenylpyruvic (*p*-HPPA) and, probably, *p*-hydroxyphenylacetic acids. In two of these cases the urines were examined further: both patients showed a large excretion of indole melano-gens; examination for urinary amines chromatographically showed high excretion of 3-methoxytyramine in each subject although excretions of metanephrine and normetanephrine were normal. In routine amino-acid chromatograms neither 4-hydroxy-3-methoxyphenylalanine<sup>3</sup> nor DOPA was detected and tyrosine excretion was not abnormal.

Duchon and Gregora<sup>4</sup> had previously examined urines from five similar subjects. In addition to HVA they noted that three other substances were excreted in large amounts. Two of these, spots Nos. 2 and 3 as described by these authors, can now be identified with virtual certainty as VLA and *p*-HPLA, respectively. The remaining spot, No. 4, which seemed to be indolic, resembles one which appeared on our chromatograms as a mixture of overlapping unidentified substances. The overall pattern of phenolic acid excretion in generalized melanoma thus seems to be rather constant and characteristic of the disease. In view of the quantity and wide variety of phenolic and indolic derivatives of DOPA excreted by each patient it can safely be assumed that the melanoma tumour tissue produced large amounts of this amino-acid.

Two aspects of the phenolic acid pattern in melanuria appear to be of particular interest. First is the excretion of large amounts of tyrosine metabolites. Both *p*-HPPA<sup>5</sup> and *p*-HPLA<sup>6</sup> have been reported to be excreted in abnormal quantities in many pathological conditions. It is usually accepted that such "tyrosylurias" can be attributed to an impairment in the oxidation of *p*-HPPA as a result of the absence of, or inhibition of, the necessary *p*-HPPA hydroxylase (for a recent summary of tyrosyluria, see ref. 7). In generalized melanoma the impairment may indicate an inadequate supply of the enzyme because of reduction in liver tissue as a result of tumour infiltration.

The second point of interest concerns the relative amounts of the various DOPA metabolites excreted. Normal urines contain relatively substantial quantities of HVA, but VPA is detectable in much smaller amounts only as a highly fluorescent hydantoin derivative<sup>1</sup> and VLA has not been detected as yet. Our subjects showed only moderately increased excretions of HVA, as did those of Duchon and Gregora<sup>4</sup>. By contrast, excretions of VPA and VLA were much more striking. The formation of HVA from DOPA could be preceded by either decarboxylation of amino-acid to amine or transamination of amino-acid to pyruvic acid. No direct evidence is available to support the second process, but the first is indicated by the increased excretion of the intermediate 3-methoxytyramine. Transamination (or the unlikely but equivalent oxidative deamination) must, however, precede the formation of VPA and probably that of VLA which is most likely derived from pyruvic acid by reduction. These findings suggest that in melanoma there is a shift in the metabolism of DOPA towards products derived by transamination rather than by decarboxylation. Although difficult to pinpoint because of the complications arising from the possibility of *O*-methylation occurring at any point along the sequences from DOPA to the various methylated derivatives, it seems most likely that an impairment occurs somewhere in the normal metabolism of DOPA as well as in that of tyrosine. A high excretion of VPA would result if oxidation of pyruvic acid (to sub-

stituted phenylacetic acid or to undetected, possibly aliphatic, metabolites) were impaired; in this event a shift towards transamination would only be apparent. On the other hand a real shift would result if decarboxylation were impaired, thus forcing metabolism through the transamination pathway.

Such an impairment might again result as a consequence of liver damage. We wish to direct attention, however, to the possibility that the metabolic pathways of tyrosine and DOPA may not be entirely independent and might well be subject to mutual interference. Enzyme systems participating in the metabolism of aromatic amino-acids seem frequently to be susceptible to inhibition by compounds structurally related to their substrates. Aromatic pyruvic and lactic acids seem to be particularly potent in this respect and members of this group have been shown to inhibit the activities of *p*-HPPA oxidase<sup>8</sup>, tyrosine transaminase<sup>9</sup> and DOPA decarboxylase<sup>10</sup>. In melanoma any of numerous inhibitory effects might be operative and these rather than, or in addition to, liver damage might account for the findings.

Although the data are as yet meagre, other observations suggest that abnormalities in the metabolisms of tyrosine and DOPA may be closely linked. It has been previously reported<sup>1</sup> that detectable quantities of urinary VLA are usually associated with a high excretion of *p*-HPLA. Furthermore, one of the most puzzling features of Medes's classical case of tyrosinosis<sup>11</sup> is the claim that the subject excreted DOPA when fed tyrosine and tyrosine and *p*-HPPA when fed DOPA. Although the experimental data cannot be accepted unreservedly because of the problems created by dietary control, identity of the DOPA isolated and apparent absence of DOPA metabolites, an explanation in terms of mutual interference seems as attractive as any other. It may be noted that if our speculations on these lines are correct, then simultaneously increased excretion of DOPA and tyrosine metabolites would not necessarily imply increased production of DOPA, although this undoubtedly occurs in melanoma.

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## Spontaneous Tumours in Howler Monkeys

DURING 1965 we conducted post-mortem examinations on 292 free ranging howler monkeys (*Alouatta caraya*, Humboldt, 1812)<sup>1</sup> and found fourteen tumours (Table 1). In a recent review of spontaneous malignant tumours in non-human primates, Kent<sup>2</sup> reported forty-nine cases in eighteen species, but none were found in *Alouatta*. Houser *et al.*<sup>3</sup> have found benign adrenal adenomas in *Alouatta villosa* of Panama.

In *Alouatta caraya* we found cortical adenomas of the adrenal glands in eleven animals. The neoplasms ranged

in size from 1 to 20 mm, and in all cases only one node was found. The tumours were encapsulated by a fibrous capsule and occasionally compressed the adjacent adrenal cortical tissue. The cells of these adenomas were arranged in a columnar pattern, similar to that of the zona fasciculata, and did not show pleomorphism or giant nuclei; occasional normal mitotic figures were present.

A testicular seminoma was found in one adult male weighing 5 kg (ref. 4). This tumour consisted of a solid whitish grey node, measuring 8 mm in diameter, which had no sharp demarcation from the normal testicular tissue. Microscopically, this case presented the monocellularity, the stromal pattern and the focal lymphocytic infiltration which are commonly found in human seminomas. The tumour cells were moderately large and polyhedral with hyperchromatic nuclei. There was moderate mitotic activity; in the peripheral portion of the neoplasm atypical mitotic figures as well as necrotic seminiferous tubules were observed. No metastatic lesions were observed in the abdominal and inguinal lymph nodes, or in the abdominal and thoracic viscera, nor were metastatic lesions seen in survey films of the skeleton. This is the fourth malignant testicular tumour on record and the second seminoma found in non-human primates.

Table 1. TUMOURS FOUND IN WILD HOWLER MONKEYS (*Alouatta caraya*)

Group	Number of animals		Males	Females
	♂	♀		
Prepuberal	43	20	0	0
Postpuberal	131	98	Seminoma (1) Alveolar lung carcinoma (1) Adrenal adenomas (5)	Adrenal adenomas (6) Renal adenoma (1)

A type of lung neoplasia found in one adult male howler monkey has not been previously reported in non-human primates. The tumour was believed to be an alveolar carcinoma; it was situated in a peripheral location and was classified as a nodular form. The alveolar walls were lined by a single regular layer of cuboid epithelial cells, and mucin-secreting activity was detected. No metastasis was found.

A renal adenoma was found in the medullary portion of the kidney of one adult female. The tumour, approximately 3 mm in size, showed tubules and cysts, lined by epithelial cells. No papillary projections were observed.

Our findings raise a number of questions. We found no neoplasms in prepuberal animals, and this is in accordance with findings of other observers with other non-human primates in studies dealing mainly with young animals. Adrenal adenomas similar to those observed in *Alouatta caraya* have also been described in *Alouatta villosa*<sup>3</sup>; it is interesting that these tumours are frequent in this genus as in man but not in the common laboratory primate, the rhesus monkey. The overall incidence of malignant tumours in the present series is less than 1 per cent, but the occurrence of two cancers in 131 adult males, which is more than one would expect in a human population of comparable age, raises the possibility that this species may be unusually prone to cancer. It also seems to be higher than the percentages reported by others in other non-human primates.

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## HAEMATOLOGY

## Basic Protein of Normal Human Plasma

BASIC low molecular weight proteins of normal human blood, designated as  $B_1$  and  $B_2$ , were recently discovered by Takahashi and Schmid<sup>1</sup>. Acidic and neutral low molecular weight serum proteins and peptides, however, have been known for many years<sup>2-4</sup>. In the present report the purification and partial characterization of one of the basic proteins,  $B_2$ , are described.

Fraction VI of pooled normal human plasma was chromatographed on a DEAE-cellulose column at pH 5.5 and  $\Gamma/2$  0.005 (ref. 5). The resulting effluent was passed subsequently through a CM-cellulose column at the same pH and ionic strength to adsorb and concentrate the proteins from this solution. Fractional elution of the second column gave the  $3S \gamma_1$  (ref. 5) and  $2S \gamma_2$  (ref. 6) globulins and the mentioned basic components. The basic compounds were further purified on a DEAE-Sephadex A-50' column at pH 8.4 and  $\Gamma/2$  0.005. Final purification was achieved by chromatography on a hydroxylapatite column at pH 6.8. The resulting  $B_2$ -preparation revealed a single component on ultracentrifugation and starch gel electrophoresis at pH 8.6 (Fig. 1).

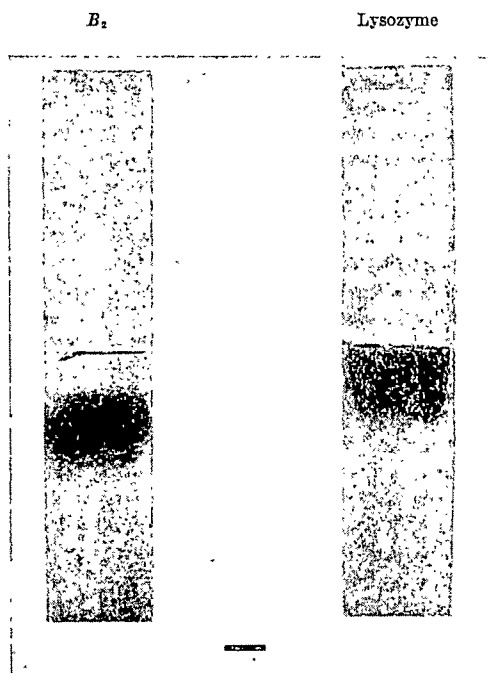


Fig. 1. Starch gel electrophoresis of  $B_2$  in pH 8.6,  $\Gamma/2$  0.01 borate buffer. Amido black was used as stain and lysozyme as reference. The protein moved toward the cathode (—).

The basic protein,  $B_2$ , was characterized in terms of some of its major physical chemical and chemical properties. Its molecular weight determined by the Yphantis procedure<sup>7</sup> was found to be approximately 9,000. On ultracentrifugation  $B_2$  sedimented at the rate of  $1.3S$ . The isoelectric point of this protein was estimated to be between pH 10.0 and 10.5. Ultra-violet absorption (Fig. 2) between 250 and 290 m $\mu$  revealed a maximum that indicated a very low content of tyrosine and/or tryptophan. Chemical analysis of  $B_2$  showed that its polypeptide moiety accounted for the total weight. Independent measurements confirmed the lack of neutral hexoses, hexosamines, and sialic acid.

We thank Dr. J. McComb, Mr. L. Larsen and their co-workers of the Division of Biologic Laboratories at Massachusetts Department of Health. This investigation

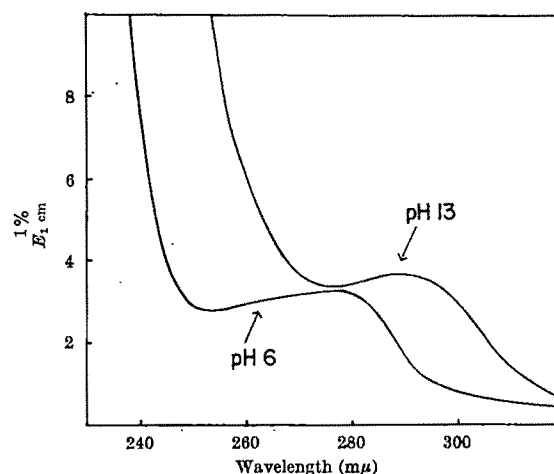


Fig. 2. Absorption curves of  $B_2$  measured at pH 6 and 13, respectively.

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Decrease in the Concentration of Haemoglobin  $A_2$  during Erythroleukaemia

DURING the past decade there have been several descriptions of abnormal haemoglobin in leukaemia<sup>1-4</sup>. The earliest reports emphasized the increase of foetal haemoglobin content in some cases of leukaemia in children and adults<sup>1-3</sup>. The occurrence of haemoglobin H in cases of erythroleukaemia and atypical chronic myeloid leukaemia was also reported<sup>4</sup>. In these cases no alteration in the level of the normal minor haemoglobin fraction, haemoglobin  $A_2$ , was noted. We have investigated an erythroleukaemic patient with a very low level of haemoglobin  $A_2$  which developed in the course of leukaemia.

A 63 year old man, first seen in 1963, had clinical and haematological findings of subacute erythroleukaemia with signs of a haemolytic component (reticulocytosis, hyperbilirubinaemia, increased osmotic fragility before and after incubation at 37° C for 24 h). Coombs test and tests for complete antibodies were negative. Haemoglobin analysis with starch gel electrophoresis with borate buffer, pH 8.6, and alkali denaturation<sup>1,5</sup> gave normal results, namely foetal haemoglobin less than 1 per cent and no alteration in the content of the normal minor haemoglobin component, haemoglobin  $A_2$  (3.5 per cent). The patient was successfully treated with corticosteroids, and clinical manifestations and haematological findings improved moderately. Nearly 1 year later, a second analysis of haemoglobin was carried out. Repeated determinations of haemoglobin  $A_2$  with different methods such as starch gel electrophoresis<sup>6</sup>, starch block electrophoresis<sup>6</sup> and DEAE-cellulose chromatography<sup>7</sup> showed a very low content, namely between 0.29 per cent and 0.5 per cent (Fig. 1). This finding was confirmed by Dr. C. Pik of the Department of Pediatrics, State University of Groningen, and also at the Central Laboratory of the

Netherlands Red Cross, Amsterdam. In the latter case the following determinations were also performed: serum haptoglobin high normal, glucose 6-phosphate dehydrogenase 64 u/g (normal range  $15.4 \pm 6.7$  u); glutathione reductase 48 u/g (normal range  $15.4 \pm 4.4$  u); pyruvate kinase 27.9 u/g (normal range  $41.7 \pm 10.6$  u). The concentration of serum iron was between 220 and 240  $\gamma$  per cent on repeated determinations during the course of his illness. The patient died 1.5 years after the onset of leukaemia.

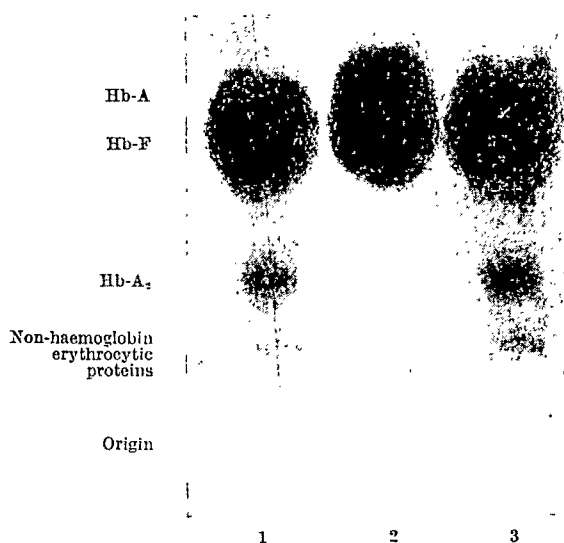


Fig. 1. Starch gel electrophoresis with borate buffer, pH 8.6. 1, Patient with Cooley's anaemia: Hb-A + Hb-F + Hb-A<sub>2</sub> (increased concentration); 2, propositus: Hb-A(Hb-A<sub>2</sub> is not visible due to its extremely low concentration); 3, normal: Hb-A + Hb-A<sub>2</sub> (normal concentration). The gel was photographed after staining with buffalo-black in methanol.

A decrease of the haemoglobin A<sub>2</sub> fraction in adults has been observed in the following states: (a) in some forms of thalassaemias, such as  $\alpha$ -thalassaemia (haemoglobin H) or in certain cases of F-thalassaemia<sup>8,9</sup>; (b) when variants of haemoglobin A<sub>2</sub> such as haemoglobin A<sub>2</sub>' or haemoglobin Flatbush are present<sup>10,11</sup>; (c) in the presence of haemoglobin Lepore in the heterozygous state<sup>12</sup>; (d) in some cases of severe and long standing iron deficiency anaemia<sup>13</sup>. (Some other conditions in which haemoglobin A<sub>2</sub> is completely absent are not included in this discussion.)

The results of analysis of haemoglobin and the presence of high values for serum iron easily excluded all the possibilities mentioned above. Another important fact in the evaluation of the decrease of haemoglobin A<sub>2</sub> in our erythroleukaemic patient is its occurrence in the course of his illness. At the beginning the percentage of haemoglobin A<sub>2</sub> was found within normal limits, that is 3.5 per cent, by the method of starch gel electrophoresis. Contrary to this, one year later the haemoglobin A<sub>2</sub> fraction decreased to a content of 0.29–0.5 per cent as measured by the method of starch block electrophoresis and DEAE-cellulose chromatography. This shows clearly that the decrease of haemoglobin A<sub>2</sub> developed during the course of erythroleukaemia. In other words, the decrease of haemoglobin A<sub>2</sub> fraction may be considered an acquired phenomenon.

A possible explanation of the decrease of the haemoglobin A<sub>2</sub> fraction in a leukaemic patient is that the leukaemic state sometimes leads to an imbalance of haemoglobin chain synthesis which results in a relative excess of  $\beta$ -chains or a compensatory production of  $\gamma$ -chains<sup>4</sup>. In our case, this imbalance led to a decrease in the production of  $\delta$ -chains resulting in a low concentration of haemoglobin A<sub>2</sub> without compensatory production of  $\beta$ - or  $\gamma$ -chains as shown by the absence of haemoglobin F and H.

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### Specificity of Sugar Carriers in Erythrocytes

The mechanism of exchange transport can be explained in various ways<sup>1,2</sup>, but it is evident that substances participating in it penetrate through the membrane using the same carriers. This type of transport can therefore be used to investigate substances which share common carriers. The specificity of sugar carriers in erythrocytes was investigated by this method and the results indicated that only some of the monosaccharides share the same carriers<sup>3</sup>. The others pertained to monosaccharides which had a lower affinity for the carriers<sup>4</sup>. The objection has been made that in experiments with sugars of low affinity to the carrier it was only their low relative concentration in the medium (that is, concentration with regard to the affinity) that could be the reason for the lack of exchange transport<sup>5</sup>. We have explored this suggestion and followed the exchange transport of glucose for L- and D-arabinose (that is, sugars with a quite different affinity for the carrier<sup>4</sup>) at the same relative concentrations.

Blood was collected in ACD solution (35 g of glucose, 11 g of trisodium citrate, 4 g of citric acid in 1,000 ml. of water). After separation of erythrocytes from plasma, 0.5 ml. of erythrocytes (containing 5.7 mg of glucose/ml.) was incubated in a solution of D-arabinose (260.6 mg/5 ml. of water), L-arabinose (20.85 mg/5 ml. of 0.9 per cent sodium chloride), and in 0.9 per cent sodium chloride. After 1, 7 and 13 min of incubation at 0° C samples of the suspension were withdrawn and erythrocytes were separated by filtration through a 'Hyflo Super Cel' layer<sup>6</sup>. After haemolysis, the proteins were precipitated and glucose determined from the difference in reduction power before and after oxidation with glucose oxidase<sup>7</sup> and chromatographically<sup>8</sup>.

In order to obtain the same values for relative concentration (with regard to the affinity for the carrier) of L- and D-arabinose the dissociation constants of these two sugars were assessed from the figure in the paper of LeFevre and Marshall<sup>4</sup> ( $K_s$  for L-arabinose 0.12 mole/l.,  $K_s$  for D-arabinose 1.5 mole/l.). The affinity of L-arabinose to the carrier is about 12.5 times higher than that of D-arabinose, and so we dissolved 12.5 times more D-arabinose than L-arabinose. To maintain approximately isotonic conditions, L-arabinose was dissolved in 0.9 per cent sodium chloride, and D-arabinose in distilled water.

Fig. 1 shows that the efflux of glucose into the L-arabinose medium was significantly higher than that into

the D-arabinose medium; in the latter case the efflux was similar to that into 0.9 per cent sodium chloride (see also chromatogram in Fig. 2). Fig. 3 shows that during the incubation in L-arabinose the intensity of glucose spots (corresponding to the amount of glucose in erythrocytes) decreases while that of L-arabinose spots gradually increases. In incubation of erythrocytes in the medium with D-arabinose this sugar rapidly penetrates into cells under our conditions (Fig. 3); efflux of glucose is only slight.

These results suggest that L-arabinose (that is, the sugar sharing the common carrier<sup>3</sup>), unlike D-arabinose (the sugar reported not to share the common carrier<sup>3</sup>), accelerates the efflux of glucose even in conditions where the relative concentrations of both sugars with regard to their dissociation constants are the same. It should be noted that as a measure of the affinity we used the dissociation constants estimated at 37° C (ref. 4) while the experiments were carried out at 0° C. A similar relation must therefore be assumed for the temperature changes of the

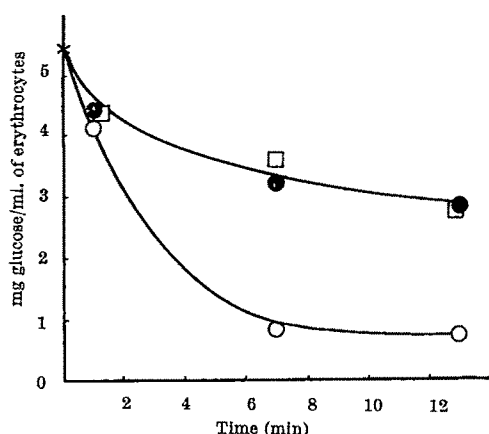


Fig. 1. Efflux of glucose into media with L-arabinose, D-arabinose, and 0.9 per cent sodium chloride. Erythrocytes (0.5 ml. containing 5.7 mg of glucose/ml.) were incubated in the following media: ○, 20.85 mg of L-arabinose/5 ml. of 0.9 per cent sodium chloride; ●, 250.6 mg of D-arabinose/5 ml. of water; □, 0.9 per cent sodium chloride. ×, Concentration of glucose at beginning of experiment. After 1, 7 and 13 min, the samples were withdrawn and glucose was determined (for details see text).

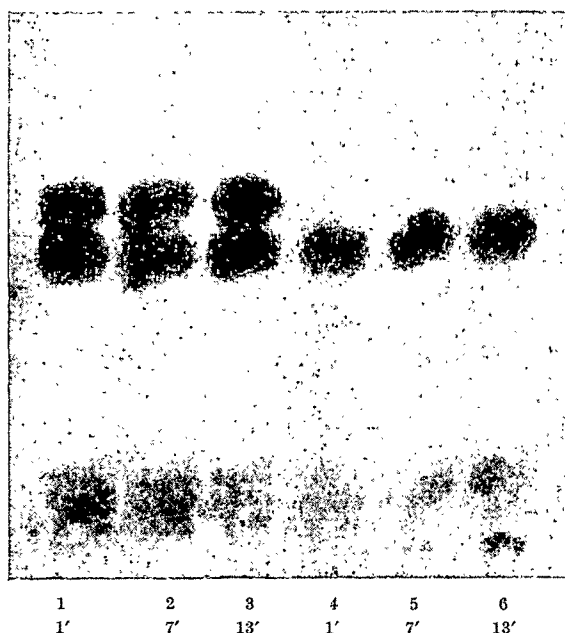


Fig. 2. Chromatogram showing the efflux of glucose in D-arabinose or into 0.9 per cent sodium chloride. 1-3, Erythrocytes were incubated in D-arabinose; 4-6, erythrocytes were incubated in 0.9 per cent sodium chloride. Analyses for the contents of sugar in erythrocytes were carried out after 1, 7 and 13 min of incubation. Upper row, D-arabinose; lower row, glucose. The conditions of the experiments are given in Fig. 1.

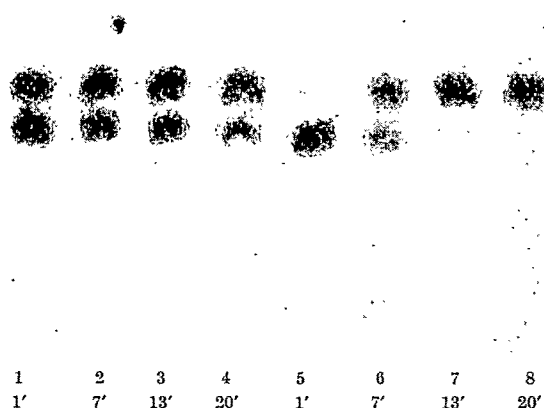


Fig. 3. Chromatogram showing the efflux of glucose into D-arabinose or L-arabinose. 1-4, Incubation of erythrocytes in D-arabinose; 5-8, incubation in L-arabinose. Analyses were carried out after 1, 7, 13 and 20 min. Upper row, arabinose; lower row, glucose. The conditions of the experiment are given in Fig. 1.

dissociation constants of L- and D-arabinose. There is no doubt, however, that although the concentration of D-arabinose was one order of magnitude higher than that previously used<sup>3</sup>, again no significant difference was found between the penetration of glucose into the media with D-arabinose and that into 0.9 per cent sodium chloride without sugars.

It is of further interest that, although D-arabinose under these conditions appeared in erythrocytes within the first minute of the incubation, the efflux of glucose was not accelerated. Provided that it is not merely a question of adsorption of D-arabinose on the membrane, this finding also indicates differences in the uptake of glucose and L-arabinose on the one hand, and of D-arabinose on the other.

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## BIOLOGY

### Estimation of Survey Efficiency for Animal Populations with Unidentifiable Individuals

IN any census of an animal population a number of individuals may remain undiscovered and this makes the estimation of population size difficult. Various statistical procedures may be used to overcome this problem<sup>1,2</sup>, but these methods usually require the identification of the individuals: that is, that animals revealed during one count should be recognized during other counts. To our knowledge there is published only one method<sup>3</sup> for estimation of the efficiency of a survey and the size of populations containing unidentified individuals, but the validity of this method has been questioned<sup>4</sup>.

We present here a brief description of two techniques for estimating the efficiency of a survey from repeated

counts of a population which do not require identifiable individual members.

In the first method, which we will call the variability method, variability in the results of repeated counts of a population is used to obtain an estimate of the efficiency of survey. We suppose that a population of constant size of  $N$  individuals is counted  $n$  times. In count  $i$  ( $i=1, 2 \dots n$ )  $X_i$  individuals are discovered. The estimator

$$\hat{p}_v = 1 - \frac{S^2}{\bar{X}}$$

can be shown to give a useful estimate of the efficiency of the survey where  $\bar{X}$  is the sample mean of the number of individuals discovered during the counts

$$\bar{X} = \frac{1}{n} \sum_i X_i$$

and

$$S^2 = \frac{1}{n-1} \sum_i (X_i - \bar{X})^2$$

We suppose the  $X_i$ 's to be independently and binomially distributed  $b_N(x, p)$ .  $E(X) = Np$  and  $E(S^2) = Np(1-p)$  and so  $\hat{p}_v$  can be used to estimate  $p$ . Approximation formulae for the expectation and variance for  $\hat{p}_v$  have been computed

$$E(\hat{p}_v) = p - \frac{1-p}{nN} \quad (1)$$

$$V(\hat{p}_v) = \frac{2(1-p)^2}{n-1} + \frac{5p-3p^2-2}{nN} \quad (2)$$

The bias and variance are estimated by substituting  $\hat{p}_v$  for  $p$  and  $\frac{\bar{X}}{N}$  for  $Np$  in (1) and (2). The bias may become noticeable in very small populations and then a corrected estimate may be obtained from (1).

The variance is only moderately dependent on the size ( $N$ ) of the population. This means that the usefulness of the method is very much increased if it is possible to subdivide the population into smaller populations (for example, in area taxation, to subdivide the area) in such a way that one can assume the size of the sub-populations to be constant. Suppose the number of sub-populations to be  $K$ . It is then possible to obtain counts and estimates  $\hat{p}_v^j$ ,  $j=(1, 2 \dots k)$  from each sub-population, and to obtain an average estimate

$$\hat{p}_v = \frac{1}{k} \sum_{j=1}^k \hat{p}_v^j$$

If the original population is subdivided into  $k$  sub-populations the variance of the efficiency estimate will be reduced by a factor of nearly  $1/k$  compared with the variance of an estimate calculated on the basis of an undivided population.

The second method, which can be called the varied observation period method, uses differences in the results of high and low speed surveys of strips or areas to give an estimate of survey efficiency. In the high speed survey each individual will be under observation for a shorter time than in the low speed survey, which causes differences in the number of discovered individuals.

One specific condition must be met: two sightings of the animal in any two distinct time intervals during the period of observation must be independent. In these conditions the probability  $P(t)$  of discovering an individual in an observation period of length  $t$  will be

$$P(t) = 1 - \exp(-\lambda t)$$

where  $\lambda$  is a certain constant.

If a survey of a strip or area with a given speed  $v$  and with efficiency  $p$  is undertaken, and the survey of the same strip or area is carried out  $n$  times,  $n \geq 1$ , then the average number  $X$  of animals discovered depends on the constant population size  $N$  and  $p$ . We have  $E(x) = Np$ . Suppose then that in one survey we use a lower speed  $v/a$ , where  $a > 1$ . We will find that the efficiency with this speed will be a specific function  $p_a(p)$  of  $a$  and the higher speed survey efficiency  $p$ , provided that the above mentioned condition is met. If  $Y$  is the number discovered in this low speed survey, then  $E(y) = Np_a(p)$ , and it is possible to obtain an estimate  $\hat{p}$  of  $p$  by solving for  $\hat{p}$  in the equation

$$\frac{Y}{\bar{X}} = \frac{p_a(\hat{p})}{\hat{p}}$$

The exact form of the function  $p_a(p)$  will depend on assumptions about the shape of the area the surveyor has under observation at any one time. We shall consider two alternatives. (a) The field of observation is rectangular. (b) The field is circular, and the animals are randomly or evenly spaced. We have computed approximate expressions for the expectation and variance of  $\hat{p}$

$$E(\hat{p}) = p + \frac{p_a(1-p)}{GnN} + \frac{1}{pG} \left( G - \frac{p^2}{2} \cdot \frac{d^2}{dp^2}(p_a) \right) \cdot V(\hat{p})$$

$$G = p \frac{d}{dp}(p_a) - p_a$$

$$V(\hat{p}) = \left( \frac{p \cdot p_a}{G} \right)^2 \cdot \frac{1}{N} \cdot \left( \frac{1-p_a}{p_a} + \frac{1-p}{np} \right)$$

$\hat{p}$  is approximately unbiased. It will often pay to choose  $n > 1$ . The function  $p_a$  has the following forms in the two cases: (a)  $p_a = 1 - (1-p)^a$ ; (b)  $p_a$  is in this case implicitly defined by

$$p = 1 - J(u)$$

where

$$J(u) = \int_0^1 \exp[-u(1-r^2)^{\frac{1}{2}}] dr \quad p_a = 1 - J(au)$$

In (b) a suitable approximation must be made in the calculation.

For smaller values of  $p$  it can be seen that (a) and (b) give approximately the same results, and thus that any specific assumptions about the form of the area of observation are relatively unimportant.

So far the population in the strip or area has been assumed to be constant. An important property of the estimator here described is that it is approximately unbiased even if the population fluctuates.

Possibly this method can also be used in other animal census techniques, so long as the observation period during which an animal can be discovered can be varied, as it is in our example, without changing other relevant aspects of the procedure.

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### Leaf Resistance in Succulent Plants

REPORTS in the literature indicate or suggest that the stomata of succulent plants open in the dark and close in the light. This conclusion is based on direct observations<sup>1</sup>, measurements of diffusion with a porometer<sup>2</sup>, and inference<sup>3</sup>. Nishida<sup>2</sup> reported nocturnal stomatal opening in several succulent species, but diurnal opening in others. Thick leaves, few stomata, and small stomatal openings make measurements of the stomatal aperture and subsequent determinations of leaf resistance difficult. The innovation of a resistance hygrometer by Wallihan<sup>4</sup> and van Bavel *et al.*<sup>5</sup> allowed the precise determination of leaf resistance to water vapour transfer in succulent plants without the complications frequently encountered with other methods. Measurements of leaf resistance are based on changes in the resistance of a lithium chloride relative humidity sensor as water vapour diffuses from the leaf to the sensor. The sensor is calibrated with standard length tubes placed over a free water surface; thus the leaf resistance can be estimated in meaningful physical units (sec/cm)<sup>6</sup>. In this communication, we present data indicating the magnitude of leaf resistance to water vapour transfer in *Kalanchoe blossfeldiana* v. Poelln. in the light and dark. *K. blossfeldiana* was chosen because many investigators have used it as an experimental plant for dark carbon dioxide fixation studies<sup>6,7</sup>.

Measurements were made in an environmental control chamber fitted with a glove-box through which the resistance meter could be manipulated. Radiant energy in the chamber was  $5 \times 10^4$  erg/cm<sup>2</sup>/sec at plant height. The plants were kept on 8 h photoperiods. Relative humidity was maintained at  $50 \pm 10$  per cent mean differential and temperature at  $\pm 0.55^\circ$  C. All measurements, except as indicated, were taken on the lower surface of young leaves.

On a thermoperiodic regime of  $26^\circ$  C during the light period and  $21^\circ$  C during the dark, leaf resistance to water vapour transfer was uniformly low in the light and high in the dark (Fig. 1). Several hundred measurements substantiated this conclusion. When the dark period temperature was lowered to  $15^\circ$ , resulting in a diurnal fluctuation of  $11^\circ$ , resistances were somewhat higher in

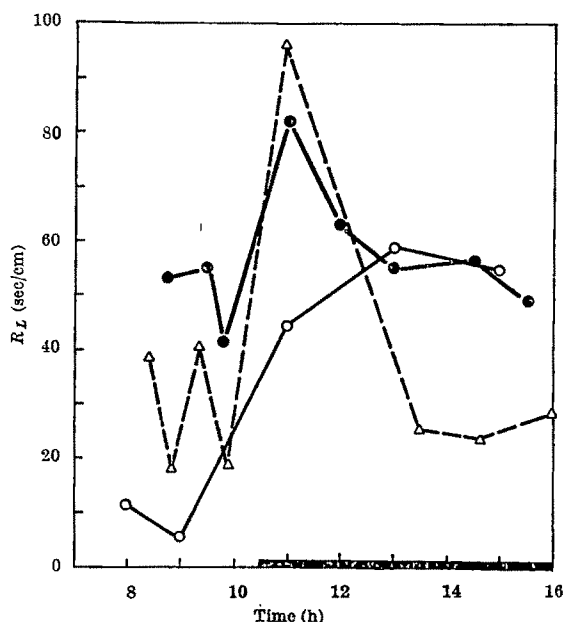


Fig. 1. Leaf resistance ( $R_L$ ) at the end of a light period of 8 h and beginning of dark period of 16 h on three thermoperiodic regimes. Points are means of six determinations from different plants. ●, Light temperature =  $26^\circ$  C, dark temperature =  $21^\circ$  C; ○, light temperature =  $26^\circ$  C, dark temperature =  $15^\circ$  C; △, light temperature =  $26^\circ$  C, dark temperature =  $26^\circ$  C.

the light and lower in the dark. An apparent night stomatal opening relative to the  $26^\circ$ – $21^\circ$  regime was therefore observed. Indications of night opening, however, were only apparent as the minimum resistances of 5–10 sec/cm measured in the light (on the  $26^\circ$ – $21^\circ$  regime) were not observed in the dark. With no diurnal fluctuation, that is, with day and night temperatures of  $26^\circ$ , the resistances were high in both dark and light. The peak resistances measured immediately after the light period were consistent and reproducible.

The mean steady resistance in the dark (that is, the points between 13 and 16 h of Fig. 1) at  $15^\circ$  was  $26.14 \pm 2.6$  sec/cm while at  $21^\circ$  and  $26^\circ$  they were  $56.7 \pm 8.1$  and  $53.1 \pm 7.2$  sec/cm respectively. The  $15^\circ$  mean resistance was significantly different from the  $21^\circ$  and  $26^\circ$  mean resistances at the 1 per cent level. It is interesting to note that the lower night resistance at  $15^\circ$  can be correlated with the reported accumulation of organic acids at low temperatures<sup>8</sup>.

Nishida<sup>2</sup> observed nocturnal stomatal opening in *K. blossfeldiana*; yet Schwabe<sup>9</sup> did not report nocturnal opening. The experimental temperature conditions of the former worker were, however, quite similar to our  $26^\circ$ – $15^\circ$  regime. Our data, therefore, agree partly with both reports.

Young leaves had significantly lower leaf resistances than older leaves (Table 1), and upper surfaces had generally lower resistances than lower surfaces (Table 2). We estimated the number of stomata on the upper and lower surfaces to be  $3,274 \pm 108$  stomata/cm<sup>2</sup> and  $4,698 \pm 118$  stomata/cm<sup>2</sup> respectively. These differences were significant at the 1 per cent confidence level. Further, we estimated the aperture of open stomata (young leaves) to be about  $2.5\mu$ . The relatively high resistances observed were accountable, therefore, by few and small stomata<sup>9</sup>. Lower resistances associated with the upper surface, however, were not correlated with stomatal density. A striking feature of these data was the greater resistances measured when compared with leaf resistances of non-succulent plants<sup>10</sup>.

Table 1. COMPARISON OF YOUNG AND OLD LEAVES ON A SINGLE PLANT

Age	Leaf pair, from top	$R_L$ sec/cm
Young	3	$12.3 \pm 0.6$
Medium	5	$15.0 \pm 2.5$
Older	7	$99.1 \pm 8.8$

Means are based on three determinations taken during the middle of the light period from the lower surface. Light =  $5 \times 10^4$  ergs/cm<sup>2</sup>/sec, light temperature =  $26^\circ$  C, dark temperature =  $21^\circ$  C, relative humidity = 50 per cent.

Table 2. COMPARISON OF UPPER AND LOWER LEAF SURFACES ON MEDIUM AGED LEAVES OF A FLOWERING PLANT

Surface	Light	$R_L$ sec/cm
Upper	+	$20.5 \pm 6.7$
Lower	+	$53.5 \pm 7.5$
Upper	–	$51.0 \pm 5.4$
Lower	–	$80.6 \pm 5.1$

Means are based on three determinations. Dark measurements were taken by interrupting the middle of the light cycle. Light =  $2.3 \times 10^4$  ergs/cm<sup>2</sup>/sec, light temperature =  $30^\circ$  C, dark temperature =  $19^\circ$  C, relative humidity = 50 per cent.

Transpiration ( $T$ ) and diffusion through the stomata can adequately be described by an equation of the form.

$$T = \frac{D \Delta e}{R_L + R_A}$$

where  $D$  = diffusion coefficient,  $\Delta e$  = gas concentration difference, and  $R_L$  and  $R_A$  = the leaf and air resistances respectively<sup>9</sup>. Under our experimental conditions we estimated  $R_A$  to be 0.5 sec/cm.  $R_A$  is therefore relatively insignificant when compared with the observed values of  $R_L$ , and transpiration in *Kalanchoe blossfeldiana* could be described by an equation of the form

$$T = \frac{D \Delta e}{R_L}$$



Transpiration (and possibly assimilation of carbon dioxide) should vary inversely with leaf resistance. Assuming that leaf resistance ( $R_L$ ) reflects stomatal micro-anatomy<sup>9,10</sup>, changes in resistance will indicate changes in stomatal aperture. These data suggest that in *Kalanchoe blossfeldiana*, unlike many plants, gas exchange is under direct stomatal control<sup>9</sup>.

*Kalanchoe blossfeldiana*, under the conditions of our experiments, did not show significant nocturnal stomatal opening as estimated from leaf resistance measurements. The minimum resistances (of 5 to 10 sec/cm) were measured in the light. Apparent nocturnal stomatal opening was observed on a thermoperiodic regime of 26° C in the light and 15° C in the dark; when compared with 26°–26° and 26°–21° regimes, the lower nocturnal leaf resistances were statistically significant. In general, leaf resistances were higher than those reported for many non-succulent plants.

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### Simple Device for obtaining Synchronous Cultures of Algae

SYNCHRONOUS cultures (populations of algae in which cell divisions and interphase preparations for division are phased or synchronized) are often used in studies of the algal life-cycle. The devices and methods used to obtain synchronous cultures of algae are as a rule rather complicated<sup>1-3</sup>, so we developed a device of simple construction, the parts of which are easy to obtain. To synchronize the cultures we chose the method in which a random culture is subjected to a regular alternation of light and dark periods of adequate duration.

Fig. 1 shows a well stirred thermostat made solely of glass (36 × 23 × 26 cm) in which two culture vessels can be placed. As culture vessels we used flat bottles of capacity 1 l. Each bottle was provided with two air-inlet tubes, one outlet tube and one tube, adjustable at variable depths, to collect samples and to dilute the suspension.

The cultures were illuminated by four fluorescent lamps through each of the two longest sides of the water bath, giving a light intensity, measured in the middle of the water bath, of 15,000 lux from each side.

To get more light and a spectrum that resembles more closely that of the Sun a high pressure mercury fluorescent lamp should be placed over each culture. This gives from above a light intensity of 5,000 lux in the centre of the thermostat. Placing three thermostats between the lamps enabled us to use three different temperatures and also culture six different organisms at the same time under the same light conditions, to yield about 900 ml. of a dense suspension from each bottle.

With this device we obtained completely synchronized cultures of *Scenedesmus obliquus*, *Ankistrodesmus falcatus* and *Chlorella vulgaris* at temperatures between 25° C and 30° C, using the culture solution of Lorenzen<sup>1</sup>. The light-dark rhythm was 14 h light : 10 h dark, and aeration was achieved with sterile air enriched with 3 volume per cent carbon dioxide. To start cultures we inoculated a small quantity (about 2 ml.) of cells from a liquid stock culture (about  $2 \times 10^8$  cells/l.) into the bottles containing 900 ml. of sterile culture medium. After three light-dark cycles the algal suspensions were dense enough to be diluted for the first time. The stock cultures were grown in slightly modified Rodhe's culture solution 8 (ref. 4). When culture vessels and culture solutions were sterilized (20 min at 120° C) no serious infections occurred during sampling and dilution. Fig. 2 shows the change of cell numbers and relative chlorophyll quantities during one light-dark cycle of *Scenedesmus obliquus*.

Table 1 gives the number of cells and the relative chlorophyll quantities in six succeeding periods of the light-dark change of this organism.

As the average number of autospores of *Scenedesmus obliquus* in these cultures appeared to be ten, we diluted

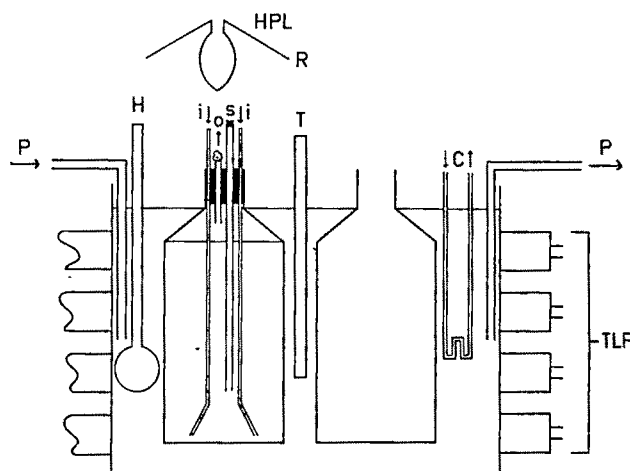


Fig. 1. Schematic representation of thermostat which contains two culture vessels. P—pump for mixing the water; H—electrical heater; T—contact thermometer; C—copper coil for cooling tapwater; i—air inlet tubes; o—air outlet tube; s—tube for sampling and dilution; TLF—fluorescent lamps; HPL—fluorescent high-pressure mercury lamp; R—reflector.

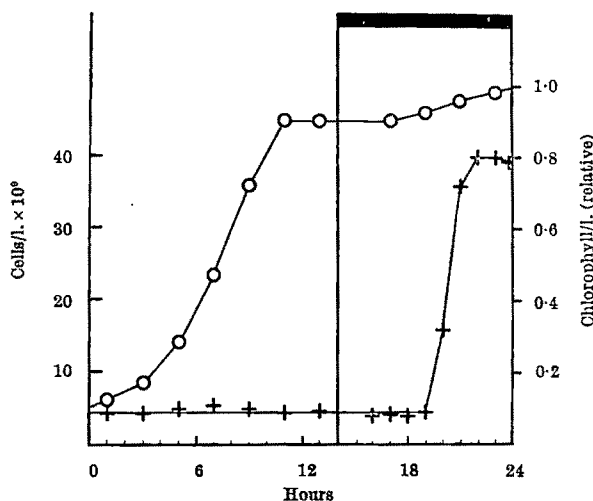


Fig. 2. Cell numbers and relative chlorophyll quantities during one light-dark cycle of *Scenedesmus obliquus* at 30° C. +, Cell numbers; O, relative chlorophyll quantities.

Table 1. CELL NUMBERS AND RELATIVE CHLOROPHYLL QUANTITIES IN SIX SUCCEEDING LIGHT-DARK CYCLES OF *Scenedesmus obliquus* AT 30° C

Date	h	Cells/l. $\times 10^6$	Chlorophyll/l.
14/10	0	4.3	0.100
	17	4.2	0.000
	24	39.4	0.006
18/10	0	4.0	0.082
	17	4.0	0.760
	24	40.0	—
19/10	0	4.0	0.090
	17	4.1	0.836
	24	42.4	—
20/10	0	3.9	0.086
	17	4.2	0.802
	24	36.0	—
21/10	0	3.9	0.090
	17	4.3	0.840
	24	38.8	—
22/10	0	3.8	0.089
	17	4.2	0.829
	24	41.6	—

tenfold with fresh, sterile culture medium at the end of each dark period.

These results are obtained by means of the extra light from the high pressure mercury fluorescent lamps ('Philips HPL 80 W'). Without these lamps and thus with the light of the fluorescent lamps ('Philips TLF 65 W 33') only, complete synchronization occurred as well, but the average autospore number was slightly lower and the chlorophyll synthesis in the dark was lower. Also we observed a temporary decrease of the amount of chlorophyll at the start of sporulation, the meaning of which is not yet clearly understood.

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### Disappearance of Bacteria from the Zone of Active Mycorrhizas in *Tricholoma matsutake* (S. Ito et Imai) Singer

IN coniferous forests in Japan, especially forests which contain *Pinus densiflora*, the edible mushroom "matsutake" (*T. matsutake*) forms fairy rings of fruiting bodies on the ground. The ring follows the mycorrhizal development of pine rootlets and grows outwards 10–15 cm every year. On the inner area of the fairy ring where the mycorrhizas have been decomposed, fruit bodies are seldom formed<sup>1,2</sup>.

Previous studies on the microflora of soils<sup>3</sup> gave little information on the interactions between mycorrhizal fungi and soil bacteria. In this communication some characteristics of bacterial population in the zone of active mycorrhizas of *T. matsutake* and the vicinity\* will be reported; the interactions between pine roots and the mycelia are described in another paper<sup>2</sup>.

A soil profile about 50 cm deep and about 100 cm wide was prepared by trenching along the line through the centre of the fairy ring and through the point IV (0,0) (see Fig. 1), where the fruiting bodies of the matsutake mushrooms occurred in 1964.

Soil samples were obtained by inserting sterilized glass tubes into the soil at various points on the profile shown in Fig. 1. This method avoided contamination from organic matter on the soil surface.

\* This area is called "Shiro" in Japanese, which means "a castle" and may be accepted as a subterranean biotic community where the mycorrhizal development plays a leading part over the soil constituents, especially soil microbes<sup>4</sup>.

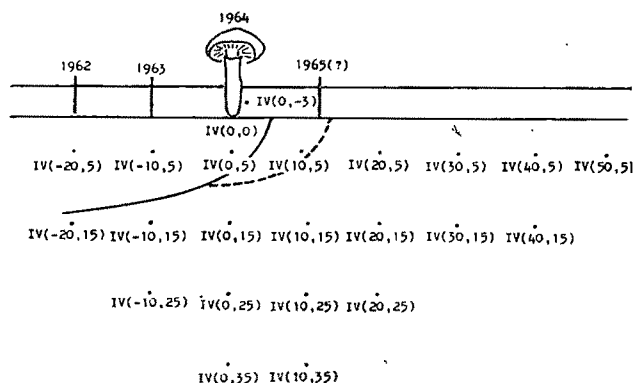


Fig. 1. Soil profile of "Shiro" in June 1965. IV (0,0) is the site of fruiting bodies in October 1964. In a sampling point designated as IV ( $x, y$ ),  $x$  indicates the distance (cm) and  $y$  the depth (cm) from IV (0,0). The solid curve illustrates visible border of white mycelial layer in October 1964, and broken one that in June 1965. The area enclosed by these two curves contains active mycorrhizas.

Since November 1964, soil has been sampled about once a month at Iwakura, Kyoto, in a forest consisting of 70–80 year old *P. densiflora*.

Isolation and estimation of bacteria were made mainly by the dilution plate method using the following media: GPYS agar†; soil extract agar<sup>4</sup>; Thornton's asparagine-mannitol agar<sup>4</sup>; nitrogen free mannitol solution<sup>4</sup>; thio-glycolate semi-solid nutrient agar<sup>4</sup>; beef extract peptone agar. Direct smearing of sampled soils on GPYS and soil extract agar slant was also done. All the isolation procedures were done within 12 h of sampling and the cultures were incubated at 30° C for 72 h. For mycelium of *T. matsutake*, 30° C is lethally high, and 72 h is not a sufficient time for it to grow<sup>7</sup>.

Neither bacteria nor actinomycetes were isolated from the point IV (0,5), right above which the fructification of matsutake occurred in October 1964; on the nutrient plates, only colonies of *Mortierella* spp. (Phycomycetes) that were commonly obtained from almost all samples were observed.

From the soil samples outside the active mycorrhizal zone [sampling points IV (10,5), (20,5), etc.; IV (0,–3) = humus layer and IV (0,15)], bacteria and actinomycetes were obtained abundantly, especially from the soil samples at IV (40,5) and (50,5) (Fig. 2b). Among them, *Pseudomonas* spp., *Arthrobacter* spp., *Agrobacterium* spp. and others were found.

From the soil through which the front of the active mycorrhizal zone had passed [IV (–10,5) and (–20,5)], bacteria and actinomycetes were seldom isolated, and remarkable aggregation and desiccation of the soil were observed. But in the soil near the centre of the ring, some bacteria and actinomycetes began to appear. The pH value of the soil of active mycorrhizas (4.6–4.7) was higher than that outside (4.1–4.4), but the water content of the former (11.0–12.0 per cent) was lower than that of the latter (15.0–19.0 per cent) (Sagara, N., unpublished data).

A seasonal change of bacterial population is another characteristic of the "Shiro". When sampled in the spring and early summer of 1965, bacteria and actinomycetes were observed to disappear from the point IV (10,5), where many of them were isolated in the autumn of 1964 (Fig. 2, left). During this period, the front of the active mycorrhizal zone advanced about 10 cm.

Similar results were obtained from the "Shiro" soils sampled at another place in Kyoto, at a place in Okayama Pref., at three places in the north-east of Japan (two of them near the northern limit of *Pinus densiflora* and one on a coastal sand dune) and at two places on the northern

† GPYS agar contains 10 g glucose; 10 g peptone; 5 g yeast extract; 100 ml. soil extract; 15 g agar and 900 ml. tap water; pH 6.8 and 5.0 (GPYS-1 and 2 respectively).

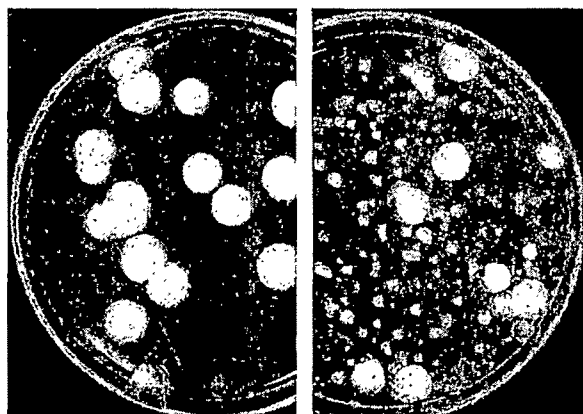


Fig. 2. *a*, Colonies isolated in June 1965 from IV (10,5) on GPYS-1 agar plate after incubation for 48 h at 30° C. Bacteria and actinomycetes are completely eliminated and only colonies of *Mortierella* spp. are observed. The same results were obtained from IV (0,5) in November 1964. ( $\times$  c. 0.6.) *b*, Colonies isolated in June 1965 from IV (40,5) on GPYS-1 agar plate after incubation for 48 h at 30° C. Bacteria, actinomycetes and *Mortierella* spp. are observed. ( $\times$  c. 0.6.)

slope of Mount Fuji (one of them in *Abies veitchii*—*Tsuga diversifolia* forest).

The fine rootlets of pine developing in the active mycorrhizal zone branch like witches' brooms<sup>2</sup>. Because the sample from the point IV (10,5) was obtained from the soil containing rootlets with these characteristics, and *Mortierella* spp., which were isolated from the soil sample, had no inhibitory effect on the growth of bacteria *in vitro*, the disappearance of bacteria, especially aerobic and heterotrophic ones as well as actinomycetes, might be closely related to the mycelial activity of *T. matsutake* or to the mycorrhizal development of pine rootlets.

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### Rate of Glucose Oxidation in Sea Urchin Eggs

ACCORDING to Bullough<sup>1</sup>, the principal process providing energy for mitosis is the oxidation of glucose during the period before prophase (antephase or preprophase). His views are not generally accepted<sup>2</sup> and cannot be extended to all instances of cell division<sup>3</sup>. On the other hand, they appear to hold for cleaving sea urchin eggs and for those of some other animals as the respiration rate of eggs increases during the period before mitosis<sup>4,5</sup>. Unfortunately, these results have not been substantiated by other authors<sup>6</sup> so that the question of the respiration cycle during egg cleavage remains open.

In order to check Bullough's concepts and the data obtained by Zeuthen<sup>4,5</sup> we have investigated the rate of glucose oxidation at different stages of the three first cleavage divisions in the eggs of *Strongylocentrotus dröbachiensis* (O. F. Müller). The work was carried out in the Murmansk Marine Biological Institute. 8,000–12,000 sea urchin eggs were placed in Warburg flasks in 4 ml. of a solution of <sup>14</sup>C glucose in sea water. The rate of oxidation of <sup>14</sup>C glucose was measured from the radio-

activity of the carbon dioxide produced with 10 per cent potassium hydroxide solution. The temperature during the experiments ranged from 6° to 7° C.

In the first experiment the eggs were placed into <sup>14</sup>C glucose solution for 40 min, at different times after fertilization, and Warburg flasks were stoppered. After 60 min more, potassium hydroxide samples (0.2 ml.) were taken to determine labelled carbon dioxide. In all, four experimental series were carried out on the eggs taken from four females. Fig. 1 summarizes the results obtained in these experiments. It can be seen that the rate of oxidation of <sup>14</sup>C glucose rises considerably during the period between divisions, and decreases during mitoses.

To find whether or not this change in the rate of oxidation of <sup>14</sup>C glucose during divisions is related to rhythmic changes in the permeability of the egg to glucose, a second experiment was carried out. Sea urchin eggs were placed into <sup>14</sup>C glucose solution 30 min after fertilization and kept there for 6 h up to the stage of eight blastomeres. Potassium hydroxide samples for radioactivity measurements were taken every 40 min, the reagent being completely replaced each time by freshly prepared solution. The results of this experiment are shown in Fig. 2. They do not differ considerably from those of the first experiment (Fig. 1): the rate of oxidation of <sup>14</sup>C glucose increased in the period between divisions and decreased during mitoses. It should be emphasized that the data obtained in the second experiments are more clearly expressed. It is particularly interesting that in this experiment the maximal increase in the rate of oxidation of <sup>14</sup>C glucose is found in the phase before prophase, thus adding weight to the hypothesis of the presence of a special phase (antephase) in cell division. A considerable rise in the rate of oxidation of <sup>14</sup>C glucose at the third division of sea urchin eggs compared with that at the first and second divisions seems to be associated with a gradual increase in the concentration of carbon-14 in the eggs.

Thus the results obtained on the rate of oxidation of <sup>14</sup>C glucose at different stages of the first three divisions in eggs of *Strongylocentrotus dröbachiensis* support the findings of Zeuthen that the respiration rate of sea urchin eggs increases in the period between mitoses, as well as Bullough's concept of the part played by glucose oxidation in providing energy for mitoses. These observations do

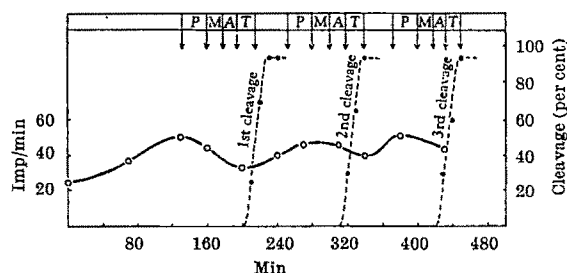


Fig. 1. Rate of oxidation of <sup>14</sup>C glucose (measured from the radioactivity of carbon dioxide) at different stages of the first three cleavage divisions of sea urchin eggs. P, Prophase; M, metaphase; A, anaphase; T, telophase.

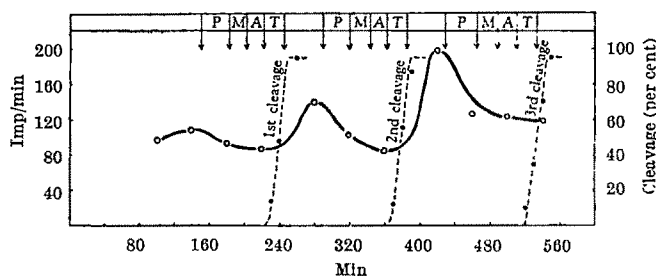


Fig. 2. Rate of oxidation of <sup>14</sup>C glucose at different stages when sea urchin eggs are kept continuously in <sup>14</sup>C glucose solutions during the first three cleavage divisions.

not contradict the hypothesis advanced by Swann<sup>7</sup> that there is an energy reservoir that fills before mitosis. On the basis of our results it can be assumed that the filling of the energy reservoir in sea urchin eggs proceeds at the expense of glucose oxidation. Finally, it seems that an increase in the rate of oxidation of glucose in the period between divisions of sea urchin eggs is related to an activation of the enzymes of the hexose monophosphate shunt, as it is known that glucose oxidation at cleavage is realized mainly by way of the hexose monophosphate shunt<sup>8</sup>. This confirms the observations that in synchronized fibroblast cultures the concentration of glucose-6-phosphate dehydrogenase (which controls the hexose monophosphate shunt) rises in the period between divisions and falls during mitoses<sup>9</sup>.

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### Kinetin-treated Flax Seedlings as Models for the Integration of the Plant Body

AMONG the many morphological experiments performed on various seedlings<sup>1</sup> a recent experiment indicated in Fig. 1 seems to be particularly suitable to further the understanding of the growth correlations which are responsible for the integration of the plant body. Seedlings of *Linum usitatissimum* when decapitated above the first leaf pair always replace the lost top, when the cotyledonary buds grow out vigorously and prevent the development of the buds which are above them in the axils of the first two leaves, because the cotyledonary buds exhibit a much more advanced embryonic development because the cotyledons have a larger area than the first leaves. Kinetin immediately applied in 1 per cent lanolin to the bud of the first leaf caused it to expand, as in other cases<sup>2-4</sup>, but the cotyledonary shoots, even in this case, grew out more strongly and finally inhibited the shoot pretreated with kinetin (Fig. 1a).

When, in addition, the root and most of the hypocotyl were removed simultaneously, only the bud treated with kinetin developed when plants were placed simply on a water surface (Fig. 1b). In spite of their initially greater embryonic differentiation the cotyledonary buds remained in this case quite small, apparently because of a lack of particular metabolites of the root. The transport of these latter could be blocked by indolyl-3-acetic acid in 0.5 per cent lanolin, placed in the form of a ring at the upper end of the hypocotyl, under the cotyledonary node. In such plants with the root left the expansion of the previously much larger cotyledonary primordia was suppressed when the first leaf bud was treated with kinetin. These results agree with the well known action of kinetin which causes, for example, accumulation of proteins and nucleic acids, renewed formation of chlorophyll in senescent leaves, and activation of auxin<sup>5-10</sup>. All these effects of kinetin are diminished if the roots are preserved.

The correlative influence of the roots enhances the obvious apical dominance, allowing in intact *Linum* seedlings a very limited expansion of the lateral buds which have been treated with kinetin. The regular dominance of the bud of the first leaf which has been treated with kinetin in decapitated seedlings without the root

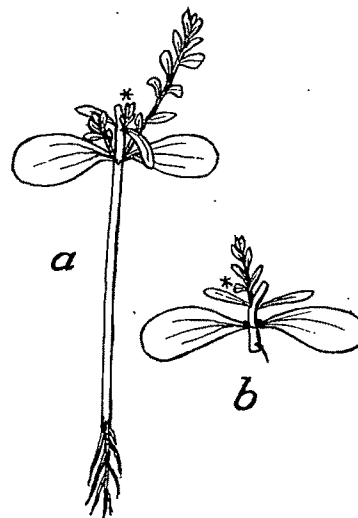


Fig. 1. *Linum usitatissimum*, decapitated seedlings, treated with kinetin paste on the bud of the first leaf, marked by (\*). a, Root and hypocotyl were left and the cotyledonary buds developed more strongly and b, these organs were removed and only the bud of the first leaf, which had been treated with kinetin, developed.

and hypocotyl shows that many other such differences in the state of the embryonic development of the buds, their position on the plant and their attachment to the conducting system can be prevented by localized application of kinetin. This does show very clearly, however, the primary importance of root activity in the correlative integration of the plant body.

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### Living Root System distinguished by the Use of Carbon-14

THERE is no adequate method to distinguish a functional root from a non-functional or presumably dead root. An electrical technique has been used<sup>1</sup> to diagnose the vitality of portions of roots of some weeds by measuring the resistance and capacitance of the tissue. Vital staining, in many cases with triphenyl tetrazolium chloride, has also been used for seedlings of grasses such as rice<sup>2</sup>. These methods, however, can only be used when the root is thick enough to allow insertion of an electrical probe into tissues or when the root is so young that a stained portion may be easily seen.

Accordingly, there is need of a satisfactory and more widely applicable method, for grasses which are grown in the field, to distinguish functional or non-functional parts of the root system.

We applied carbon-14 to the leaves of seedlings of Italian ryegrass (*Lolium multiflorum* Lam.) and traced the translocation of labelled assimilates to various parts of the root system, to ascertain whether they were readily translocated to non-functional roots, because carbohydrates are metabolites of living cells of the tissue.

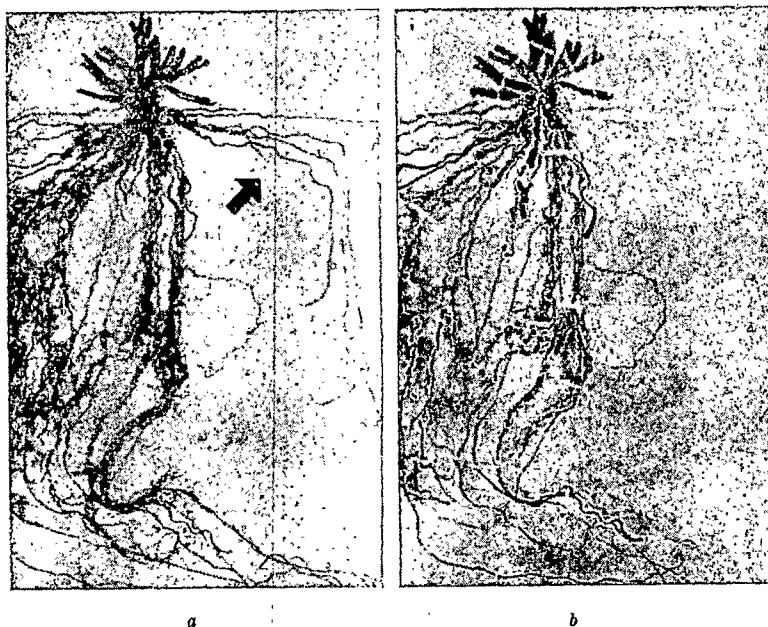


Fig. 1. Distribution of carbon-14 in the root system of 3-month-old Italian ryegrass seedlings. (a) The plant material and the part of root which was killed throughout its length is indicated by an arrow; (b) autoradiograph of the same plant.

Seedlings of Italian ryegrass were grown in small pots in glasshouse conditions and, after 3 months, plants were removed and parts or whole lengths of some roots were killed in boiling water before replacement in the pots. Carbon-14 (20  $\mu\text{C.}/\text{plant}$ ), in the form of carbon dioxide, was applied to the plants in the pots, which were placed in a plastic, air-tight, chamber and the plants were fed for 3 h at 22°–25° C in natural light. Plants were then taken out of the pots and washed thoroughly in running water. After sufficient freeze drying to permit autoradiography, each piece of plant material was mounted on a sheet of paper and exposed to X-ray film. After 2 days satisfactory autoradiographs were obtained.

Autoradiographs revealed that labelled assimilates were not readily transferred to the dead parts of the root system. This is more marked in whole root lengths which had been killed as is shown in Fig. 1, in which the dead part of the root system is arrowed (a). The autoradiograph of the same plant (b) shows few images in the dead roots and, in the living parts of the roots, images are distributed evenly throughout the whole length, but images around the root tips develop rather strongly.

This suggests that assimilates cannot readily move down to the non-functional roots; our technique may be applicable to a growing plant on the field.

In the next trial, we have applied this technique to an Italian ryegrass sward to observe its effect on older plants. Italian ryegrass was planted in spaces 20 cm apart in November 1964 on a field of volcanic acid soil. On May 18, 1965, a bottomless plastic chamber large enough to cover four plants simultaneously, 30 cm wide, 50 cm long and 30 cm high, was placed on the sward and the edges of the side wall of the chamber were fixed to the soil surface by a trench, in which was slightly acid water; this made the chamber air-tight. Two cocks of the chamber were connected to a carbon dioxide generator and an air pump by means of rubber tubing, so that the gas circulated through the chamber with a velocity of about 1 l./min.

Carbon-14 (100  $\mu\text{C.}$ ) was applied to each plant for 1 h at 25°–30° C. After feeding, excess labelled carbon dioxide was absorbed by dilute alkali solution, the chamber was removed, and the roots were taken out of the soil with an iron core sampler 10 cm in diameter and 10 cm deep. Blocks of soil were thoroughly washed and the tops were detached just below the crown. All detached roots were killed by placing in boiling water for 1 min, washed, dried and mounted on a sheet of paper after each individual root had been trimmed to a length of 3 cm as shown in Fig. 2. After exposure to X-ray film for 7 days, the autoradiographs were developed.

We counted the number of roots in the sample and the number of these which developed images in the autoradiograph and calculated the ratio of the latter to the former. There was a large range of blackness among the images, but the following results were obtained: the mean number of roots/plant was  $267.5 \pm 58.2$ ; the mean number of roots with images was  $243.5 \pm 46.6$ ; the percentage of roots with images was  $91.7 \pm 3.7$  per cent. This revealed that only 8 per cent of all the roots had lost their function by the middle of May while the rest of the roots were still functional. This large number of functional roots also seemed to

correspond with vigorous vegetative growth of tops which yielded  $15.61 \pm 3.19$  g on a dry weight basis.

There are some problems to be solved with this technique. One of them concerns the rate of translocation of assimilates to underground organs which depends on the age of the individual leaf and tiller of the shoot system<sup>3</sup> as well as on the growth stage of the plant. Flower initiation, in general, seems to retard this downward translocation, for at this stage of reproduction most photosynthetic products tend to move to the ear. This upward translocation is detrimental to our technique, so some treatments, for example removal of flower organs, are necessary before the application of carbon-14.

Another problem concerns the distribution of translocated assimilates within the root tissue. As in the case of the seedling, we observed assimilates distributed evenly throughout the root, but the same distribution also occurs

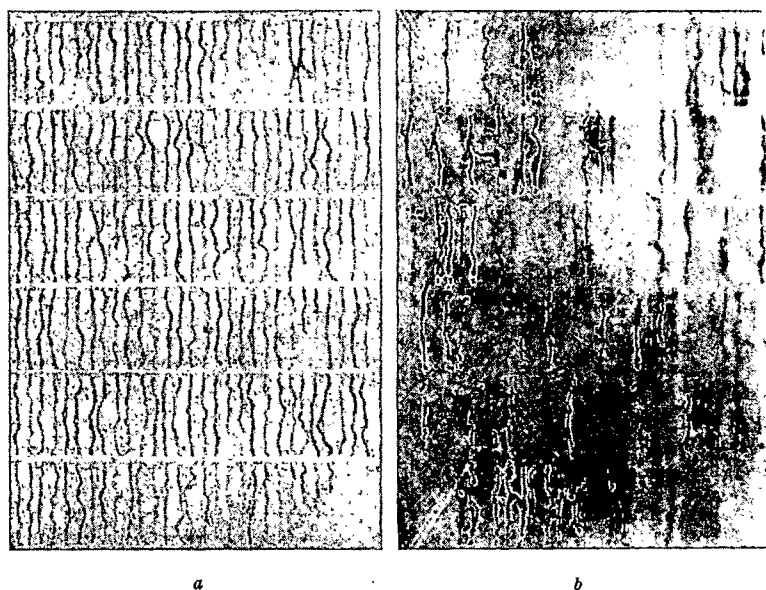


Fig. 2. Distribution of active carbon-14 in the root system of older Italian ryegrass on the field. (a) Roots which were trimmed to 3 cm long; (b) autoradiograph of the same sample.



in an older root. If the distribution in the root tissue is uneven, assimilates can accumulate in the lower part of roots, even if the upper part of the root such as those 3 cm long in this trial shows no image on an autoradiograph. In this case, we suppose that it is better to collect much longer roots.

We believe that this technique provides an approach to the understanding of the function of the roots particularly in perennial herbs.

We thank R. D. Williams of the Grassland Research Institute, Hurley, for advice.

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<sup>1</sup> Greenham, C. G., and Cole, D. J., *Austral. J. Agric. Res.*, **1**, 103 (1950).

<sup>2</sup> Aimi, R., and Fujimaki, K., *Proc. Crop Sci. Soc. Jap.*, **27**, 21 (1958).

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## AGRICULTURE

### Definite Record of *Fomitopsis annosa* in Australia

For many years *Fomitopsis annosa* (Fr.) Karst (= *Fomes annosus*) has been closely investigated in other countries because it is a facultative parasite of plantation grown softwood timbers which kills trees and causes heavy losses from heart rot. In Scandinavia it has been reported to cause losses of forest yield which vary from 30 per cent to 90 per cent, and in south-east Europe it has been described as one of the most devastating diseases in primeval forests of conifers<sup>1</sup>. It appears to be much less important as a parasite in Great Britain and the United States, although it is still an important cause of heart rot, especially in plantations.

Until last year it was believed here that Australia was free from *F. annosa*. Its presence was reported during the last century in both Queensland and New South Wales<sup>2</sup>. The latter collection has been cited but not confirmed in identity from the remains housed at Kew. There was considerable doubt as to the correctness of these identifications, and P. H. B. Talbot (personal communication, 1961) also concluded that no authentic evidence of its occurrence here was available. In June 1964, however, A. W. Gardner of the Queensland Department of Forestry at Atherton, North Queensland, sent me four fruit bodies collected from dead stumps of hoop pine (*Araucaria cunninghamii* Ait.) which I tentatively identified as *F. annosa*.

The general appearance and microstructure of the fruit bodies were very similar to a previous comprehensive description given<sup>3</sup> and also to specimens received in our herbarium as *F. annosa* from England, the United States, Portugal and India. Basidiospores were not seen, but all other characters corresponded closely. Cultures were isolated from some of the fruit bodies and agreed with another description<sup>4</sup> including the presence of the highly characteristic and unusual oedocephaloid conidiophores. P. H. B. Talbot (personal communication, 1965) agrees that the Atherton fungus is identical with *F. annosa*, and both fruit bodies and cultures have also been submitted to American workers for confirmation of these. C. S. Hodges, jun., who is making an intensive investigation of this species, states (personal communication, 1965) that it is morphologically identical with *F. annosa* as it occurs in the United States and elsewhere.

Despite these confirmations it is not certain that this fungus has the same parasitic abilities as, or is even conspecific with, the destructive European forms. From its location it seems to be endemic and its occurrence within the tropics is unusual for *F. annosa*, but reports give no

evidence of parasitic effects. It may be related to the form found in temperate rain forests in New Zealand on the closely related timber *Agathis australis* (kauri) or to the Philippine form<sup>5</sup> as *Trametes insularis* on *Pinus insularis*. The New Zealand form does not appear to be parasitic on exotic pines, but its physiological and taxonomic relationships are now being investigated in the United States. Unfortunately, the extreme rarity of clamp connexions in *F. annosa* precludes the normal tests for conspecificity, but it is hoped that other methods may be used.

This record may not therefore constitute any serious threat to the extensive plantings of exotic softwoods in Australia, but it emphasizes the importance of an intensive search for this fungus in, or around, established plantations, especially in areas where tentative earlier records were obtained, such as "Richmond River, N.S.W."<sup>6</sup>. After careful investigation, only one earlier record has been confirmed, at Mallanganee, N.S.W., 1919, collected by J. B. Cleland on hoop pine.

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<sup>2</sup> Cooke, M. C., *Handbook of Australian Fungi*, 135 (Williams and Norgate, London, 1892).

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## PSYCHOLOGY

### Time taken to change the Speed of a Response

CRAIK<sup>1</sup>, in seeking to understand the nature of reaction times, questioned whether they were due to stimuli having to pass through a long chain of synapses between sense organ and effector, or whether some "condensed" time lag due to a decision process occurred in one part of the chain. He argued that if the latter was the case, the process would be subject to serious interference from subsequent stimuli unless protected by a form of switching mechanism or "gate". Subsequent experimental work<sup>2</sup> has indicated that the reaction time to a signal ( $S_2$ ) arriving during the reaction time to a previous signal ( $S_1$ ) is longer than when  $S_2$  arrives well after the reaction to  $S_1$ , and the theory (the "single-channel hypothesis") has been advanced that the arrival of  $S_1$  raises a "gate" which is not lowered until the reaction time to  $S_1$  has ended. Reaction to  $S_2$  is thus delayed by the time elapsing between the arrival of  $S_2$  and the end of the reaction time to  $S_1$ . Clear exceptions have been found only when it has been reasonable to regard subjects as able to group  $S_1$  and  $S_2$  into a single unit and respond to both together. Typically, this occurs only when they are less than about 0.1 sec apart, and not always then.

The present communication describes what appears to be a type of exception not previously reported. Subjects were presented with vertical lines rising 1.5 in. from a baseline on a paper band revolving on a kymograph drum behind a screen in which was a vertical slit 10 mm wide. The lines were spaced so as to appear at irregular intervals of 2-3 sec. One group (A) was told that as soon as a blue line appeared they were to draw a line of the same length in the slit and then return to the baseline, making the whole movement smoothly and without hurrying. After forty-five practice lines they were told that occasionally a red line would appear, in which case they should make the movement as rapidly as possible. A second group (B) made their normal responses to the blue lines as rapidly as possible and their occasional responses to the red lines at leisure. For each group the test series consisted of 140 blue lines interspersed with

72 red lines, eight standing alone and eight following blue lines at each of the intervals 25, 50, 75, 100, 150, 200, 250 and 300 msec. A third group (*C*) were given the same instructions as group *A*, except that when a red line occurred they were to stop their movement and pause before returning to baseline. There were three subjects in each group.

The results relevant to the single-channel hypothesis are set out in Fig. 1. They consist of all cases in which  $S_2$  arrived during the reaction time to  $S_1$  and separate responses were made to both. Cases were excluded in which the response to the red line was wholly substituted for that to the blue—a kind of grouping effect which sometimes occurred when the interval between  $S_1$  and  $S_2$  was less than 100 msec. According to the hypothesis the points for each group should lie on a straight line sloping at  $45^\circ$ . Those for group *B* are in very fair agreement with prediction, and this was so for each of the subjects individually: the rank-order correlations ( $\tau$ ) between the two measures plotted in Fig. 1 were respectively  $+0.47$  ( $P < 0.001$ ),  $+0.38$  ( $P < 0.001$ ) and  $+0.28$  ( $P < 0.02$ ). These correlations are high when it is remembered that they are inevitably reduced by any variation in  $S_2$  arising from sources other than single-channel delays. The results for group *A* show no systematic trend and the correlations for all three subjects were very small:  $-0.02$ ,  $-0.05$  and  $-0.06$ . Two members of group *C* behaved like those in group *B* and produced correlations of  $+0.42$  ( $P < 0.001$ ) and  $+0.32$  ( $P < 0.01$ ): their shorter mean reaction times are understandable in that the decision to stop a response is probably simpler than one to change its speed. The third subject in group *C* behaved like those in group *A* with a correlation of  $+0.07$ .

The results shown in Fig. 1 suggest an important refinement of the single-channel hypothesis. The central processes following  $S_1$  were concerned with the initiation of a phased pattern of muscular action, which had to be modified in response to  $S_2$ . Clearly, the extent of this

modification was very different for groups *A* and *B*. The latter, in order to slow a movement down, would have had to change the pattern of muscular innervation substantially, bringing antagonists into play in order to arrest the rapid motion, and it is therefore understandable that the delays found were similar to those observed in a previous study in which movements had to be reversed<sup>2</sup>. For group *A*, however, the speeding up seems likely to have involved merely an intensification of the pattern already in operation. Group *C* should at first sight all have behaved like group *B*, but it is possible that the subject whose performance was like that of group *A* managed simply to discontinue his movements instead of actively arresting them. If so, it seems reasonable to suggest that the decision process protected by the "gate" is the initiation of a fresh pattern of action. Thus a signal to bring other muscles into play is excluded, while signals conveying instructions merely to increase or reduce the activity of those already in action can get through.

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<sup>1</sup> Craik, K. J. W., *Brit. J. Psychol.*, **38**, 142 (1948).

<sup>2</sup> For reviews see: Bertelson, P., *Quart. J. Exp. Psychol.*, **18**, 153 (1966). Welford, A. T., *Brit. J. Psychol.*, **43**, 2 (1952); *Acta Psychol.* (in the press).

<sup>3</sup> Vince, Margaret A., *Brit. J. Psychol.*, **38**, 149 (1948).

### Social Organization of Rats in a "Social Problem" Situation

In general, animals which are kept together form habits of responding to each other which are mostly regular and predictable. Part of the growing interest in the social behaviour of humans and animals is to discover more about the nature of these patterns of responding and the extent to which they vary between different types of animal, in order to build up some sort of "complete picture". Despite the potential usefulness of social experiments in the laboratory with all kinds of animals, the lower vertebrates show only rudimentary capacities for social organization. It is not then surprising that relatively little work has been done on small mammals such as the rat, because rats do not appear to be especially influenced by each other's behaviour. Where there have been experiments designed to study social behaviour in the rat they have been mainly concerned with dominance and aggression; gregariousness; social facilitation; competition and co-operation. Many of these appear to have been designed specifically to test, in an "all or none" fashion, the existence or non-existence of social psychological concepts assumed to be appropriate to a variety of animals, and in particular to the higher primates including man. Studies of co-operation<sup>1</sup> and "altruism"<sup>2</sup> provide examples of this thinking. The question arises as to how much this sort of experimentation has tended to over-emphasize assumed behavioural similarities between animals, rather than using situations thought to be flexible enough also to demonstrate important behavioural differences. One way of attempting to counterbalance this possible limitation is to use well tried techniques previously free from social psychological considerations, and to employ groups of animals in them instead of individuals. With this consideration in mind we have now completed a series of thirty pilot studies in which the social organization of the rat was investigated. Our work is based on a report of Mowrer<sup>3</sup> in which he described a situation which involves groups of rats in a "social problem"—that is, animals were trained to feed themselves by pressing a lever. When groups of three trained rats were placed in the apparatus together, hierarchies of performance emerged. One animal (of each group) did most of the work, the other two

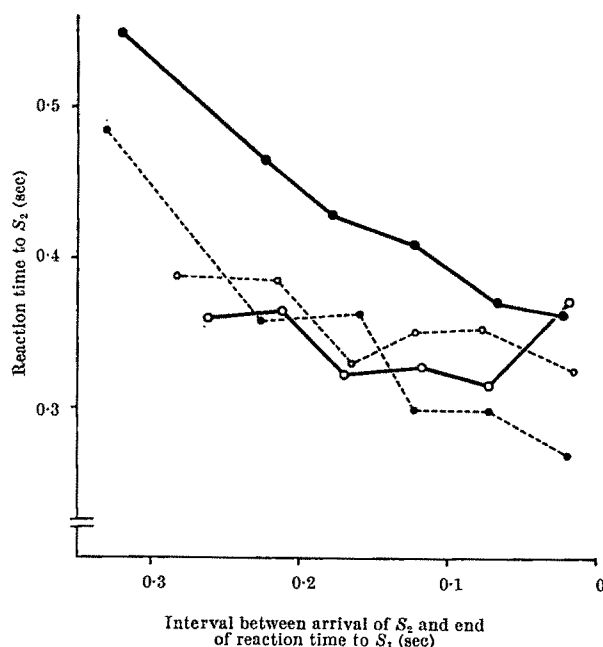


Fig. 1. Times taken to modify the speed of a response.  $\circ$ — $\circ$ , Group *A* (slow to fast).  $\bullet$ — $\bullet$ , Group *B* (fast to slow).  $\circ$ — $\circ$ — $\circ$ , One subject in group *C* (slow to stop) whose performance resembled that of group *A*.  $\bullet$ — $\bullet$ — $\bullet$ , Two subjects in group *C* whose performances resembled that of group *B*. Each point is the mean reaction time to  $S_2$  for a range of intervals between the arrival of  $S_1$  and the end of the reaction time to  $S_1$ . The ranges (from right to left) in msec were 0–49, 50–99, 100–149, 150–199, 200–249 and 250 and over. The numbers of readings contributing to each point for groups *A* and *B* vary from 7 to 38 (mean 20); those for the single subject in group *C* from 2 to 14 (mean 8.2) and for the two subjects in group *C* from 7 to 21 (mean 13.7).

most of the eating! Additionally, the solution was said to persist indefinitely. Apart from the obvious fascination of this as a situation, the investigation of group situations using instrumental conditioning techniques seemed a neglected yet potentially fruitful source of material on the interaction of individual behaviour patterns.

All our pilot studies have been carried out with one strain of black hooded Norway rats. The apparatus was a 'Perspex' box 2 ft. long and 18 in. high, with lever and food tray attachments 3 in. from the caged floor of the box, which could be rearranged so that either both tray and lever were together at one end of the box (as in the early pretraining phase) or were at opposite ends (as in the experimental situation). Each single depression of the lever delivered one small pellet of the animals' standard diet into the food tray.

Initially, we used groups of three rats which had been individually trained, and recorded that after an average of four trials of 30 min a "division of labour" similar to that reported by Mowrer could be observed. The development of one such hierarchy in terms of the number of lever pressings per subject per 30 min experimental session is typically presented in Fig. 1. Our next step was to set up a series of experiments to investigate the effect of variables which might both account for and/or influence this type of group structure; for example, the variables age, sex, group size (from 2 to 5 subjects), whether the groups were littermates or non-littermates and the effect of equating the three rats of a group for size, weight and standard of performance reached during the individual training stage. In all these experiments "hierarchies" of performance emerged which were very similar to those observed in the initial series.

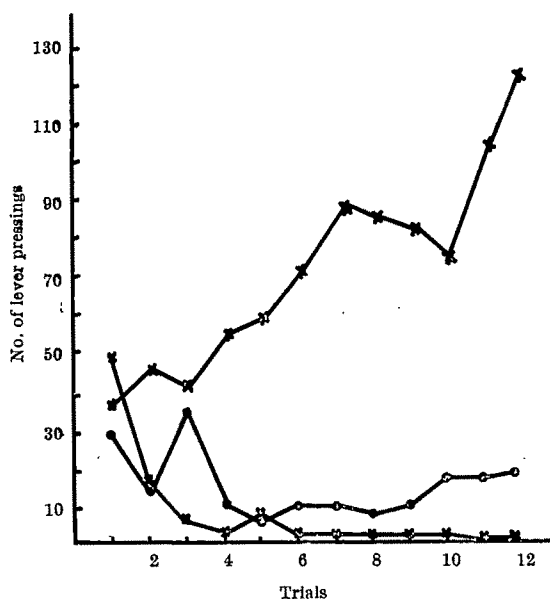


Fig. 1. Number of lever pressings per subject per 30 min session.

In addition, the same order of performance was maintained in all experiments throughout their duration, that is, most of these were run for 30 min a day, for 12 days. Similarly, the same structure was maintained on a series of retest trials after a lapse of several weeks.

Another series of experiments aimed at manipulating the organization itself. This series arose primarily as a means of trying to find answers to two main questions. First, what makes one animal start regularly pressing the lever? Second, what maintains this animal in its level of performance when so little food is forthcoming? One way of looking at the first of these questions was to deprive

members of a group of food differentially during the individual training stage, and alternatively, after a "hierarchy" had been formed. Another way was to use different schedules of reinforcement for the three animals during the individual training stage. Using six groups of animals for each condition we were unable to demonstrate any clear relationship between either the degree of hunger deprivation, or different schedules of reinforcement, and lever pressing performance during experimental sessions.

Further, it was of obvious general interest to observe the effects of running groups of three worker rats together and groups of three non-workers (or "parasites" in Mowrer's terminology). Again, these groups formed similar hierarchies of performance, the main difference between them being that the worker groups were much faster in all activities than the non-worker groups. When all these animals were subsequently retested in their original groups they behaved as in their initial experiments. This raised the interesting question of group membership, that is, whether animals adopted and maintained a status within their particular experimental group which is a result of the interaction between three particular rats. One way of testing this was to observe whether a rat would behave as a worker in one group, in one series of trials, and as a non-worker in a parallel series of trials. We did in fact observe this situation in several groups of animals of both sexes. Specific group membership then does appear to play a part in the formation of this type of social organization.

Finally, we have investigated the effect of training our animals in groups from the start, instead of using an initial individual training stage. Three main general conclusions emerged: (a) group training took appreciably less time than individual training to reach a comparable performance; (b) we observed the same sort of group structure as observed in the other experiments; but (c) that the structure appeared to be more flexible to the extent that a second animal in an experiment sometimes took over the bulk of the work load after a few trials. In other words it looked as though group trained animals did not develop such a fixed structure of performance for any particular group as did individually trained animals. In all other respects, however, they looked very similar.

The situation we have been investigating has in general proved flexible enough to raise a whole variety of questions relating to experimental studies of social behaviour in the laboratory. One factor which has struck us most forcibly in all our experiments is just how difficult it is to interpret our results within any general framework of social behaviour. Terms like co-operation and dominance-submission appear to have no useful application as yet. One of the most interesting aspects of this work is that it has led us to re-examine some of the terminology used to describe and explain social behaviour with respect to this and other animals. On the other hand, by careful examination of individual performances in a large number of experiments before training we have been able to make successful predictions about the status of individual members within our group situation. Worker animals, for instance, seem to be characterized at the early training stage by a greater activity and speed with which they learn and perform the task.

We hope that further investigation of this sort may point the way to an explanation of rat behaviour in group situations and thus indicate something of the quality of their adaptive capacity in social learning situations.

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<sup>1</sup> Daniel, W. J., *J. Comp. Psychol.*, **34**, 361 (1942).

<sup>2</sup> Rice, G. E., and Gainer, P., *J. Comp. Physiol. Psychol.*, **55**, 123 (1962).

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 6

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Gottmann (Paris): "The Growing City as a Social and Political Process. I, City Form and Function in a Changing Society".\*

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 6 p.m.—Dr. H. D. Anthony: "The Study of Scenery—2".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Emil Kekich: "Commerce" (last of four Cantor Lectures on "Some Aspects of the U.S.A. To-day").

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 6 p.m.—Prof. A. Williams: "British Ordovician Shelly Faunas. I, Paleogeographic Setting and the Principal Faunas".\*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. B. J. Hardy: "Some Problems in the Design and Use of Power Cables".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION, AND THE INDUSTRIAL MARKETING RESEARCH ASSOCIATION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. J. A. Nicoll: "A Technique of Long Term Forecasting"; Mr. B. N. P. Hutcheon: "Market Research—Forecasting for the Chemical Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Alwyn A. Ruddock: "The Earliest English Voyagers Across the North Atlantic".

## Tuesday, February 7

CHELSEA COLLEGE OF SCIENCE AND TECHNOLOGY (at Manresa Road, London, S.W.3), at 5.30 p.m.—Prof. P. M. S. Blackett, F.R.S.: "Continental Drift".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. S. W. A. Kuper: "Malignant Cells in Tissue Fluids". (Eighth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 6 p.m.—Prof. A. Williams: "British Ordovician Shelly Faunas. II, Faunal Distributions in Time and Space".\*

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Prof. A. T. James: "The Biosynthesis of Unsaturated Fatty Acids by Higher and Lower Plants".

PLASTICS INSTITUTE, LONDON SECTION (at Imperial Chemical House, Millbank, London, S.W.1), at 6.30 p.m.—Mr. E. W. Langley: "Hazards and Dangers in the Plastics Industry".

## Wednesday, February 8

PLASTICS INSTITUTE (at the Institution of the Rubber Industry, 4 Kensington Palace Gardens, London, W.8), at 2.30 p.m.—Mr. R. A. Horsley: "Environmental Stress Cracking".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—The Earl of Halsbury: "The Effects of Decimalization of the Currency".

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 5 p.m.—Dr. S. W. A. Kuper: "The Laboratory Diagnosis of Bronchial Carcinoma".\*

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Gottman (Paris): "The Growing City as a Social and Political Process. II, The Bonds of Scale and Density".\*

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Dr. P. Grootenhuys: "The Use of Visco-Elastic Materials to Control Vibrations".

SOCIETY OF INSTRUMENT TECHNOLOGY, AUTOMATION COMMITTEE (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Mr. A. H. Parker: "Automatic Testing Applied to Aircraft Electronic Systems"; Mr. E. S. Rothery: "Techniques of Satellite Launcher Checkout".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. P. P. Scott: "The Nutritional Requirements of Domestic Animals".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Brunel University, Woodlands Avenue, London, W.3), at 6.30 p.m.—Mr. H. A. Codd: "Digital Computers in Road Traffic Control".

## Thursday, February 9

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.20 p.m.—Mr. J. B. Roberts: "Monte Carlo Methods for Solving Random Vibration Problems".\*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. P. H. Fowler, F.R.S.: "The Charge Spectrum of the Primary Cosmic Radiation".\*

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. P. J. Dickinson: "The Japanese Oil Industry".

UNIVERSITY OF LONDON (at the Institute of Child Health, London, W.C.1), at 5.30 p.m.—Dr. K. Hellmann: "Naturally Occurring Anticoagulants and Fibrinolysins". (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (in the Engineering Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. P. N. Rowe: "Engineering Research and Undergraduate Teaching" (Inaugural Lecture).\*

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (joint meeting with the Liverpool Section, in the Department of Metallurgy, The University, Liverpool), at 7 p.m.—Mr. D. J. H. Sandford: "Plastics Applications in the Motor Industry".

## Friday, February 10

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Prof. J. N. Murrell: "Theoretical Aspects of Photochemistry".

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. H. Jackson: "Some Features of the Gaelic Place-Names in Britain".\*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. J. A. Fraser Roberts, F.R.S.: "Genetic Advice to Patients".

## Saturday, February 11

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Alfreid Leutscher: "Epping Forest—Its History and Wildlife".\*

BRITISH MICROCIRCULATION SOCIETY (at the Royal Postgraduate Medical School, Du Cane Road, London, W.12), from 11 a.m. to 6 p.m.—Papers in the general fields of Microcirculation and Biorheology.

## Monday, February 13

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (joint meeting with the Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Prof. A. E. Alexander: "The Role of the Surfactant in Heterogeneous Polymerization".

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 6 p.m.—Prof. A. Williams: "British Ordovician Shelly Faunas. III, Implications of Faunal Distributions".\*

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 6.15 p.m.—Dr. M. K. Towers: "Cardioversion and Control of Arrhythmias".\*

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN INORGANIC CHEMISTRY—The Registrar, The University, Manchester 13, quoting Ref. 6/87 (February 10).

LECTURER (preferably particularly interested in the fields of power electronics, applied mechanics or hydraulics) IN ENGINEERING SCIENCE—The Registrar, University of Durham, Old Shire Hall, Durham (February 10).

LECTURER (man or woman, experienced educational psychologist) IN EDUCATION—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (February 11).

LECTURER (with good academic qualifications in biology, successful teaching experience and preferably a research degree in education) IN EDUCATION—The Registrar, The University, Liverpool (February 11).

SENIOR RESEARCH FELLOW IN AGRICULTURAL SCIENCE IN THE DEPARTMENT OF BIOLOGY to undertake and supervise research into biological problems of direct relevance to the establishment, maintenance, and utilization of improved grassland on hill farms in the Lancaster region—The Secretary, University of Lancaster, Bailrigg, Lancaster, quoting Ref. L. 199, C (February 11).

LECTURER OR ASSISTANT LECTURER IN THE DEPARTMENT OF PHILOSOPHY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (February 13).

DIRECTOR (with special interests in fisheries research) of the Institute of Marine Biology and Oceanography, Fourah Bay College, The University College of Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 15).

LECTURER (with postgraduate experience in the theory of electrical or optical properties of solids, or in elementary particle theory) IN PHYSICS—The Deputy College Secretary, Westfield College (University of London), Kidderpore Avenue, Hampstead, London, N.W.3 (February 15).

LECTURER IN SOCIOLOGY—The Secretary of the University Court, The University, Glasgow (February 18).

ASSISTANT LECTURER OR LECTURER (preferably specialized in the field of insect physiology) IN ZOOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (February 20).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with the degree of Ph.D. or equivalent research experience and normally below the age of 30) IN CHEMISTRY, PHYSICS, ENGINEERING (including chemical engineering), METALLURGY, PHARMACOLOGY or related subjects—The Registrar, The University, Sheffield, 10 (February 20).

LECTURER (preferably with relevant teaching experience) IN SOCIOLOGY OF EDUCATION—The Secretary, University of London Institute of Education, Malet Street, London, W.C.1 (February 20).

LECTURER (with teaching experience, good academic qualifications in physics, and preferably a higher degree in education) IN EDUCATION—The Registrar, The University, Keele, Staffordshire (February 20).

LECTURER IN GEOGRAPHY—The Secretary, The Queen's University, Belfast, Northern Ireland (February 22).

LECTURERS OR ASSISTANT LECTURERS (2) IN PHYSICS (for one of these posts applicants should have research experience in low temperature physics)—The Registrar, The University, Nottingham (February 22).

READER OR SENIOR LECTURER (with a higher degree in mathematics, experience in university teaching and research, and with special interests in either pure mathematics or applied mathematics) IN MATHEMATICS at the University College of Townsville, University of Queensland—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Townsville, February 24).

LECTURER IN THE DEPARTMENT OF CHEMICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (February 25).

LECTURER (with a good honours degree and experience in one of the following areas: theory or application of microwave semi-conductor devices, electromagnetic wave propagation as applied to waveguides or antennas, radar systems) IN THE DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING—The Registrar, The University, Leeds, 2 (February 27).

ASSISTANT LECTURER IN GENETICS—The Secretary, The Queen's University, Belfast, Northern Ireland (February 28).

BIOMETRICIAN (recent graduate in mathematics or statistics interested in probability theory, statistics, numerical analysis and computer science) to assist in the design of experiments and the analysis and interpretation of data—The Secretary, Institute for Research on Animal Diseases, A.R.C., Compton, Newbury, Berkshire (February 28).

LECTURER IN MEDICAL STATISTICS—Dr. Alice Stewart, Unit of Social Medicine, University of Oxford, 8 Keble Road, Oxford (February 28).

SENIOR LECTURER OF LECTURER (veterinary surgeon) in the DEPARTMENT OF VETERINARY ANATOMY—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (February 28).

SENIOR RESEARCH FELLOW (with a veterinary qualification) to join a team undertaking research into respiratory diseases of poultry in a new building at the Veterinary Field Station in Wirral, Cheshire—The Registrar, The University, Liverpool, 3, quoting Ref. RV/387 (March 1).

LECTURER (qualified in the field of petrology) IN GEOLOGY at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 3).

READER/SENIOR LECTURER IN NUMERICAL ANALYSIS in the DEPARTMENT OF MATHEMATICS—The Academic Registrar, Loughborough University of Technology, Loughborough, Leicestershire, quoting Ref. 4/G (March 9).

READER IN ANIMAL ECOLOGY in the Department of Zoology—The Secretary of Faculties, University Registry, Broad Street, Oxford (March 11).

CHAIR OF PURE MATHEMATICS at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 17).

LECTURER OR ASSISTANT LECTURER in the DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Leeds, 2 (March 31).

CHAIR OF APPLIED OPTICS in the DEPARTMENT OF APPLIED PHYSICAL SCIENCES—The Registrar (Room 39, O.R.B.), The University, Reading.

CHAIR OF SOCIAL SCIENCE AND HUMANITIES and HEAD OF THE DEPARTMENT—The Academic Registrar, The City University, St. John Street, London, E.C.1.

TEMPORARY LECTURERS (2) (pure or applied mathematicians) in the DEPARTMENT OF MATHEMATICS—The Secretary, University College London, Gower Street, London, W.C.1.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

The Gas Council. Research Communication GC 127: 57th Report of the Joint Refractories Research Committee-1965-66. Pp. 16. Research Communication GC 128: Pilot-Plant Investigations of the Desulphurisation of Light Distillates. By Dr. J. A. Lacey and S. K. Mukherjee. Pp. 14. Research Communication GC 129: Metallurgical Aspects of High-Temperature Reformer Furnace Alloys. By Dr. R. G. Baker and Dr. W. L. Mercer. Pp. 16. Research Communication GC 130: A New Wind Generator for the Laboratory Assessment of Balanced-Flued Appliances. By K. J. D. Brady and D. J. Kerac. Pp. 27. Research Communication GC 131: Flow Visualisation Applied to Flues Research. By E. W. G. Dance. Pp. 25. Research Communication GC 132: Studies of Flow Patterns and Convection in Rapid Heating Furnaces Using Model Techniques. By W. E. Francis, Mrs. B. E. Moppett and G. P. Read. Pp. 14. Research Communication GC 133: Prediction of Performance of Heating Systems Under Practical Usage Conditions. 2: Direct Circulating Systems. By W. J. Bennett and Mrs. W. R. Bramwell. Pp. 12. Research Communication GC 134: Cyclic Reforming with the Gas Council Silica-Supported Nickel Catalysts. By W. E. H. King. Pp. 18. Research Communication GC 135: Safety Shut-Off Valves for Automatic Gas Burners. By P. G. Atkinson and D. J. Moppett. Pp. 10. Research Communication GC 136: Catalysis—Art or Science? By Dr. G. M. Dixon and Dr. F. E. Shephard. Pp. 11. (Papers to be presented at the 32nd Autumn Research Meeting of the Institution of Gas Engineers, 15th and 16th November, 1966.) (London: The Gas Council, 1966.) [1511]

Flora of Tropical East Africa. Edited by E. Milne-Redhead and R. M. Polhill. Tecophilaeaceae. By Susan Carter. Pp. 6. 1s. 8d. Berberidaceae. By R. M. Polhill. Pp. 4. 1s. 8d. Tamaricaceae. By D. R. Hunt. Pp. 4. 1s. 8d. Pittosporaceae. By Dr. G. Cufodontis. Pp. 13. 2s. 3d. Juncaceae. By Susan Carter. Pp. 11. 2s. (London: Crown Agents for Oversea Governments and Administrations, 1966.) [1811]

Bulletin of the British Museum (Natural History). Geology. Vol. 13, No. 5: New Gymnosperms from the Tico Flora, Santa Cruz Province, Argentina. By Sergio Archangelsky. Pp. 259-295+8 plates. (London: British Museum (Natural History), 1966.) 32s. [1811]

University of Aberdeen: Department of Agriculture. School of Agriculture. Miscellaneous Publication No. 5 (September 1966): Index of Scientific and Economic Studies of Particular Significance to the Pig Industry of the United Kingdom, Part 5. By J. Ward. Pp. xx+76. (Aberdeen: The University, 1966.) 7s. post free. [1811]

Agricultural Research Institute of Northern Ireland. Thirty-Ninth Annual Report, 1965-1966. Pp. 36. (Hillsborough, Co. Down: Agricultural Research Institute of Northern Ireland, 1966.) [1711]

Ministry of Health and General Register Office. Report on Hospital In-Patient Enquiry for the year 1962. Part 1: Tables. Pp. xiii+203. (London: H.M. Stationery Office, 1966.) 22s. net. [1711]

Building Research Station Digest 76 (Second Series): Integrated Daylight and Artificial Light in Buildings. Pp. 8. (London: H.M. Stationery Office, 1966.) 4d. [1811]

### Other Countries

World Health Organization. Technical Report Series, No. 343: WHO Expert Committee on Dependence-Producing Drugs—Fifteenth Report. Pp. 18. (Geneva: World Health Organization; London: H.M. Stationery Office, 1966.) 2 Sw. francs; 3s. 6d.; \$0.60. [1011]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tribophysics, 1965-66. Pp. 1-1+18. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1966.) [1011]

Organization for Economic Co-operation and Development. Development Assistance Efforts and Policies of the Members of the Development Assistance Committee—1966 Review. Report by Willard L. Thorp, Chairman of the Development Assistance Committee. Pp. 186. (Paris: Organization for Economic Co-operation and Development; London: H.M. Stationery Office, 1966.) 10 francs; 15s.; \$2.50. [1011]

Bibliographien des Deutschen Wetterdienstes. Nr. 19: Agrarmeteorologische Bibliographie 1964. Bearbeitet von Maximilian Schneider. Pp. xviii+226. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1966.) [1011]

Annals of the New York Academy of Sciences, Vol. 130, Article 3: Interdisciplinary Investigation of Mucus Production and Transport. By S. Jakowska and 22 other authors. Pp. 869-973. (New York: New York Academy of Sciences, 1966.) \$4. [1111]

National Science Foundation. Surveys of Science Resources Series, NSF 66-25: Federal Funds for Research, Development, and Other Scientific Activities, Fiscal Years 1965, 1966, and 1967, Vol. 15. Pp. x+196. (Washington, D.C.: Government Printing Office, 1966.) \$1.25. [1111]

United States Department of the Interior: Geological Survey. Professional Paper 650-C: Geological Survey Research 1966, Chapter C. Pp. v+269. (Washington, D.C.: Government Printing Office, 1966.) \$2.25. [1111]

Institut Royal Météorologique de Belgique. Bulletin Mensuel. Observations Ionosphériques, Septembre. Pp. 26. Publications, Série A, No. 60: One Year of Ozone Measurements at Léopoldville (Congo), 1958. By D. Stranz. Pp. 49. Publications, Série A, No. 61: Bases Météorologiques du Calcul des Apports Thermiques au cours de la Période Estivale. Par R. Dogniaux. Pp. 42. (Bruxelles: Institut Royal Météorologique de Belgique, 1966.) [1111]

Australian Academy of Science. Year Book, June 1966. Pp. 97. (Canberra: Australian Academy of Science, 1966.) [1411]

Smithsonian Institution: Bureau of American Ethnology. Bulletin 197: An Analysis of Sources of Information on the Population of the Navaho. By Denis Foster Johnston. Pp. v+220. (Washington, D.C.: Government Printing Office, 1966.) \$2. [1411]

Contributions from the New South Wales National Herbarium. Flora Series. Flora of New South Wales by Various Botanists. Produced under the Direction of K. Muir. No. 24: Flagellariaceae. By O. D. Evans. No. 25: Restionaceae. By L. A. S. Johnson and O. D. Evans. Pp. 28. No. 27: Xyridaceae. By O. D. Evans. No. 28: Eriocaulaceae. By O. D. Evans. Pp. 12. No. 34: Xanthorrhoeaceae. By Alma T. Lee. Pp. 42. (Sydney: Government Printer, 1966.) [1411]

Albert Schweitzer—Conférences du Congrès International des Écrivains-Médecins, Debrecen, 1966. Pp. 211. (Debrecen: Éd. Debreceni Orvostudományi Egyetem, Hongrie, 1966.) [1411]

Kungl. Sjöfartsstyrelsen, Stockholm. Lovö Geomagnetic Observatory—Year Book 1963. Pp. 34. (Stockholm: Kungl. Sjöfartsstyrelsen, 1966.) [1411]

Publications of the Dominion Observatory, Ottawa. Vol. 30, No. 6: Bibliography of Seismology—Items 18999-19426, September-December, 1964. By F. E. Langill. Pp. 185-208. \$1. Vol. 32, No. 6: Record of Observations at Meadbrook Magnetic Observatory, 1964. By A. B. Cook and G. A. Brown. Pp. 205-254. \$0.25. (Ottawa: Queen's Printer, 1966.) [1411]

United Nations. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. (General Assembly. Official Records: Twenty-First Session, Supplement No. 14, A/6314.) Pp. iii+153. (New York: United Nations, 1966.) \$2.50. [1611]

Growth and Distinction: The University of California 1958/1966—Report of the President. Pp. 32. (Berkeley: University of California, 1966.) [1611]

Heparin and Lipid Metabolism: An Annotated Bibliography. By Prof. Myer M. Fishman. Pp. iv+95. (River Edge, New Jersey: Technical Service Laboratories, 1966.) \$5.50. [1611]

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions. ANARE Scientific Reports. Series A (IV) Glaciology. Publication No. 83: Glaciological Studies in the Region of Wilkes, Eastern Antarctica, 1961. By W. F. Budd. Pp. 152. (Melbourne: Antarctic Division, Department of External Affairs, 1966.) [1611]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Chemical Research Laboratories, 1965-66. Pp. 146. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1966.) [1611]

Memoirs of the Southern California Academy of Sciences, Vol. 4 (October 25, 1960): Comparative Osteology and Evolution of the Lungless Salamanders, Family Plethodontidae. By David B. Wake. Pp. 111. (Los Angeles: Southern California Academy of Sciences, c/o Los Angeles County Museum of Natural History, Exposition Park, 1966.) [1611]

Union Internationale Contre le Cancer—International Union Against Cancer. Research Commission Committee on TNM Classification. Clinical Stage Classification and Presentation of Results. Malignant Tumours of the Cervix Uteri, Corpus Uteri and Ovary. (Clinical Trial 1967-71.) Pp. 10. Malignant Tumours of the Skin, including Melanoma. (Clinical Trial 1967-71.) Pp. 10. (Geneva: Union Internationale Contre le Cancer—International Union Against Cancer, 1966.) [1711]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1798-B: Fluvial Sediment in the Little Arkansas River Basin, Kansas. By C. D. Albert and G. J. Stramel. Pp. v+30+plates 1 and 2. \$0.60. Water-Supply Paper 1798-C: Sedimentation in Brownell Creek Subwatershed No. 1, Nebraska. By James C. Mundorf. Pp. v+49+plate 1. Water-Supply Paper 1823: Sedimentation and Chemical Quality of Surface Water in the Heart River Drainage Basin, North Dakota. By M. L. Maderak. Pp. v+42+plate 1. \$0.55. (Washington, D.C.: Government Printing Office, 1966.) [1811]

Bulletin of the American Museum of Natural History. Vol. 134, Article 1: Hybridization in Meadowlarks. By Wesley E. Lanyon. Pp. 1-26+plates 1-8. \$2. Vol. 134, Article 2: A Contribution to the Herpetology of West Pakistan. By Sherman A. Minton, Jr. Pp. 27-184+plates 9-36. \$5. (New York: American Museum of Natural History, 1966.) [1811]

East African Common Services Organization. East African Virus Research Institute Report, January to December 1965. Pp. iii+44. (Entebbe: East African Virus Research Institute, 1966.) [1811]

India: Council of Scientific and Industrial Research. Annual Report of the Regional Research Laboratory, Jorhat, 1964-65. Pp. 57. Industrial Processes of the Regional Research Laboratory, Jorhat, Assam. Pp. 40. (Jorhat, Assam: Regional Research Laboratory, 1966.) [1811]

Norsk Polarinstitutt. Skrifter Nr. 139: The Eskimos of Northeast Labrador: a History of Eskimo-White Relations, 1771-1955. By Helge Kleivan. Pp. 195. (Oslo: Norsk Polarinstitutt, 1966.) 35 Kr. [2111]

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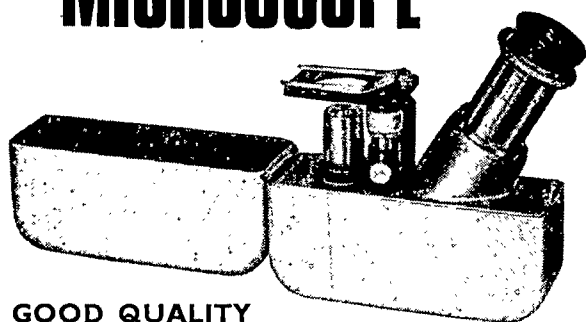
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### APPOINTMENTS VACANT

#### UNIVERSITY OF LEEDS THE ASTBURY DEPARTMENT OF BIOPHYSICS

Applications are invited for the post of Assistant Lecturer or Lecturer to assist in the teaching of the physical chemistry of biological macromolecules and to carry out research in this general field. Applicants must be chemists or physicists with experience of biological macromolecules. The post is tenable immediately but the starting date could be postponed until October 1 if necessary. The successful applicant will be required to assist in teaching to undergraduates and to postgraduate students but ample time will be provided for research in a new building (occupation date June, 1968) with modern equipment. Salary on the scale £1,105 to £1,340 for an Assistant Lecturer or £1,470 to £2,630 (efficiency bar £2,270) for a Lecturer.

Applications (three copies), stating age, qualifications and experience and the names of three referees, should reach the Registrar, the University, Leeds, 2 (from whom further particulars may be obtained) not later than February 28, 1967. (469)

#### UNIVERSITY OF STRATHCLYDE

#### DEPARTMENT OF NATURAL PHILOSOPHY

#### LECTURESHIP

Applications are invited from physicists with theoretical or experimental research interests in plasma physics or lasers for appointment as LECTURER in the Department of Natural Philosophy.

The successful candidate will be required to take part in undergraduate teaching and to participate in the programme of research in the Department.

Salary scale: £1,470 to £2,630 per annum plus F.S.S.U. with placing according to experience and qualifications.

Application forms and further particulars (quoting 2/67), can be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.1, with whom applications should be lodged by February 28, 1967. (476)

#### UNIVERSITY OF WATERLOO DEPARTMENT OF BIOLOGY WATERLOO, ONTARIO, CANADA

Applications are invited from students for post-graduate studies leading to M.Sc. or Ph.D. Areas of research include freshwater biology, taxonomy, microbiology, physiology, genetics, and palynology. Teaching Fellowships to the value of \$2,600 for 8-month academic year, plus summer stipend of \$1,000 available.

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(560)

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(530)

# Pharmaceutical Research



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## **TECHNICIAN FOR DRUG METABOLISM STUDIES**

The successful applicant will have had a number of years' experience in a similar field and be familiar with analytical, biochemical or drug metabolism techniques.

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Applicants must have a Ph.D. in botany, a number of years of research experience in systematic botany and some administrative experience. It is expected that this appointment will be made in the salary range \$14,000-\$20,000.

Résumés should be submitted to the  
Bio-Physical Sciences Program,  
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(524)

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### SENIOR RESEARCH ENTOMOLOGIST RESEARCH ENTOMOLOGIST

For research in Biological Control of Tsetse Flies by the Method of Sterile Male Release

An A.R.C.C.A. research group of five graduates with a strong supporting staff and good field facilities has been working for the past three years, together with a team seconded from the U.S.D.A., on the field and laboratory development of this new technique. Both chemosterilant and radiosterilant treatments have been successfully proved and the project is at the Pilot Scheme stage on an island at Lake Kariba. There will shortly be a vacancy for an experienced research glossinologist in this group. Salary in the Principle Research Officer scale £2,136 to £3,000 according to qualifications and experience. An appointment may be offered in a higher scale for candidates of exceptional accomplishments. A three-year contract is offered with family return fares and mid-tour leave to Cape Town. A secondment for a shorter period of not less than a year would be considered. The post is available from March 1, 1967.

A vacancy will also occur for a newly qualified research entomologist to join this team at M.Sc. or Ph.D. level.

There are no application forms, but career details and the names of three referees should be sent to one of the undermentioned addresses:

The Secretary, Agricultural Research Council of Central Africa,  
P.O. Box 3397, Salisbury, Rhodesia. P.O. Box 215, Lilongwe, Malawi. P.O. Box 2218, Lusaka, Zambia. (X438)

## RESEARCH ASSISTANTSHIPS IN PHYSIOLOGY

Applications are invited for RESEARCH ASSISTANTSHIPS from qualified students wishing to work towards the M.Sc. or Ph.D. degrees. Stipend range \$2,300 to \$3,600 per annum.

Write for further information to: Dr. D. H. Copp, Head, Department of Physiology, University of British Columbia, Vancouver 8, B.C., Canada. (341)

## UNIVERSITY OF STRATHCLYDE

THE ANDERSONIAN LIBRARY

SUB-LIBRARIAN

Applications are invited for the above post from Honours graduates with a Librarianship qualification, preferably experienced in the field of Science and Technology.

Lecturing ability on aspects of Information Retrieval and a capacity for Readers' Adviser work essential.

Salary scale: £1,470 by £90 to £2,010 by £85 to £2,180 by £90 to £2,630 per annum, with F.S.S.U. benefits.

Forms of application (quoting 11/67), can be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.1. (475)

## UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF METALLURGY

LECTURESHIP IN CHEMICAL  
METALLURGY

Applications are invited for a Lectureship in Chemical Metallurgy in the Department of Metallurgy. Candidates should be Physical Chemists with an interest in Thermodynamics and Electrochemistry and should be willing to develop those interests within the context of Extraction Metallurgy. There is an active Research School and the successful applicant would be expected to carry out and supervise research in this field.

Salary scale: £1,470 to £2,630 per annum plus F.S.S.U.

Application forms and further particulars (quoting 73/66), can be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.1, with whom applications should be lodged by February 11, 1967. (477)

## UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

DEPARTMENT OF CHEMISTRY

Applications are invited for the post of ASSISTANT LECTURER in the Department of Chemistry. Candidates should have research experience either in inorganic chemistry or in physical chemistry. Salary scale according to age and experience in the range £1,105 by £1,340.

Applications, together with the names of three referees, should reach the Registrar, University College, Cathays Park, Cardiff, from whom further particulars may be obtained, by February 15, 1967. (359)

## UNIVERSITY OF READING

RESEARCH ASSISTANT required in high pressure/high temperature experimental unit Geology Department. Honours degree in Geology or Chemistry. No previous experimental petrology experience necessary but an interest in crystalline rocks and/or phase equilibria studies essential. The Assistant will carry out research towards a higher degree. Salary £600 by £25 to £650 per annum.

Write, quoting M3 to Assistant Bursar (Personnel), University of Reading, Berkshire. (452)

## NO MONEY TO SPEND

NOBODY can say that Mr. Johnson's budget for the coming fiscal year is calculated to win friends among scientists in the United States. It is much more likely to increase the irritation of those who have been saying, for several months now, that too much of the federal government's support for scientific activities of one kind or another is being spent on the attainment—and even, occasionally, the failure to attain—short term objectives. Congress is accustomed to deal more harshly with civil than with military projects in research and development. The Department of Defense can usually get a fair wind for the development of a new missile, but the Mohole project can be cancelled after a few desultory committee meetings. (Whatever the faults of Mohole may have been, it should now be clear that Congress would have been wiser to find some other way of saving money than to assume direct control of the affairs of what should be an independent agency.) Then there are the figures which show that expenditure on research and development in the United States has now decisively levelled off. Between 1967 and 1968, the budget estimates for military research and development amount to an increase of 7.2 per cent, but the amount to be spent on civil activities (including space flight) will be almost unchanged. In the circumstances it is no wonder that the physicists have taken a somewhat masochistic pleasure in pointing out (*Physics Today*, 20, 99; 1967) that the trend of expenditure on research in physics now lags a long way behind the target the physicists were encouraged to design for themselves only a year ago.

But what is to be done? There are two issues to be decided—the level of expenditure on research and development appropriate to an advanced society like that in the United States, and the proportion of this that should be spent on basic research. So far there are only rules of thumb and the dictates of arbitrary shifts of public policy to decide how much to spend and where. Even expenditure on military research and development is not as direct a consequence of what the military planners consider to be military necessity as they would like to think. A great many projects, for example, cannot be attempted for lack of men. For the research and development budget as a whole, men and their availability are also crucial. The host of decisions which go to make up a complete budget of the kind now published in the United States is tantamount to a decision about what proportion of the country's skilled manpower can be devoted to creative research and development. In the circumstances, it is meaningful to protest that the administration may have made wrong choices about the allocation of its resources. In the United States, it is remarkable how many of the projects now being undertaken in the belief that they will bring industrial advancement of some kind or another are being financed from federal money.

Why cannot industrialists make their own decisions in these matters? On basic research, the best criterion so far for deciding how much should be spent is that there should be enough to keep educators enlivened and their students provided with problems on which to cut their teeth. One of the cogent objections to the present arrangements for financing basic civil research in the United States is that a large proportion of the money available comes from what are called mission-oriented agencies. In the nature of things, these are not equipped to make disinterested decisions about the pattern of work and education in universities and similar establishments. It could not fail to be a benefit if some of their largesse were channelled through institutions like the National Science Foundation more able to keep closely in touch with what is happening in the laboratories. That way, there might be less complaint and, more important, some hope of constructing the volume of case-law necessary if objective yardsticks about the pattern of spending are eventually to be designed.

## CLOSING THE STABLE DOOR

THE statement on information exchange groups by the Commission of Biological Editors which appears on page 547 is reasonable and predictable, if a little late in the day. It amounts to a declaration of contrary interest by an influential group of biological journals. If the IEG scheme had still been in full flood, this statement would have given the organizers and the members cause to reconsider what they were about. In the event, the National Institutes of Health have had the good sense to see that the experiment could not flourish without creating contradiction, and the groups are to be discontinued. That is a welcome development, but it does not follow that the statement is redundant. The horse may never have the strength to bolt, but it is only sensible to shut the stable door. The statement will put clearly on record the case for hoping that other experiments similar to IEG will not in future be attempted. It will also draw attention to the need for channels of communication in science with those attributes which made the IEG experiment attractive. Because the IEG seem to be coming to an end, there is no case for treating the long-term questions less urgently.

The case against IEG is now familiar, and needs no rehearsal. The biological editors are wise, however, to work from the assumption that the activities of the exchange groups were indistinguishable from publication in the more orthodox sense. There is no point in beating about that bush. Publication is broadcast writing, and the circulation of preprints to the large and often anonymous membership of some of the exchange



groups is publication in every sense that matters. The fact that IEG communications are circulated only within a chosen circle is no defence against the charge of publication but merely evidence that the method of publication is defective. Certainly the methods of the IEG are enough like publication to make a mockery of the phrase "personal communication" used as a euphemism in referring to information gleaned from circulating preprints. The biological editors would have been within their rights if they had insisted on some demeaning alternative to distinguish the communications of the IEG from the personal letters which are a traditional part of scholarship of all kinds—"impersonal communication", perhaps, or "postal circular". Certainly they are on safe ground, and they will carry most working scientists with them, in saying that they have no wish to connive at multiple publication. That is the essence of their case against the IEG.

The question will arise of where *Nature* stands, and it should be plain already that this journal has the fullest sympathy with the statement which the biological editors have put out. But the rules which the biological editors have drafted are not easily applicable to journals like this. For one thing, there is no regular means by which a general journal can know what is circulating on the IEG network. Indeed, even the journals which have decided to adhere to the statement by the biological editors can only hope consistently to enforce their own rules through the supposedly secret knowledge of people who are also on the books of the IEG. It is also important that *Nature* has a special interest in publishing material which commands attention outside the branch of science with which it is chiefly concerned, so that the region of potential conflict with the IEG (which must be narrowly defined if they are to function at all) should be less serious than for most journals. At the same time, a journal like this, whose function is in part to provide working scientists with a sense that they know what is going on, cannot wholeheartedly endorse the view that the IEG would be unobjectionable if they circulated "very brief notices of current research". The truth is that preliminary accounts of research in progress are as much in need of publication for the world to see as are the more formal contributions to the literature. But all this raises a host of almost legalistic questions. In the long run, the only defence is to beat the IEG at their own game. Fortunately, there is every reason to expect that about the middle of this year, *Nature* will be operating consistently with a time lag of a few weeks, and without skimping on the care spent in sending communications to referees. It will be harder for monthly journals to follow suit, but obviously they should try.

Improving the service which the journals provide is obviously important, and the statement by the biological editors draws attention to some of the more obvious steps which could be taken, although there is a good chance that many of the common complaints against the journals would melt away if more of them had full-time technical assistance. But mere mechan-

ical improvements are not enough. One of the most serious defects of the published literature is that it has become obsessively concerned with the compilation of a historical record, and too little concerned with communicating ideas and information in such a way as to refresh and to enliven. Indeed, one of the reasons that the IEG system has given such offence to the established journals is the implication that material sent for publication would be brought by some other means to the attention of those most likely to find it interesting. In part, of course, the fault lies with the journals, which have often taken too solemn a view of their function. Fortunately, they have it in their own power to decide whether the literature is to be read now or only by posterity.

## MANNING THE COMPUTERS

THE advice which the British Government has been given on the training of people to run computers is probably sound but certainly unimaginative (see page 539). It is comforting, of course, to know that there is not much reason to worry about the training for the less demanding jobs—operating computers and writing programmes for them. The computer manufacturers will look after that. But the advice which the working group at the Department of Education and Science has provided on the impending shortage of systems analysts and systems designers, as they are called, is much less convincing. The working party may be right, but its arguments are a little pedestrian and therefore unconvincing.

The immediate problem is to know precisely what is meant by a systems analyst or designer. The working group does not help by saying that the terms are intended as a "generic rather than an exact description". The point seems to be that there is a great need among potential users of computers for men and women who can devise ways for making the best use of computers, and who can do so with such flair that they carry other people with them. Evidently, systems analysts are evidently the evangelists of the computer age. But is it not therefore in the nature of things that they will work themselves out of their jobs? Will the need of them be as conspicuous and particular when there is a more sensitive appreciation throughout British industry of the ways in which computers can bring rationality and better performance to many industrial and commercial organizations? Exact predictions are obviously impossible, but the working group may well have been too wooden in making a simple linear extrapolation into the seventies. Courses there should be, of course, but in the long run it may be more important that undergraduates in a variety of disciplines should be given an opportunity to learn at first hand what it is like to have to plan the efficient use of a large computer. The truth is that if systems analysis is as valuable as the working group believes, it should be made a leavening throughout society.

## NEWS AND VIEWS

### Natural Gas

CHOOSING an awkward moment, Phillips Petroleum Exploration have announced a new find of natural gas in the North Sea. The announcement came a few days before the Gas Council and the successful oil companies—Shell-Esso and Amoco—were due to resume negotiations about the price to be paid for the gas the companies have found. The new find, which Phillips put at  $87 \times 10^6$  cu ft per day (twice as much as from any previous find in the North Sea), may strengthen the hand of the Gas Council in the negotiations. The Chairman of the Gas Council, Sir Henry Jones, has been consistently optimistic about the amount of gas which the North Sea field would produce, and is bound to feel vindicated by this latest find. Sir Henry believes that supplies of up to  $4,000 \times 10^6$  cu ft per day may be possible if more wells are drilled; the wells already drilled, he says, could supply between  $2,500 \times 10^6$  and  $3,000 \times 10^6$  cu ft per day. The Ministry of Power still quotes a figure of  $1,000 \times 10^6$  cu ft per day, although the Phillips find may induce it to think again.

The battle over price now enters a new and fascinating phase. For the first find, by British Petroleum, the Gas Council has agreed to pay 5d. a therm (1 therm = 100 cu ft) for  $100 \times 10^6$  cu ft per day. This contract was awarded as a stop-gap measure, with an eye towards encouraging other companies to explore. Those who were encouraged would have been naive to expect comparable rewards, and in fact the original Gas Council offer to Shell-Esso and Amoco represents 1.8d. per therm for  $500 \times 10^6$  cu ft per day. This offer, described as "woefully inadequate" by an Amoco spokesman, now begins to look more reasonable. The oil companies cannot offer to sell the gas direct to industry unless they can satisfy the Ministry of Power that it has first been offered to the Area Gas Boards at a reasonable price. The minister, Mr. Richard Marsh, is presumably to be the judge of what is reasonable. In the past week, agreement on a compromise figure of between 2.25 and 2.75d. per therm has seemed likely, but price is not the only factor. The load factors of the pipelines, rate of build-up of supplies and the duration of supplies have also to be negotiated.

To compete with fuel oil, the Gas Council says that it must supply gas at the factories at a rate of 4d. a therm, which implies an onshore price of about 2d. to 3d. Low prices, it says, would provide a great stimulus to the British economy, and would help to bring down the price of other forms of energy. It points to the situation in the United States, where natural gas accounts for a quarter of heat generation, and one fifth of electricity generation, and natural gas from Texas and Louisiana sells at 1.7d. per therm. The Ministry of Power, on the other hand, has also to consider the National Coal Board, to which fears of unemployment are a powerful emotional weapon, and which is unwilling to accelerate the process of eliminating uneconomic pits. The Gas Council, however, has a final trump card; it points out that increas-

ing use of natural gas at the expense of oil would favourably affect the British balance of payments.

### Men for the Machines

THE British Government has been provided with a prediction of the balance between the supply and demand for skilled men to operate computers which is partly cheerful and partly alarming. The report of the Interdepartmental Working Group now published by the Department of Education and Science and the corresponding bodies in Scotland and Northern Ireland (*Computer Education*, H.M.S.O., 4s. 6d.) says that on present tendencies there will be a "crucial shortage" of systems analysts and systems designers in 1970. The group seems, however, to have satisfied itself that those who use and manufacture computers will be able to train on the job the programmers and machine operators they are likely to need.

The estimates of future demand are based on forward projections of the numbers of computers likely to be in service in the United Kingdom in 1970. The 1,000 computers in service in Britain in 1965 are expected to be joined by a further 2,000 by 1970. Although there is likely to be a need for an extra 19,000 programmers by 1970, the computer manufacturers trained 4,000 people in 1964, are expected to have trained 6,000 in 1966, and appear confident that they will be able to meet all foreseeable needs and also to make up for wastage. Machine operators seem equally easy to come by and to train quickly. The computer manufacturers seem also confident of being able to recruit and train the maintenance men they will need in the next few years. But where systems designers and systems analysts are concerned, the group has calculated that there will be a need for an extra 12,000 of them by 1970, and that at least 500 of those will have to be senior people. Even now, the group considers, employers are finding it so hard to recruit systems analysts that existing computers may be used less efficiently than they might be.

The potential shortage of systems analysts provides, in the language of the report, "one specific urgent call on the educational system over the next few years". The group would like to see an integrated programme of education and training with successive periods of experience at work and in full-time instruction at some college or university. It suggests that students on courses should usually be sponsored by potential computer users. The report points out that the Industrial Training Boards could do something to help, principally by providing cash incentives to industry, but it is also recognized that the training of the more senior people thought to be necessary will have to come by greater provision of postgraduate courses at universities and elsewhere. The group considered that planning of courses on this pattern, at present being discussed with universities, was a hopeful sign but was unlikely to cater for the whole demand.

In practice the group was dismayed to find that the postgraduate courses at present available with what is called a "substantial computer element" are not fully used by students. In 1965, for example, there were 165 postgraduate students on 19 courses at various universities and colleges of advanced technology. This represented an increase of nearly 25 per cent compared

with the population of these courses in 1964 but was nevertheless much less than the estimated optimum capacity of all the courses which amounted to 260 or thereabouts. Indeed, the working group has estimated that the same courses could have accommodated roughly 400 people in 1965 if they had been stretched to full capacity.

## Office for Information

SUPPORT by the British Government for the development of information services in science is growing steadily. The Office for Scientific and Technical Information (OSTI) emerged as an independent unit under the Department of Education and Science from the fragmentation of the Department of Scientific and Industrial Research in 1964-65. Last year (1966) the expenditure of the office amounted to £300,000. A scientific staff of 13 is now employed. For the time being, the office seems to have adopted as an article of faith the view that it should wherever possible work closely with similar organizations abroad and particularly in the United States. Much of its activity so far has consisted of the application to British circumstances of information services already being developed in the United States.

Most of the activity of OSTI consists of letting contracts to outside bodies. Most often this means universities, but research associations, learned societies, and industry are also eligible for assistance. As part of the plan to collaborate with information services abroad, OSTI is supporting the trial in Britain of the MEDLARS information retrieval service devised by the United States National Library of Medicine, and the corresponding system in chemistry sponsored by *Chemical Abstracts*. But the office has also helped with grants to the University of Sheffield for research on the automatic detection of structural similarities among chemical structures (£17,500 for the three years), the formation of an information centre for high temperature processes at the University of Leeds (£13,555 over three years) and what is called a National Reprographic Centre for Documentation at the Hatfield College of Technology (£32,691). The terms of reference for OSTI seem to be generously wide. They have, for example, allowed it to provide a grant for the support of *Physics Abstracts*, at present published by the Institution of Electrical Engineers. The intention is that the grant should enable the abstracting journal to investigate new techniques of compilation and dissemination. *Physics Abstracts* is also supported on a continuing basis by the United States Government by means of a grant through the American Institute of Physics.

OSTI policy is determined largely by the Advisory Committee for Scientific and Technical Information under Dr. F. S. Dainton, Vice-Chancellor of the University of Nottingham. The committee, eleven strong, consists of six academics, three industrial scientists, a librarian and an independent consultant linked with a scientific journal. The committee seems to meet about three times a year and includes assessors from other Government departments including research councils, the Ministry of Technology, and the Atomic Energy Authority. Although the committee is advisory, it has the responsibility for deciding how resources

should be divided. As yet, fortunately, the budget seems to have kept up with the flow of good ideas.

## Design for Brittle Materials

ENGINEERS have long fought shy of using brittle materials. Ductile materials are better understood, more reproducible, and have the overwhelming advantage of being able to relieve stress by expansion if the design stress is accidentally exceeded. Some brittle materials, of course, are in common use—concrete, glass, and cast iron—but they are normally used in bulk at low stress. This reluctance to use brittle materials will, however, have to be overcome, for some of them possess properties—strength at high temperatures, and corrosion resistance—which are important in hypersonic aircraft, spacecraft and gas turbines. This group of materials includes tungsten, chromium and compound materials, ceramics and glasses. A new publication by K. W. Mitchell of the Fulmer Research Institute, commissioned by the Inter-Services Metallurgical Research Council, examines the criteria for designing in brittle materials (H.M.S.O., 12s.).

If it were necessary simply to design components which will not fail under normal conditions, the problem would be relatively easy. But in practice, of course, weight and cost have also to be considered. For ductile materials, designs are commonly based on mean or average stresses calculated by normal methods of stress analysis, but for brittle materials a more detailed examination of the stress distribution is desirable since the design must provide for unexpected loads. Conventional theories of elasticity are not strictly applicable except as a first approximation, and failure stresses must be defined in terms of a statistical distribution for the material in the condition and the size needed. There is a great shortage of published information about the properties of brittle materials: companies, which have gained their knowledge by practical experience are reluctant to divulge it freely. Then little is known about the mechanism of failure of brittle components; apart from testing at constant temperature, it is necessary to examine the effect of rapid heating and cooling, which may set up thermal stresses in the material. Surface condition is also important, since surface cracks increase stresses locally. The problems of lubrication and wear resistance are mentioned in this report but not considered in detail. And throughout this field, there seems a need for more information, and not only about successful designs: reports of failures can prevent others repeating them.

## Changes at Harwell

THE United Kingdom Atomic Energy Authority, committed to diversification, is to undertake responsibility for two new research centres at Harwell. The new centres, announced on February 6 by Minister of Technology Anthony Wedgwood Benn, will be responsible for Non-Destructive Testing and Ceramics Research. When fully established the centres will cost £200,000 and £420,000 per year respectively, and five-year plans have been drawn up for both projects.

The Ceramics Research Centre is intended to help and encourage industry to make the best possible use of ceramics. Although widely used for industrial processes at high temperatures, ceramics are not well understood. The iron and steel industry uses more ceramics than most, and is likely to be the chief beneficiary if the centre can make radical improvements in manufacture, fabrication or use, but other industries also use ceramics in smaller quantities. Dr. F. J. P. Clarke of the Ceramics Division at Harwell will be the first head of the centre. Initially work will be concentrated on developing refractory oxides, and on methods of producing high quality oxide ceramics and special graphites.

THE Atlas Computer Laboratory of the Science Research Council is embarking on an experiment to test the usefulness of multiple access computer systems. According to the Science Research Council, the project will cost £95,000. By the beginning of 1968 it is planned that there shall be twenty teletype consoles linked to the Atlas computer through an intermediate satellite machine built by the General Electric Co., Ltd. (U.K.). In the planning of the experiment, careful attention is being given to the quality of the system, and the planners are particularly anxious to avoid peripheral troubles of the kind which have occasionally afflicted other multiple access systems—inadequacy of teleprinter links, for example. The satellite computer is intended to take over much of the housekeeping work associated with multiple access systems; experience with Project MAC at the Massachusetts Institute of Technology is said to have shown that roughly a third of the time spent by users of multiple access systems goes on comparatively humdrum activities such as editing and retrieving information. Obviously it is not sensible to bother the big machine with chores like these. In practice the multiple access experiment is intended to compete with the other users of the Atlas computer. At present keep that machine more than busy. Every job taken on, two are turned down. The laboratory is co-operating with International Business Machines Corp., Ltd., in a study of the problems which arise when multiple access is used by many users of the multiple access system. The Atlas laboratory is part of a group concerned with the study of the problems known as ASCOT,

The laboratory itself seems now to have settled down in the building at Culham, Berkshire, which it has occupied since the middle of 1964. At present the computer is running three shifts on five days a week and the weekends are likely to be used as well in the course of this year. The computer itself is now in service for between 95 and 98 per cent of the available time, and half of the unplanned shutdowns seem to be due to failures in the 48K core store with which the machine is provided. The experience of the past few months has shown that, in a typical week, something like 2,500 jobs may be undertaken at the laboratory. The principal customers are universities, and there are 250 university projects on the book, most of them in mathematics, the physical sciences and engineering. The laboratory also works for some industrial users and for government research laboratories. It has, for example, been concerned with numerical forecasting for the Meteorological Office.

FEDERAL support for science was the theme when Dr. Frederick Seitz, President of the National Academy of Sciences, dedicated the Space Sciences Building at the University of Arizona in Tucson. Dr. Seitz gave the impression that research workers were beginning to go short of money, and the war in Vietnam was not the only reason for this. For a start, expenditure on basic science was not growing at the rate of 15 per cent per year which many people felt was necessary. The figure of 15 per cent, he said, applied only to basic sciences, and only for a limited period, perhaps a decade or so more.

The second difficulty was that of big science. The Mohole project, said Dr. Seitz, had been scientifically sound, but had been cancelled because of the escalation of costs and the intrusion of political issues. Dr. Seitz said that he hoped the project would be reinstated; a permanent restriction on deep-sea drilling would not serve the interests of science. As for the 200 GeV accelerator, which scientists in the United States had asked for, Congress had not yet felt ready to act, while in the U.S.S.R. a 70 GeV accelerator was almost finished and in Europe a 300 GeV machine seriously contemplated. If the decision on the American machine were delayed beyond 1967, the position of the United States in high energy physics would be put in serious jeopardy.

A third factor, according to Dr. Seitz, was the growing geographical concentration of scientific expenditure. Centres of excellence, he implied, carried with them their own dangers in a country in which so many decisions are made on the basis of proportional representation. "It is difficult to believe that Congress will agree indefinitely both to expand the budgets for science and to concentrate the money increasingly in a few geographical areas." Congress might well take the matter of geographical distribution into its own hands. On space research, Dr. Seitz hoped that the frenzied atmosphere of competition could be replaced by some form of international co-operation to ensure that the scientific aspects of the exploration could be handled in a less peripheral way.

## More about Mohole

THE complaint about the way in which the U.S. Congress has cut off Project Mohole without a shilling is also one of the themes of the annual report of the President of the Carnegie Institution, Mr. Caryl P. Haskins, for 1965-66. Mr. Haskins pointed to the way in which the federal government has grown to be a dominant influence in the sponsorship of research, and to the way in which a situation of this kind has arisen. Policy will become a matter for "public and indeed political judgment and decision". Mr. Haskins said that the dangers had become particularly acute in 1966 and that the way in which the Appropriations Committee of the House of Representatives had refused the National Science Foundation \$19.7 million for Project Mohole was ominous. For one thing, he said, there has been financial loss as a result of the cancellation but, more important, real damage has also been done to the pattern of research in the United States. Although Mohole had begun badly, it had "recently been much more consistently planned". Mr. Haskins also complained at the way in which the National Science Foundation has repeatedly been denied by Congress the sums of money for which it asked in its budget. The foundation, he said, is the only agency of the government free to put its resources where they can be best applied.

## Trouble about Stonehenge

ASTRO-ARCHAEOLOGISTS seem to be a quarrelsome lot. The controversy in *Nature* about whether Stonehenge was a computer for predicting eclipses, and if so what kind of computer, has been sustained ever since the publication by Professor Gerald S. Hawkins of an article on the subject in 1963 (*Nature*, 200, 306). Last week, for example, Drs. Cotton and Martin from the University of Melbourne argued that Professor Hawkins's scheme, like that of Professor Hoyle, is too elaborate. But Professor Hawkins himself (Smithsonian Institution Astrophysical Observatory, Special Report 226) has now taken Sir Norman Lockyer to task for having published "Some Questions for Archaeologists" in 1906 (*Nature*, 73, 280) which, so Hawkins says, "should never have been permitted into print by the editor of *Nature* who was at that time Sir Norman himself". Hawkins goes on to refer to Lockyer's other work on Egyptian chronology, mythology, religion and the calendar, and says: "Such an extensive effort was premature, and he did not proceed at an appropriate scholastic pace by publishing step by step in the scientific journals and thus benefiting from the appraisal and criticism of other scholars." Despite the unfortunate context in which they are found, several of the suggestions in the 'Dawn of Astronomy' seem to be valid when judged in terms of the criteria of this paper. The measurements of the great temple of Amon-Ra at Karnak are sufficiently accurate to establish that this mammoth temple is aligned to the midsummer sunset. His suggestion that this alignment was used as a secret marker to enable the priests to predict the rising of the Nile is an interesting one. The temple of Isis at Denderah seems to be aligned to the rising of Sirius, and the alignment is confirmed by the inscriptions at the temple. The smaller temples at

Karnak could well be aligned to Canopus, the second brightest star in the sky". It is only natural to ask whether Professor Hawkins will be travelling next to Egypt, not to Stonehenge and the Hebrides.

## Finfish Preserved

It is well known that an army marches on its stomach, and it is nearly as well known that the United States Army hopes to fill its stomach with irradiated food. The verbatim record of the review of the United States Food Irradiation programme, held last year by the Joint Congressional Committee on Atomic Energy, shows that of the three agencies charged with fostering what is called the commercialization of food irradiation, the army is the most keen to start work. One army witness spoke of the potential demand of the army for 15 million pounds of irradiated ham a year and 7.4 million pounds of frankfurter sausages, and the same witness pointed out that the difficulty of handling conventionally preserved foods in Vietnam implies that rapid progress with food irradiation would now be particularly opportune. In the event, however, everything will depend on how soon the much discussed Pilot Irradiation Plant is built, and how quickly the Food and Drug Administration can satisfy itself about the safety of irradiated foods so as to be able to approve applications for irradiation licences as a matter of routine. At the hearings there was very little talk of the allegedly toxic effects of the chemical products of irradiating carbohydrates such as have been reported by Professor F. C. Steward and his colleagues (*Nature*, 208, 850; 1965). Dr. Charles L. Dunham did, however, suggest that there are probably similarities between the effects produced by irradiation and the caramelization of sugar. "I have a feeling that we are dealing with the same sort of thing here."

In the long run the most important part of the hearings may be the cost-benefit study which has been carried out by a private organization for the Atomic Energy Commission. The commission had asked for a particular study of a number of perishable commodities, where such advantages as there may be in food irradiation should show up as an actual saving of food which is prevented from decay. The preservation of New England finfish seems to offer the best chance of economic saving, chiefly because there is a growing demand for edible fish in the north-eastern states. The study calculates that a processing plant on the pier at Boston would be able to prevent roughly 5 per cent of the catch from going bad, and that this would be economically attractive because the cost of processing would be less than 2 cents a pound. (The study included a survey among housewives throughout the United States of willingness to buy irradiated foodstuffs, and produced the cheering result that 57 per cent of them would do so.) Unfortunately, irradiation enthusiasts, the only ones who seem to come well out of the cost-benefit study, are papaya, for which it seems that the savings from an increase of shelf-life would be about offset the development of new varieties of most foodstuffs, however, the savings are comparatively great, with fish, for example, and crabs, for example, and in a central



## Science Research Council

THE appointment of Professor B. H. Flowers as Secretary of the Science Research Council, in succession to Sir Harry Melville, has now been confirmed (see *Nature*, 213, 3; 1967). Professor Flowers has been given leave of absence for five years from his post as head of the Physics Department at the University of Manchester. He says that he intends to return to research at the end of his tenure of office. He will remain chairman of the Computer Board.

## Continental Drift

To celebrate its elevation to the status of a university college, Chelsea College of Science and Technology has inaugurated a yearly special lecture, to be called the Chelsea Lecture. The first was given on February 7 by Professor P. M. S. Blackett, on the subject of Continental Drift.

Suspensions about continental drift were not new, said Professor Blackett, but there were now many pieces of evidence which, taken together, made the theory absolutely convincing. The presence of corals, salt and fossils in regions in which they could not possibly have originated all showed a similar trend of movement. Glaciation over large areas of Africa, India, South America and Australia could only have occurred if they had once been much closer together; there was not enough water in the world to make an ice-cap large enough to cover them in their present geographical positions. Rock magnetism had proved a powerful tool, and classic studies of faults such as the Great Glen in Scotland, the San Andreas Fault in California, and the Great New Zealand Fault had shown that relative movement between land masses could and did occur. The rate of movement rarely reached 6 cm/y, and was more usually between 1 and 5 cm/y. More evidence was supplied by the Mid-Atlantic Ridge, placed equidistant between the coasts of Africa and South America. On the ridge itself, there are active volcanoes, but farther out the volcanoes are older, indicating a movement of material away from the centre.

To provide an explanation, Professor Blackett admitted, was harder; convection currents in the Earth's core, and much slower currents in the mantle itself, had been proposed. These could explain why sedimentary rocks, laid down on the ocean floor, were sometimes found on the tops of mountains.

## Compulsion on Pesticides

THE existing voluntary system for controlling the use made of pesticides in Britain will be replaced by a compulsory scheme if the Advisory Committee on Pesticides and Other Toxic Chemicals has its way. In a published *Review of the Present Safety of the Use of Toxic Chemicals in Agriculture* (H.M.S.O., 6s.), the committee points out that the voluntary Pesticides Committee has been found to "work as comprehensive as possible" but "there can be nothing

to prevent a toxic substance being put on the market by a manufacturer not wishing to participate in the voluntary scheme, nothing to restrict the import by potential users of toxic materials, and nothing to ensure that the labels used by manufacturers for marking their materials, on which the effectiveness of the voluntary scheme depends, are as accurate as the considerations of safety would demand. The advisory committee is particularly concerned that the British Government should have powers to act "rapidly and effectively" if the need should arise.

By all accounts, the principal manufacturers of pesticides in Britain now support this conclusion, largely on the grounds that they would prefer to know precisely where they stand. Nevertheless, the introduction of a compulsory scheme would be an important break with the precedents of the recent past. No doubt manufacturers of pharmaceuticals will now be asking themselves whether the existing voluntary arrangements for the approval of drugs under the Dunlop Committee may be replaced by a compulsory system.

On pesticides, the advisory committee recommends that it should be made an offence to sell, supply or import any pesticide product for use in agriculture, gardening or food storage which has not been licensed by the appropriate government department. Pesticides would be tested for mammalian toxicity, carcinogenicity, residue persistence and effects on reproduction and on wild life. It would be for manufacturers applying for a licence to vouch for the safety of their products. The advisory committee considered and rejected the possibility of providing an independent check by government laboratories, largely on the grounds of expense.

## Aflatoxin

DR. J. McL. PHILP of Unilever Research Laboratories at Colworth House has pointed out that a recent item in *Nature* (212, 1512; 1966) may have given a false impression of the toxic properties of aflatoxin. Dr. Philp and his colleagues were among the first to show that aflatoxin, produced by a mould called *Aspergillus flavus* on common foods such as peanuts, maize and rice, was responsible for death in turkeys, and gave rise to liver cancer in rats. It is now known that aflatoxin produces liver damage in many animals, including pigs, cows, rats, monkeys and turkeys. In some cases it has been shown to produce liver cancer, but it is not justifiable to suggest that it produces liver cancer in all those animals for which it produces liver damage. In man, aflatoxin has not so far been implicated either as a source of liver damage or of liver cancer, although the possibility cannot be excluded.

## Zinc for Healing

It appears that zinc deficiency may be very common in human beings. According to the U.S. Department of Agriculture, more than half of the states have zinc deficient soils. Although zinc deficiency has been found widely among domestic animals, there has not so far been much evidence of a specific effect of zinc deficiency in man. An article in the *Lancet* (i, 121;

1967) shows that zinc can accelerate wound healing in human beings when given by mouth. Two groups of healthy young airmen were operated on to drain the pilonal sinus. This operation gives a uniform wound which can be used as a measure of healing capacity. Ten of the subjects were given zinc sulphate by mouth in capsule form three times daily, while ten were not given zinc. The treated airmen showed a marked acceleration of wound healing: their wounds became covered with epithelium and formed no further scab after only 45 days, while the wounds of the control subjects took an average of 80 days to heal. The effect was more marked in the second half of the healing process—the phase of epithelialization. The wounds in the patients given zinc were also cleaner, pinker and healthier than those of the controls.

The obvious question is how zinc acts to make healing quicker. Zinc is known to be concentrated in the skin and epithelia. It takes part in a number of enzyme systems, some of which are likely to play a part in wound healing. Zinc may be incorporated into these systems. One interesting finding in connexion with this piece of research is that the amount of zinc in the urine increases markedly after wounding.

## Preclinical Institute

A new preclinical institute, which will eventually accommodate about 360 students as well as research workers and other staff, was opened on October 31, 1966, at the University of Bergen, Norway. The departments of biochemistry, physiology and anatomy are housed in one block and this is connected to a second block containing the teaching laboratories, auditoria, a museum and a reading room.

In the research block a special feature—unusual in a research institute—is the arrangement of laboratories around a central area which contains special facilities such as service and instrument rooms, and X-ray and tissue culture units, which are thus easily accessible from all laboratories. The biochemistry department has four principal laboratories, each with about eight research workers engaged particularly in projects in molecular biology. Of the five general laboratories in the physiology department one is specially equipped for electrophysiology. Each of the fifteen scientists here has his own office. In the department of anatomy there are laboratories for cytology, histology, embryology, comparative anatomy and dental anatomy. The illustration unit, staffed by an artist and two photographers, is available to all three departments.

## Bacterial Interactions

from a Correspondent in Microbiology

PRACTICAL means of prolonging the steady state of exponential growth of bacteria followed rapidly on the fundamental studies of Monod. Today, continuous flow culture techniques are becoming increasingly important in the study of microbial and cell culture physiology. The use of this technique for investigating population changes within a single bacterial species under the influence of a constant environment has complemented earlier observations from batch cultures, where growth is influenced by a changing set of

conditions. Such studies have clearly demonstrated the interplay of mutation rates and selective processes in the changing character of populations; in particular, the maintenance of exponential growth conditions has revealed the phenomenon of periodic selection. But pure cultures, by definition, are free from the selective pressures of biological competition which operate naturally.

The application of continuous cultures to the study of mixed bacterial populations has posed considerable technological problems, but recent work at the University of Oregon Dental School augurs well for their use in determining interactions between different bacterial species. R. B. Parker (*Biotech. Bioeng.*, **8**, 473; 1966), in an article which contains full details of the mechanical system, describes a two-stage continuous culture unit in which steady state pure populations are fed into a common mixed culture vessel. This equipment has made possible the investigation of interactions between at least six species, and the results of a tri-culture mixture composed of *Staphylococcus aureus*, *Streptococcus salivarius* and *Veillonella alcalescens* are used to illustrate the potentialities of the system. The established conditions of the mixed culture vessel (the "ecostat" condition) are such that the dilution rate,  $D$ , exceeds  $u_{\max}$ , the specific growth rate constant.

The generation times of each species are altered markedly from those characteristic of pure cultures. Thus, *V. alcalescens* and *S. salivarius* show reciprocal stimulation while inhibitory interaction seems to be directed against *S. aureus*. Moreover, under the ecostat conditions, the generation time of *S. salivarius* falls to the remarkably low value of 8.1 ( $s=0.5$ ). Although the residence time of bacteria in the ecostat is short when  $D > u_{\max}$ , this regime assures a steady state and there is no oscillation of population density as a result of interaction. These conditions, however, are far from being natural for mixed populations, and the results of experiments with increased residence times will be of great interest, for under those conditions the experimental system will most closely approach the natural ecosystem. Apart from providing necessary fundamental analyses of microbial interactions, studies of this sort could eventually have important repercussions in experimental pathology and industrial fermentation.

## Ribosomes—Structure and Function

by a Correspondent in Molecular Biology

REMARKABLY little concrete information is available so far about the structure of the ribosome and the part it plays, in physical terms, in the synthesis of polypeptide chains. A systematic attempt to identify which features of the ribosome are essential to its integrity and function has now been made (J. Mol. Biol., **22**, 145; 1966). Heat treatment with formaldehyde results in the loss of the messenger binding site, and the ribosome becomes inactive. Some cross-linking of the ribosome to formaldehyde, the ribosome dissociates into the subunits at high concentrations. The messenger binding site is

after treatment with nitrous acid, which inhibits this function and also causes dissociation of the 70S ribosomes into their 50S and 30S sub-units. Dinitrofluorobenzene is a more specific reagent to distinguish between the aliphatic amino groups of the proteins and the aromatic amino groups of the nucleotides, with which it does not react. With ribosomes there is a slow and limited reaction, indicating that few protein amino groups are accessible. The ribosomes remain integral and fully capable of binding messenger. They will not, however, catalyse the incorporation of amino-acids, and it appears that the specific binding site of transfer RNA is destroyed.

Moore has also examined the effect of perphthalic acid, a reagent which is known to oxidize adenine and cytosine to their N-oxides. This treatment prevents messenger binding and ultimately dissociates the ribosome into its sub-units. Moore concludes that nucleotide amino groups are vital to the association of the ribosomal sub-units, which could involve base pairing, and to the binding of messenger. The latter function, however, is not expected to involve base pairing, since it cannot be supposed that complementary sequences to any given stretch of messenger will usually be available. Indeed, Moore reports that poly-C, pre-treated to saturation with formaldehyde, will bind satisfactorily to the ribosome in competition with poly-U. The nature of any interaction between the bases in the ribosome and sugar or phosphate groups in the messenger remains open to speculation.

Some extraordinary observations on the function of ribosomes are reported by Takeda and Lipmann in the current issue of *Proc. U.S. Nat. Acad. Sci.* (56, 1875; 1966). Active ribosomes from *B. subtilis* have been prepared, but their stability—specifically that of the 30S sub-units—is poor, and the response to poly-U as messenger is relatively low. It is, however, possible to prepare active hybrids with sub-units of *B. subtilis* and *E. coli* ribosomes; 50S *B. subtilis* and 30S *E. coli* sub-units when mixed give good activity, and activity substantially higher than that of homologous *E. coli* ribosomes was achieved when 70S *B. subtilis* ribosomes were mixed with *E. coli* 30S particles. The presence of hybrids was demonstrated by the use of labelled preparations of the latter. It is also of interest that ribosomes from the two species show a distinct preference for interaction with their own sRNA in terms of phenylalanine incorporation.

Another relevant article comes from the laboratory of Marmur (Morrell *et al.*, *J. Mol. Biol.*, 6, 258; 1967). The 5S RNA, which has no transfer function and can be isolated from ribosomes, has recently been provoking much interest and speculation; Morrell *et al.* have isolated this species from *B. subtilis* in a high state of purity, and find that it resembles its analogue from *E. coli* in its properties. DNA hybridization experiments have shown that the 5S RNA is complementary to 2.5-3.0 per cent of the genome, compared with 0.5-1.0 per cent for tRNA. Competition experiments with ribosomal RNA have demonstrated that 5S RNA is a distinct species, and that it is not a tRNA nor a specific fission product of tRNA, which it has so far been considered. The molecule of 5S RNA is a small molecule, but it is yet to be determined whether there is vital,

## Parliament in Britain

In reply to a question in the House of Lords on January 30, the Minister without Portfolio, Lord Shackleton, said that the Atomic Energy Authority, in conjunction with industry, had completed a design study for a combined nuclear electricity generating station and flash distillation desalination plant which could produce 60 million gallons per day of fresh water and 400 megawatts of electricity. The estimated cost of the water from such a plant in Britain, with prevailing interest rates and with the "ground rules" adopted for calculating nuclear costs and allocating plant costs between electricity and the water produced, would be between 4s. 6d. and 6s. per 1,000 gallons, excluding any treatment and delivery charges.

On January 30, the President of the Board of Trade, Mr. D. Jay, and the Parliamentary Secretary, Board of Trade, Lord Walston, announced that the Monopolies Commission had been asked to investigate restrictive practices in the professions which might be contrary to the public interest. The intention was that the Commission should conduct as comprehensive a survey as possible of all professions, omitting from consideration practices which were expressly authorized by Statute or Royal Charter.

In reply to questions in the House of Commons on January 30, the Minister of Public Building and Works, Mr. R. Prentice, said that at the end of 1966 the Directorate-General of Research and Development employed a staff of 231; there were 53 vacancies and the total costs amounted to £1,960,000. The directorate had issued 28 publications besides a monthly statistical bulletin and substantial contributions to the scientific and technical press. Proposals had been accepted for the new Construction Industry Research and Information Association and would shortly be put to the full membership of the Civil Engineering Research Association for ratification. The new association would have a grant from public funds of about £200,000 in its first full year, depending on the amount which the industry subscribed, giving it a budget of about £400,000, of which about three-quarters had already been promised.

In a written answer in the House of Commons on January 31, the Minister of State, Department of Education and Science, Mr. G. Roberts, stated that last year some 15 per cent of overseas students at United Kingdom universities and a few colleges of further education were financed from British official sources. About 22 per cent of university students from developing countries were financed by their own governments or government agencies. Of the 71,000 overseas students in the United Kingdom, some 32,000, including about 24,000 from developing countries, were covered by the Government's decision to increase fees, but no additional cost would fall on those receiving British Government grants or on those who had already embarked on courses supported by the governments of developing countries. Mr. Roberts gave the following figures for United Kingdom students studying in universities in 1964: United States, 1,959; Canada (1963), 687; France, 1,158; Germany, 342; for overseas students studying in the United Kingdom, the corresponding figures are 1,405, 657, 71 and 269; half of these students, however, are attending colleges of further education.

### University News: City University, London

DR. A. F. BROWN, at present reader in natural philosophy in the University of Edinburgh, has been appointed to the second chair of physics as from April 1.

### London

DR. L. HOUGH, at present reader in organic chemistry in the University of Bristol, and chairman of the Carbohydrate Group of the Chemical Society, has been appointed to the chair of chemistry in Queen Elizabeth College in succession to Professor H. Burton. The title of professor of cell biology has been conferred on Dr. E. J. Ambrose and the title of professor of biochemistry of drug action has been conferred on Dr. L. A. Elson, both in respect of their posts at the Institute of Cancer Research: Royal Cancer Hospital.

### Manchester

THE University of Manchester Institute of Science and Technology has created a third chair in chemistry. First holder of the chair will be Professor D. W. J. Cruickshank, at present Joseph Black professor of chemistry at the University of Glasgow. Professor Cruickshank is a theoretical and mathematical chemist, with a particular interest in crystallography and computing, and is treasurer of the International Union of Crystallography.

### Appointments

DR. A. B. ARONS, professor of physics at Amherst College, has been appointed president of the American Association of Physics Teachers, in succession to Dr. M. Phillips, professor of physics in the University of Chicago.

### Announcements

THE Dental Committee of the Medical Research Council has recently been reconstituted under the chairmanship of Professor B. Cohen, head of the Department of Dental Science in the Royal College of Surgeons of England. The other members of the committee are: Professor H. J. J. Blackwood, Sir Robert Bradlaw, Professor A. I. Darling, Professor D. G. Evans, Professor R. L. Hartles, Professor A. D. Hitchin, Professor N. H. Martin, Professor A. E. W. Miles, Professor A. G. Everson Pearse, Professor G. L. Slack and Dr. A. M. Thomson.

A JOINT National Academy of Sciences-National Academy of Engineering advisory committee to the U.S. Department of Commerce's Environmental Science Services Administration (ESSA) has been formed under the chairmanship of Dr. V. E. Suomi, of the University of Wisconsin's Center for Space Science and Engineering. The executive secretary is K. P. Howard, of the National Research Council's Division of Earth Sciences. The committee will review ESSA programmes in sciences, engineering, and services for content and relevance in scientific and engineering quality in relation to the agency's service objectives.

THE Civil Engineering Research Association estimates that its income for 1966 increased by £45,000 to £194,000, and expenditure increased to £234,000. £33,500 of this was of a non-recurring nature, some spent in the association's move to new offices near the Institution of Civil Engineers. The association has now started 87 research projects and has produced a complete set of abstract and keyword cards for all its publications, on the system developed by the American Engineers Joint Council.

THE National Reference Library of Science and Invention has been formed as part of the Department of Printed Books in the British Museum. It will eventually be housed in a new building on the South Bank of the Thames, and is expected to provide fully open access to a stock of 800,000-1,000,000 volumes covering the natural sciences and their associated technologies. At present the library

is being built up in two parts: the Holborn Division, until April 1966 known as the Patent Office Library, and the Bayswater Division.

A BUSINESS meeting of the British Mycological Society will be held in the School of Pharmacy, University of London, on March 31 at 10.30 a.m.

A SHORT course on the "Principles of Power Protection" will be held in the University of Bradford during March 29-31. Further information can be obtained from the Registrar, University of Bradford, Bradford 7.

THE fifteenth annual colloquium on the "Protides of the Biological Fluids" will be held in Bruges during May 3-7. Further information can be obtained from Dr. H. Peeters, Protides of the Biological Fluids Colloquium, P.B. 71, Brugge, Belgium.

THE Institute of Navigation, with the Deutsche Gesellschaft für Ortung und Navigation, the Institut Français de Navigation and the Istituto Italiano di Navigazione, is sponsoring a conference in Paris on April 26, 27 and 28, 1967. The subject of the conference will be "Automation as applied to the Conduct of Craft by Sea and in the Air".

A CONFERENCE on "The X-Ray Analysis of Biological Materials", arranged by the X-Ray Analysis Group of the Institute of Physics and the Physical Society, will be held in the University of Oxford during April 13-14. Further information can be obtained from Dr. E. J. W. Whittaker, Department of Geology and Mineralogy, Parks Road, Oxford.

A CONFERENCE on "Non-Newtonian Flow through Pipes and Passages", sponsored by the British Society of Rheology, is to be held at Shrivenham during September 19-21. Papers are invited for the conference, and a synopsis of not more than 200 words should be sent to Dr. M. F. Culpin, 8 Broadway, Pontypool, Monmouthshire, by May 1.

A ONE-DAY colloquium on "Combustion and Exhaust Problems associated with the Internal Combustion Engine", sponsored by the British Section of the Combustion Institute, will be held at the Thornton Research Centre, Chester, on April 7. Further information can be obtained from Professor W. G. Parker, Department of Chemistry, University of Aston in Birmingham, Birmingham 4.

A SYMPOSIUM on the "Biological Role of Indolealkylamines", sponsored by the U.S. National Institute of Mental Health, will be held at the College of Physicians and Surgeons of Columbia University, New York, from May 10-12. Further information can be obtained from Dr. E. Costa, Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York 32, N.Y., 10032.

A ONE-DAY meeting, organized by the Mass Spectroscopy Group, will be held in the Geology Department of the University of Manchester on April 5. The meeting will be open to all and will be devoted to the mass spectrometry of inorganic solids including spark source, isotope ratio and Knudsen cell studies. Further information can be obtained from R. M. Elliott, Associated Electrical Industries Ltd., Scientific Apparatus Department, Barton Dock Road, Urmston, Manchester.

A CONFERENCE to review current methods by which scientists can determine the body composition of animals without injuring them, organized by the American Council in co-operation with the National Institute of Agriculture; National Institute of Health, Education and Energy Commission, will be held in St. Louis, Missouri during May. Further information can be obtained from Dr. S. S. Kistner, Department of Biological Sciences, University of Missouri, Columbia Building, Columbia, Missouri.

# Biological Journals and Exchange Groups

In the second half of 1966 there were two important developments affecting the experiment supported by the U.S. National Institutes of Health for the rapid circulation of biological communications within Information Exchange Groups (IEG). For one thing, the National Institutes of Health decided not to continue with the experiment, at least in the form in which it had been established (see *Nature*, 212, 865 and 867). A little earlier, the Commission of Biological Editors had discussed a declaration of policy by a number of biological journals that communications circulated by the IEG would not be accepted for publication (see *Nature*, 212, 4). The statement which follows is the text of that declaration as it has now been agreed within the Commission of Biological Editors.

In the course of a meeting in Vienna on September 10 and 11, we considered some fundamental questions of journal policy in their relation to material that has been distributed by an Information Exchange Group (IEG).

The Commission recognized the value of the Information Exchange Groups as a medium for rapid exchange of informal suggestions, comments, queries, criticisms and general discussion among groups of scientists who share a common interest in a particular field, provided that such memoranda are not intended for publication. This was indeed the primary, original purpose of the IEGs. In order to make this purpose clear the Commission recommended that each IEG memorandum should state on its front page that the memorandum is not intended for publication and is not to be quoted in published papers.

The circulation of an IEG memorandum that is identical (or nearly identical) with a paper simultaneously submitted for publication in a journal can cause much trouble and confusion. The paper may undergo drastic revision before acceptance by the journal; in that case many workers in the field will read the earlier unrevised version, and may fail to read the published paper. The confusion that has arisen in some such cases is unfortunate.

Moreover there are objections to the circulation by an IEG of manuscripts already accepted by journals; that is, the distribution of preprints by an agency entirely independent of the publisher of the scientific paper. This raises questions concerning possible violation of copyright.

## Journal Policies

In view of these considerations the Commission of Editors proposed that its member journals adopt the following policies:

(1) No paper will be considered for publication if that paper, in essentially the same form, has previously been released as an IEG memorandum. Papers may not be submitted simultaneously to a journal and to IEG, nor may papers already accepted for publication in a journal be submitted to an IEG.

(2) IEG memoranda are not to be cited as such in a journal. An author may refer to the information contained in an IEG memorandum as a "personal communication" and the editor may require the author to include the inclusion of reference to the IEG.

(3) IEG memoranda did not advocate the discontinuation of their continuation

as a means of informal communication among scientists with common interests. The policy statement embodied in item (1) is essentially an extension and a restatement of a long-standing policy that has been embraced by nearly all scientific journals; it is designed to prevent multiple publication of the same paper. Editors have operated for some years on the assumption that IEG memoranda were personal communications, not publications. Gradually this distinction has become blurred, since some of the IEGs have distributed many hundreds of copies of papers simultaneously submitted to journals. Such wide distribution of preprints by an agency independent of the publishers of the journals where the papers will appear is in many ways tantamount to publication, and thereby becomes a violation of the rule against simultaneous publication of research results in more than one medium.

## Further Comments

Recently the U.S. Public Health Service has announced, in a letter by Dr. Eugene A. Confrey (*Science*, 154, 843; 1966), that the IEG programme as at present conducted will be terminated early in 1967. In view of this decision, further comments are in order.

All of us recognize that we face a crisis in scientific communication. The volume of the literature has become overwhelming. The need of each scientist to learn promptly of work by others that bears closely on his own problems is increasingly insistent. The IEGs have provided such a means of rapid exchange of information among certain groups of workers with closely related interests; they also provided a forum for controversy and discussion, without the inhibiting influences that would attend formal publication. Scientists moving into new fields of research could make their activities known to others at an early stage, long before publication. All these purposes, and others, can be served by the IEGs, and by similar groups that may arise in future.

By well established practice many scientists circulate advance copies of manuscripts that have been accepted for publication to a limited group of colleagues who share their interests. This practice obviously serves the advancement of science, and no journal regards it as a violation of copyright if the number of copies so circulated is fairly small. Such manuscript copies may fail to reach other workers whose research might profit greatly if copies were available to them. Circulation of very brief notices of current research among members of an IEG



or some similar group might serve to establish communication in cases of this sort.

As editors we recognize the responsibilities of the scientific journals for speeding the process of publication and the distribution of journals after publication. We wish to maintain high standards of careful but prompt reviewing before a paper is accepted; but the interval from acceptance to publication should be as short as possible. Some journals have reduced this interval to two months, sometimes even less, while maintaining high standards of publication; this, of course, requires the co-operation of authors in careful preparation of manuscripts and prompt return of proofs. We recognize the responsibility of the journals to make every effort to shorten publication time.

Distribution of published journals by air can make scientific findings available all over the world within a few days of publication, whereas journals sent by surface mail may take two months or more to reach Asia or Australia from Europe or America. The obstacles to circulation of journals by air are not technical but financial. IEG memoranda, as a result of being sent by air mail, reached investigators throughout the world almost simultaneously; this was one of the great merits of the IEG experiment. The overseas copies of some journals are already distributed by air; the editors of others are eager to follow their example, if they can solve the financial problems involved. We believe that the rather moderate costs of such rapid distribution will be far more than repaid by the resulting stimulus to the progress of science, and the strengthening of communication among the members of the world-wide scientific community. We believe that the International Scientific Unions should play an active part in promoting such rapid communication.

In summary: (A) We recognize the value of the IEGs, and of similar groups that may be expected to arise in future among scientists with related interests, in promoting rapid communication of material not intended for publication. If the scientists themselves wish to form more such experimental groups, and to find ways of meeting the costs of operating them, such groups may well become more numerous and more varied in future. (B) The journals listed below will not consider manuscripts for publication if preprints, of essentially identical content, are to be distributed, in substantial numbers, by an agency independent of the author or of the publisher of the journal. (C) We recognize that editors and publishers of scientific journals must make every effort to accelerate publication and distribution of accepted papers.

The following journals have subscribed to this general statement of policy: *Archives of Biochemistry and Biophysics*, *Biochemistry*, *Biochemical Journal*, *Biochimica et Biophysica Acta*, *Carbohydrate Research*, *Clinica Chimica Acta*, *European Journal of Biochemistry*, *Journal of Biological Chemistry*, *Journal of Clinical Investigation*, *Journal of Lipid Research*, *Journal of Molecular Biology*, *Journal of Nutrition*, *Molecular Pharmacology*.

The following members of the Commission were present at the meeting in Vienna and voted to approve these policies: J. T. Edsall (*J. Biol. Chem.*), J. C. Kendrew (*J. Mol. Biol.*), H. Neurath (*Biochemistry*), E. C. Slater (*Biochim. Biophys. Acta*), W. V. Thorpe (*Biochem. J.*).

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## Molecular Messengers

by

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At the British Association meeting at Nottingham in September 1966, the Zoology section held a symposium on the regulating mechanism of living cells. Most often this seems to depend on the transfer of specific molecules from one place in the cell to another and from one cell to another. The survey which follows is partly based on the discussion at Nottingham.

ONE of the well established sources of evidence for the role of chemical communication in animal organization lies in the field of early development. Spemann's classical concept of the organizer, capable of inducing the overlying ectoderm to differentiate into neural tissue, carried the implication that some form of inducing agent is transmitted from the organizer region to the responding cells. The nature of the substance involved remained obscure, and in the intervening years the exploration of this concept has become an increasingly complicated exercise. For one thing, the action of the primary organizer is a special case of the widespread inductive relationships that operate between the parts of embryos, and which are brought into action at diverse stages of development. Moreover, it is now apparent that the observed phenomena of induction, and the supposed existence of a range of inducing substances or evocators, cannot by themselves provide a complete account of the events of embryonic differentiation.

R. J. Goldacre discussed this problem at the Nottingham symposium. He remarked that one illustration of the inadequacy of the concept of chemical diffusion as a framework of reference for developmental data was that books about it have usually been very thick. This, perhaps, is not necessarily a fault, for it is slim form and facile generalization which are often symptomatic of immaturity in biological research. The thick book is a challenge on which much can be built, but it is one that we understand why it has to be so unwieldy.

### Differences between Cells

A fundamental difficulty in understanding differentiation, as Goldacre expressed it, is that cells are able to respond differently to the same stimulus. They must already be different before they can be different. It is why these differences are so important in molecular genetics, and why they are so important along which the animal develops.

as was outlined by R. A. Cox during this same session. The general trend of thought is sufficiently familiar. Reproduction and development depend on the transcription of the linear sequences of the nucleotides of genes into the linear sequences of the amino-acids of protein molecules. The stored genetic information is supposedly carried from nucleus to cytoplasm by messenger RNA, the latter having had built into it a nucleotide sequence corresponding to that present in the gene. Translation of this sequence into an amino-acid sequence takes place by interaction of the messenger RNA with the ribosomes—the amino-acids being introduced into the reacting system through the mediation of transfer RNA.

### Gene Transcription

Seen from this point of view, cells may be thought of as becoming different during early development because of some form of interference with their transcription systems. Some genes are presumably switched into action at particular stages, while others are caused to become inactive. That many biological agents are able to do this is well known, and Cox gave some examples of this. Actinomycin can inhibit the transcription of the gene; the fidelity with which the messenger RNA is translated is affected by streptomycin; while the tetracycline family of antibiotics appears to inhibit the binding of transfer RNA to the messenger RNA-ribosome complex. Puromycin inhibits protein synthesis by replacing transfer RNA and forming a bond with the growing protein chain, which thus terminates with a puromycin residue. Chloramphenicol appears to act at the stage where an amino-acid is added to the growing chain. Our knowledge of these matters has advanced in a spectacular way, yet—as Cox emphasized—many details of the mechanism of protein biosynthesis are unknown. Moreover, important though it is to know that the transcription of the genes can be controlled, much remains to be learned about the mechanism of this control, and it is just this that is the crucial problem of early development. Each cell in a developing individual seems to have the same complement of identical chromosomes, as was demonstrated, for example, in the well known experiments of Briggs and King. These proved that in the blastula and early gastrula of amphibian embryos the nuclei still retained the full potentialities of the fertilization nucleus. Later the potentialities of individual nuclei become reduced, but this reduction takes place side by side with cell differentiation, and is not the causal factor that determines this differentiation. It would seem rather that differentiation must result when gene transcription becomes switched on or off at particular stages.

### Difficulties about Diffusion

In theory, evocator substances might provide one of the causal factors in the determining of nuclear responses if they could be supposed to operate through diffusion gradients that interact with cells possessing different thresholds. Goldacre argued, however, that such gradient theories must be inadequate. Among the difficulties that they encounter are the fact that, contrary to a widespread belief, diffusion over distances of the order of cell dimensions is very fast; gradients would therefore be difficult to maintain. Moreover, the growth and development of the embryo are executed with great precision; adjacent cells may be sharply differentiated from each other, while tissues become separated by abrupt boundaries. A further difficulty is that the control of biological activities is currently envisaged as being based on negative feedback, and for this the diffusion theory makes no provision at all. If cells had to depend solely on diffusion mechanisms for their regulation they would have no means of telling what other cells were the source of the information that they were receiving. It is difficult to visualize order becoming established in these circumstances; chaos would seem to be the more likely sequel.

Goldacre suggested that a solution to some of these difficulties could be found in the view that the development of a fertilized egg into a multicellular organism is regulated by interaction between the cells of the developing embryo, effected through specific cell-surface connexions. These connexions could make it possible for cell activities to be based on calculations by the cells of what has to be done, and on continuous perception by them of how much has been achieved<sup>1</sup>.

### Cell-to-cell Interaction

Among the illustrations quoted by him in support of this view is the result of dissociating embryos into single cells. The separate cells first move about by amoeboid movement, performing licking movements on one another, and appearing to recognize one another by surface coding factors. As a result they form temporary connexions at first, and then permanent ones later, pulling apart initially if the correct position is not found. Thus the embryo becomes reconstituted, and it can resume normal development once its cells have resumed their correct pattern of contacts. Experiments with models have also been illuminating. Artificial electronic cells have been brought into interaction through appropriate communication channels, and it has been possible to show that the originally identical artefacts may, as a result of such interaction, become different, with a polarity becoming generated in the system.

The concept of the interaction of cells by specific cell-to-cell connexions implies that a transmitting cell can be fed back information from a receiving cell to inform it whether or not the influence that it has transmitted has taken effect. This helps us to understand how it is that fertilized eggs can develop into viable adults despite experimentally devised obstructions that they may meet on the way. When the developing organism consists of only a few cells, it may be possible for more than half of these to be removed without development being fatally impeded. Later, the organism may be able to compensate for extensive limb amputations. This goal-seeking flexibility is like that of a guided missile. Both organism and missile attain their targets because negative feed-back makes it possible for them to compare "what is" with "what ought to be", and then to correct for the difference.

### Cancer Cells

Goldacre concluded by examining the possible bearing of these ideas on the problems of cancer<sup>2</sup>, which appears to involve failures of self-regulation resulting from the breakdown of intercellular communication. Cells in isolation tend to multiply indefinitely as, for example, do mammalian cells in tissue culture, but the growth of cells in the mammalian body is regulated by some form of inhibition. Goldacre found it significant, therefore, that the only regular difference that has so far been demonstrated between tumour cells and corresponding normal cells lies, not in enzyme content or in fine structure, but in the adhesiveness of the cell membrane. Cancer cells, unlike normal cells, do not show inhibition of movement when they make contact with normal cells; instead, they continue to move over them. Moreover, both carcinogenic viruses and chemical carcinogens produce an immediate loss of this contact inhibition when they transform normal cells into malignant ones. These facts suggest that modification of the cell membrane, with consequent interference with cell communication and with the self-regulation of growth, may be an important element in the development of malignancy.

Goldacre was thus concerned in part with factors producing a breakdown in the control of genetic transcription. K. U. Clarke, in a discussion of gene activity in insects, dealt with the problem of avoiding this breakdown. Insects are animals in which development occupies a large proportion of the life span. Thus the decoding of the genetic information that is needed to bring the life

pattern to expression must take place under conditions in which the animal will experience marked fluctuations of food supply, temperature, humidity, and photoperiod. These fluctuations must make it difficult for stability in growth and development to be maintained, and particularly so in small poikilotherms.

### Effect of Environment

Taking as a starting point the pattern of gene transcription already outlined, Clarke classified under three main headings the possible ways in which the environment can modify this transcription during the growth and development of an insect. One possibility is that the overall rate can be speeded up or slowed down. Another possibility is that transcription may be completely stopped, as happens during diapause, when the animal, in response to changes in day length, establishes a state of low energy flow which anticipates the onset of harsh winter conditions. A third possibility is that alternative information can be called forth from the genotype; this happens, for example, in the pupa of the European butterfly *Araschnia levana*, which develops a light colour in cool conditions and a blue colour in warm ones.

As an indication of the mechanisms whereby such changes in transcription may be controlled, Clarke considered the possibility that protein synthesis might be regulated in relation to the intake and utilization of food. In *Locusta migratoria*, swallowing stimulates stretch receptors in the pharyngeal wall, and trains of nerve impulses are conducted from these to the brain. This results in the release of cerebral neurosecretion from the corpus cardiacum and the circulation of this material in the haemolymph<sup>3</sup>. Further, when the animal is intact and is feeding normally, the cells of the midgut are found to show cytological characteristics of active protein synthesis, whereas if the nervous pathways are cut, or food withheld, the appearance of the cells shows that protein synthesis is minimal<sup>4</sup>.

Clarke's argument was that this relationship between protein synthesis and neurosecretory activity, initially demonstrated in *Calliphora*<sup>5</sup> and now found to exist also in *Locusta*, could be a basis for the regulation of growth. But this relationship might itself be influenced in two possible ways. One of these he saw as the control of gene activity by feedback of one of the gene products; this would be acting as a repressor substance, in accordance with the pattern that is now thought to be widespread in animal cells. The other possibility is that the repressor substances can themselves be suppressed by a hormone which, incidentally, would probably be a neurosecretion. The rate of release of such a hormone might be determined by the pattern of activity in the nervous system; a pattern that would itself be determined by the physiological condition of the animal and the environmental stimuli received by it.

### Temperature and Development

Such a system would have consequences that could help us to understand the influence of temperature on the growth and development of insects. We can suppose that the upper thermal limit of the animal is the temperature at which the gene products are being used at a maximum rate, with feedback maximally suppressed. There can be no reserves to provide for the higher rate of protein turnover that would be evoked at still higher temperatures. Above this thermal limit the system must therefore fail and the animal die. Towards the lower thermal limit the use of the gene products will be minimal, so that their repressive feedback action will be maximal. On Clarke's hypothesis this feedback could nevertheless be suppressed by hormone release; some growth and development certainly do occur at low temperatures, the restrictive effects of which are thus to some extent offset.

Clarke pointed out further that study of the biology of related species of insects shows repeatedly that the

main difference between them is thermal tolerance. The system that he was proposing (admittedly to some extent speculative) would allow changes in temperature tolerance to be achieved by relatively few changes in the genotype, leaving most of the genetic information untouched. Genetic adaptation to different climates could thus be readily accomplished, and this is just what is commonly observed in insects.

Goldacre had introduced the theme of molecular messengers as guided missiles. This was taken up again by E. J. W. Barrington in the context of hormonal regulation. He pointed out that the power of hormones to regulate cell activities depended on their capacity to interact in one way or another with the cells that they were regulating, and that this capacity depended not only on their own properties but also on the properties of their target cells, which must be precisely matched with the properties of the hormonal molecules. This is a subtle and flexible relationship, as is well shown in the vertebrates where, despite the complex evolutionary history of these animals, the characteristics of the hormones have remained more uniform than might have been expected. Many of these hormones must have been in existence early in the history of the group, yet the vertebrate endocrine system has always remained available to assist in the establishment of new patterns of adaptive regulation. Some contribution to flexibility of adaptation has doubtless been made by the addition of new hormones during the later history of the vertebrates. An example is progesterone, which regulates pregnancy in mammals. But the main contribution to flexibility has apparently come from the continuous adaptation and re-adaptation of already existing target cells, and from the bringing of new target cells into functional relationship with already existing hormones.

### Thyroid Hormones

He illustrated this argument by reference to the thyroid hormones, which are present throughout the vertebrates, always in the same molecular form. Thyroxine has a growth-promoting action that is demonstrable in fish as well as in mammals, and that is shown in responses of individual parts of the body as well as in total body growth. For example, thyroxine promotes the rate of development of certain dermal bones of the skull in trout alevins if the animals are immersed in thyroxine solution immediately after hatching, while the invagination of the lateral line system is also accelerated.

An extreme example of this kind of effect is seen in amphibian metamorphosis, which depends on the integration of complex morphological, physiological and biochemical changes throughout the body of the larva. This integration is secured by a precisely adjusted relationship between increasing blood titres of thyroid hormones and the capacity of individual cells to respond to them. Metamorphic changes can be induced in frog tadpoles if thyroid hormones are administered to them at an early stage of their life, but these metamorphic changes may be inadequate in extent, and may occur in the wrong sequence, if the cells do not receive the correct amounts of hormone at the correct time. For example, immersion in the wrong concentration of thyroxine may produce a frog without fully developed limbs. If, however, we try to imitate nature more accurately, by gradually increasing the amount of thyroxine that is administered, then we can achieve something that is more like a normal frog<sup>6</sup>.

### Hormones and Enzymes

A particularly striking example of the adaptation of target cells to a hormone is seen in the response of the skin of the tadpole to the local application of thyroxine in a cholesterol-thyroxine pellet. The effect of the hormone on the area of skin overlying the developing fore-limbs is to make it thinner, in preparation for the emergence of the limbs at the climax of metamorphosis. But the area of skin immediately adjacent becomes thicker in

readiness for the assumption of terrestrial life. Two opposite responses are thus given by regions of tissue lying side by side, and superficially indistinguishable. Presumably what thyroxine is doing here and in similar situations is to stimulate or inhibit the production of certain enzyme systems that are involved in growth and maturation<sup>7</sup>. This seems to follow from Tata's finding that the regression of the tadpole tail, which can be evoked by the hormone acting on the isolated tail *in vitro*, is accompanied by increased synthesis of protein<sup>8</sup>. This presumably represents the increased synthesis of certain hydrolytic enzymes that contribute to the regression of the caudal tissues.

### Embryonic Differentiation

This does not, of course, explain what is the basis of the adaptation of the target cells, although it can be assumed in general terms that it depends on the ability of their genetic transcription systems to react in ways that have been determined already in earlier stages of development. We are driven back, in other words, to the problems of embryonic differentiation discussed by Goldacre. He had emphasized the significance of feedback control. Recent findings<sup>6,9</sup> indicate that this plays an important part in metamorphosis, mediated in this instance through the blood stream, and involving both negative and positive feedback.

It is known that early in the life of the amphibian tadpole, before the onset of the visible changes of metamorphosis, the pituitary is able to produce thyrotropin, which can stimulate thyroid activity. At this early stage its stimulating effect is minimal. This is supposedly because its output is kept at a low level as a result of the thyrotropin-secreting cells of the pituitary being highly sensitive to negative feedback from the thyroid gland. However, the neurosecretion of the hypothalamus also enters into the regulating system. This secretion reduces the sensitivity of the thyrotropin-secreting cells to thyroxine, but its effect is at first small because the hypothalamus is only poorly differentiated. According to Etkin's analysis, the effect steadily increases because the thyroxine exerts a positive feedback action on the hypothalamus; the effect of this is to increase its output of neurosecretion, so that there is a progressive increase in desensitization of the thyrotropin-secreting cells. This positive feedback action is by its very nature self-accelerating; thus it rapidly builds up the hypothalamic neurosecretory activity to a point at which large quantities of thyrotropin are released, and the thyroid gland is raised to the high level of activity needed to bring about the metamorphic climax.

### Feedback Systems

It is not clear whether this positive feedback relationship is peculiar to amphibians, or whether the events of metamorphosis are extreme specializations of processes that occur also in other vertebrates. But the principles of organization revealed in amphibian metamorphosis must be widely applicable. This symposium made clear that at all stages of development, and at all levels of complexity, from the single cell to the advanced metazoan, molecular messengers operating through feedback systems are of paramount importance in developmental and physiological regulation.

<sup>1</sup> Goldacre, R. J., and Bean, A. D., *Nature*, **186**, 294 (1960).

<sup>2</sup> Goldacre, R. J., *Proc. Second Intern. Congr. Medical Cybernetics, Naples, 1964* (in the press).

<sup>3</sup> Clarke, K. U., and Langley, P. A., *J. Insect. Physiol.*, **9**, 287, 363, 411, 423 (1963).

<sup>4</sup> Clarke, K. U., and Gillott, C., *J. Exp. Biol.* (in the press).

<sup>5</sup> Thomsen, E., and Møller, I., *Nature*, **183**, 1401 (1959).

<sup>6</sup> Etkin, W., in *Physiology of the Amphibia* (edit. by Moore, J. A.), 427 (Academic Press, New York, 1964).

<sup>7</sup> Tata, J. R., in *Actions of Hormones on Molecular Processes* (edit. by Litwack, G., and Kritchevsky, D.), 58 (Wiley, New York, 1964).

<sup>8</sup> Tata, J. R., *Dev. Biol.*, **13**, 77 (1966).

<sup>9</sup> Etkin, W., *Neuroendocrinology*, **1**, 45 (1965).

## BOOK REVIEWS

### OLYMPIANS OF CHEMISTRY

Nobel Lectures in Chemistry 1901-1921

Including Presentation Speeches and Laureates' Biographies. Pp. xii+409. (Amsterdam, London and New York: Elsevier Publishing Company, 1966. Published for the Nobel Foundation.) 160s.

THIS finely produced book commemorates those chemists who gained the Nobel Prize in the first twenty-one years of the present century. Here indeed are riches for the historian of science! Here is pleasant reading for all who would be reminded of the great men whose names frequented the chemistry text-books of old: Nernst, Emil Fischer, Grignard, Haber, the redoubtable van't Hoff and the rest. The lives and scientific work of eighteen men and one woman are recorded. It is interesting to note that no fewer than nine of these were German; four (counting Madame Curie as French) were French; three (counting Rutherford as British) were British; and one each came from the United States, Sweden and Holland. These figures emphasize in a striking way how devoted and imaginative were Germans during the nineteenth century in all branches of chemistry.

The whole book is in English. The chapter devoted to each prizeman is divided into three parts. First there is the translation of the "presentation" speech, given by a Swedish scientist of renown, recounting carefully and concisely the main discoveries in chemistry that the recipient had made. This is in most cases done well and with the minimum of irrelevance. Then comes the prizeman's lecture, in which one of his great pieces of work is recounted at length. Finally there is a short biographical account of the man, giving dates and places, telling what he was like both in the laboratory and at home. Thus for Emil Fischer, who got the prize in 1902, the presentation speech occupies four pages, his lecture on "syntheses in the purine group and sugars" gets fifteen, and the biography four.

The three British prizemen in the book are Ramsay, Rutherford and Soddy. Ramsay's lecture was, of course, on the rare gases of the atmosphere; Rutherford's was on the chemical nature of the alpha particles from radioactive substances, and Soddy's on the origins of the conception of isotopes. It would be presumed on an occasion like a Nobel prize-giving that the recipient would have done his homework well, and certainly with these three, and, indeed, with most of the others too, this is true. Although these lectures are of chemistry in a bygone day, they still make excellent reading. If one was awarding marks one would, I think, give the highest to Soddy, whose fully documented account of the work on the elements he named isotopes, in which he himself took a hand, is a model of scientific exposition. Several of the organic lectures run this one close.

The reader misses one thing: photographs of the prize-winners. The book has a few photographs and diagrams, but it would have been improved if the reader of today might see or see afresh what these Olympians were like. They were great men. They raised chemistry from an almost pre-natal sleep to a healthy and wonderful adolescence. It is meet in a work like this, which tells us so much about them, that we should be reminded of what they looked like. The excellent paper of the book could take photographs directly, thus avoiding the special insertion of photographs required by some books. And, at the price asked for this handsome and carefully edited volume, this request is not churlish. A. S. RUSSELL

## TOP PEOPLE IN PHYSICS

### Nobel Lectures in Physics 1901-1921

Including Presentation Speeches and Laureates' Biographies. Pp. xii + 498. (Amsterdam, London and New York: Elsevier Publishing Company, 1967. Published for the Nobel Foundation.) 160s.

WITH the benefit of hindsight, the wonder is not how well the Nobel Committee chose but that there were so many giants from whom to choose. The innocent reader of this anthology of the Nobel prizewinners in physics between 1901 and 1921 must be forgiven if he asks how the committee could have put a foot wrong. Natural enough to start off with Röntgen (who was unfortunately allowed to collect his medal without delivering the now customary lecture), although even in 1901 it must have been hard to make Lorentz, Zeeman, the Curies and Rayleigh wait until the succeeding three years. To those taught physics in Britain, it is salutary to be reminded that Professor Phillip von Lenard was awarded the Nobel Prize in 1905 for his work on cathode rays, and that the differences of interpretation between him and the school at Cambridge around Thomson were still flourishing when von Lenard came to speak at Stockholm. "We have no evidence that electricity is a special *material* with inertia; it appears to be simply a *state*—a state of the ether . . ." But the following year, in 1906, it was J. J. Thomson's turn to collect a prize and to make a firm declaration of the atomicity of negative electricity. And then, of course, came Lippman (for the development of colour photography), Marconi and Braun, van der Waals, Wien, Gustaf Dalen (who developed automatic regulators for lighthouses and lightships), Kamerlingh Onnes, von Laue, the two Braggs, Barkla (whose influence on the development of atomic theory is much neglected), Planck (rather late in the day, in 1918), Stark, Guillaume (the Swiss who developed the steel alloys of the invar type used widely as stable yardsticks) and finally—also rather late in the day—Einstein in 1921. Plainly the first Nobel committees were more conscious of technology than their successors have been but, even without the claims of the technologists, it is easy to understand why Rutherford had to go among the chemists for his prize.

The style in which the Nobel prizewinners address the Stockholm gathering is revealing. It is a continuing surprise to see how ready the speakers are to deliver a solid professional paper. Perhaps von Lenard explained why when he began saying that "I assume that you would prefer me to tell you what others could not tell you". But some speakers let it slip that they consider the occasion a singular honour, others are pleasantly anecdotal, and the account by van der Waals of how he came to be interested in heat by reading Clausius on the subject is a particularly valuable way of showing how far back go the roots of what is now called physics. The younger Bragg is urbane. Einstein, awarded the prize for "his services to theoretical physics and especially for his discovery of the law of the photo-electric effect", talked unabashed about general relativity and the need for a unified field theory. There are no jokes. But for all their variety, the first two decades of addresses by physics prizewinners must have given the selectors a conviction of their own correctness.

The success with which the Nobel committees made their first choices necessarily prompts the question of whether their task is anything like as easy now that the scale and the pace of scientific work have changed enormously. No doubt a part of the problem is imaginary. History can frequently make people into giants, and giants into demi-gods, and it is necessarily difficult to imagine that each new research student carries the white tie of the Nobel presentation ceremony in his lab coat pocket. But the problem is also partly real. The Nobel system has done well to survive into the sixties without letting scale get the better of it, but the award of the

prizes is bound to seem more and more arbitrary as time goes on. The reputation of an exclusive club depends not merely on the reputation of its members but also on its success in persuading the most eligible people to membership.

JOHN MADDOX

## SERUM PROTEINS

### Serum Proteins in Health and Disease

By Georges Sandor. Translation editor Einhart Kawerau. Pp. xiii + 768. (London: Chapman and Hall, Ltd., 1966.) 252s. net.

THIS is a work on the serum proteins which begins at the beginning. It permits the reader to re-live the extraordinary developments in a field which is now so immense that there are few people left who—like me—can span the history from the past to the present day. As this book demonstrates, much falls into place, and we can understand a subject more deeply if we can follow its evolution. One has to be grateful to the author, who is the Head of the Physical Chemistry Laboratory, Protein Division of the Institut Pasteur in Paris, who has with singular authority assembled this erudite survey.

The book is divided into three parts: (1) The normal serum proteins; (2) their physiology and pathology; and (3) the serum proteins in fluids other than blood. The first part begins with a historical introduction with 342 references, many of them belonging to the nineteenth century. There is a somewhat Victorian flavour attached also to a section on the "euglobulins" which are in fact coprecipitates of  $\alpha$ - and  $\beta$ -globulins in differing proportions. There are chapters on the antibodies (170 references), structure and denaturation of serum proteins (235 references), and a superb chapter on their qualitative and quantitative characteristics, well illustrated and supported by 643 references. The second part of the book is concerned with the origin of antibodies and of the other serum proteins, the role of the reticulo-endothelial system, the major aberrations in  $\gamma$ -globulin synthesis, the lipoproteins, the glycoproteins and the physiological and pathological changes of the serum albumin level. Throughout there is maintained the historical background and the development of the theme up to the present state of knowledge, the support by numerous useful tables, by illustrations, and by copious references. The last part deals with proteinuria, the role of the serum proteins in oedema and effusions, and in the cerebrospinal fluid.

This is an unusual book with a refreshing individuality, and it should be of value to a wide circle of natural scientists and medical men.

H. LEHMANN

## INFRA-RED

### Infra-red Physics

By J. T. Houghton and S. D. Smith. Pp. xiv + 319. (Oxford: Clarendon Press; London: Oxford University Press, 1966.) 60s. net.

IN the several specialized texts on the techniques and theory of infra-red spectroscopy which have appeared in recent years, the emphasis has usually been on the experimental and chemical aspects of the subject, while the physical phenomena associated with infra-red radiation have generally been treated as a part of solid state theory. In this volume, the authors have discarded these somewhat artificial boundaries, and in doing so have succeeded in presenting a unified account of the fundamentals of both the theory and practice of chemical and physical infra-red spectroscopy in the spectral range from 0.8 $\mu$  to 1,000 $\mu$ .

The volume begins with a brief account of the basic principles of the classical and semi-classical treatments of



the interaction of radiation with matter to provide the necessary background for the discussion in the succeeding chapters. This is followed by an account of the infra-red spectra of molecules, for which the reader would find it useful to have read *Introduction to Quantum Mechanics* by Pauling and Wilson. The authors have written an excellent comprehensive description of the optical properties of semi-conductors which is illustrated with well chosen data and diagrams. The theory of the optical properties of metals has been making great progress in recent years, which can be seen, for example, from *Proceedings of the International Colloquium on the Optical Properties of Metals and Alloys*, Paris, 1965, much of which is due to the application of the theory developed for semi-conductors. It is therefore a pity that metals have been specifically omitted from the discussion. Dare we hope that, for the next edition, the authors will be persuaded to extend their account to include metals?

The rest of the book is concerned with experimental techniques and applications; a description of infra-red detectors is followed by a clearly presented discussion of the various dispersive systems available, which is remarkable for the wealth of useful information contained in so few pages. In contrast, the section on interference filters confuses the reader by compressing too much information into too few pages. The difficulties of applying conventional microwave techniques to the far infra-red spectral region (50–1,000 $\mu$ ) are well illustrated, though perhaps too much space is devoted to these methods at the expense of the grating and interferometer techniques that are producing results.

In general the book is well presented, with clear diagrams and a useful quantity of data. There is a good selection of references.

The book provides a link between the elementary undergraduate texts and the more specialized texts on molecular and solid state theory of infra-red spectroscopy. It should therefore be useful to final year students, and have a strong appeal to research workers to whom it is highly recommended.

R. W. G. CLARKE

## SANDWASPS OBSERVED

*Comparative Ethology and Evolution of the Sandwasps*  
By Howard E. Evans. Pp. xvi + 526. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1966.) 120s. net.

THIS book is probably the most detailed comparative behavioural study of a large group of wasps that has ever been published, and it is written, moreover, by a worker who has made a very large number of personal observations. The insects studied are not all the sandwasps (Sphecidae) but one large subfamily, the Nyssoninae, the members of which nearly all nest in sand and constitute a fairly compact group of about 1,000 species with a considerable range in structure and habits. They include the genus *Bembix*, which has long been famous for its elaborate nesting behaviour and which is now made even more interesting because a comparative study of many species (more than a dozen in the western United States) shows how some of the more specialized features such as day to day provisioning of the larvae could have been evolved. As the author remarks (p. 495), it is difficult to reach any certainty about the evolution of instinctive behaviour. It is certain, however, that it is easier and more profitable to speculate about a wide range of species of somewhat different habits and living in different habitats than it was for such early workers as J. H. Fabre, who were not usually familiar with more than one or two species in each genus.

It is clear that even in this long book the author could not have made a detailed survey of structure as well as

of habits, and indeed structure is not neglected. There is a considerable section devoted to the classification of the genera and to their fossil history in Chapter 13. Yet I feel myself that more work of the conventional type on the comparative morphology of the group would have been justified. In *Bembix*, for example, where there is such an interesting range of specific behaviour, very little attempt has been made to group the apparently rather uniform wasps within the genus. The unexpected correlations between structure and behaviour which are often discovered in this way appear to supply the best though far from conclusive test for any phylogenetic speculation. The very few identified fossils at least have the value of proving that insects like modern Gorytine (relatively unspecialized members of the group) occurred in the Eocene and Miocene. It is difficult to overestimate the support that such fragmentary facts lend to a conventional classification.

Apart from the comparative and evolutionary study of behaviour, the work is also a mine of facts. The habitats, nests, oviposition type, cocoons, prey and parasites of a great many species are listed and often illustrated by excellent photographs or by drawings. Although the American fauna forms the backbone of the study, observations from all over the world are brought together and a large literature is summarized. Moreover, to many entomologists the work will also be a revelation of how a study of comparative ethology should be made on a group which is eminently suitable for the attempt. It is not always easy to find nesting sandwasps, and even when found they demand prolonged observation under daunting climatic conditions. Dr. Evans has been studying them for ten years, but it is a matter for admiration how much he has done, how clearly he has described it, and to what interesting though well supported theories he has been led.

O. W. RICHARDS

## MICE IN LABORATORIES

*Biology of the Laboratory Mouse*

Edited by Earl L. Green. Second edition. Pp. 700 + 3 plates. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1967.) \$16.50.

THE mouse is by far the most important laboratory mammal, not least because the existence of many inbred and genetically homogeneous strains provides a unique basis for quantitative studies in virtually every field of biology and medicine. The first edition of the *Biology of the Laboratory Mouse* was published in 1941 under the editorship of G. D. Snell. After the lapse of a quarter of a century, we now have the long awaited second edition. Its editor is Dr. Earl L. Green, the director of the Jackson Laboratory at Bar Harbor, Maine, U.S.A., and the successor to Dr. C. C. Little, who founded that famous institution. The team of thirty-one contributors to this volume includes some of the foremost authorities in their respective fields. The scope of the work is indicated by the chapter headings: the laboratory mouse; breeding systems; keeping records; husbandry; nutrition; nomenclature; nuclear cytology; mutant genes and linkages; multiple factor inheritance; radiation genetics; reproduction; early embryology; anatomy; teratogenesis; genes and development; physiological characteristics; blood and blood formation; blood coagulation; inherited metabolic variations; endocrine variations; pigmentation; acute responses to ionizing radiation; responses to drugs; genetics of tissue transplantation; cell, tissue and organ culture; lifespan and ageing patterns; characteristic tumours; transplanted tumours; constitutional diseases; infectious diseases; immune functions; neural, sensory and motor functions; and patterns of behaviour. There is also a useful bibliography of techniques. In

general, the contributions are authoritative and well written, and each is followed by an extensive list of references. The book is well illustrated and excellently produced, and there is a good index of 43 pages. Quite apart from genetical libraries, for which this book is, of course, an absolute essential, it is difficult to see what biological or medical laboratory could afford to be without a copy. As a work of reference, it is clearly invaluable, and at least one person not unfamiliar with the mouse has made several interesting discoveries in thumbing through this volume.

H. GRÜNEBERG

## CONNECTIVE TISSUE ENDOCRINOLOGY

### Hormones and Connective Tissue

Edited by Gustav Asboe-Hansen. (Scandinavian University Books.) Pp. 431. (Copenhagen: Ejnar Munksgaard, 1966.) 103 D.kr.

DR. ASBOE-HANSEN is at the centre of a team of physicians, surgeons, anatomists and dermatologists who join together in a unique experiment in collaborative research on connective tissue metabolism. In 1954 he edited a group of papers *Connective Tissue in Health and Disease* which introduced the work of the group, but which by the inclusion of reviews by other experts provided a volume holding within its covers all the growing points of a rapidly expanding subject. During the intervening thirteen years, connective tissue studies have advanced quickly, and Dr. Asboe-Hansen's present volume demonstrates not only the quantitative development in Copenhagen but also the change in emphasis there. The broad coverage of the earlier volume has been replaced by the treatment in depth of a single aspect.

The seventeen chapters cover a variety of aspects of connective tissue endocrinology. The first two deal with the effects of a broad spectrum of hormones on tissue mucopolysaccharides and on the formed elements of blood. Another seven chapters deal with the specific effects of individual hormones on selected tissues, whereas a further group is concerned with more clinical aspects of connective tissue metabolism.

During the past decade, considerable evidence has been provided from which the primary and secondary structure of the collagen fibre has been deduced. It might appear surprising, therefore, that a book of this calibre fails to include any correlation between chemical structure and function and a discussion as to show this is determined by environmental and especially by hormonal influences.

Unfortunately metabolic studies on connective tissue components have not kept pace with structural studies, and research in the first of these fields is still very much at the observational level. Many of the contributions to the present book, therefore, consist of experimental observations which appear to have little relationship with one another. Such a compilation does not lend itself to easy reading. It is not a book to be read at bed-time, but the solid meat between its covers should be a useful source of information for those wishing to learn how individual hormones may affect the tissues in which they are interested.

If one has to make any criticism of the subject matter of the book, it has one fault in common with any publication originating in a single institute or group of workers. Because of the circumscribed nature of the work undertaken by the various collaborators, their approach is uniform. A large proportion of the references quoted are Scandinavian and the work of one or two other schools engaged in similar studies is virtually ignored. If, however, the volume is accepted for what it is—a survey of the work of a highly active and energetic research group in relation to selected studies elsewhere—

it should prove to be of great use to other workers in the field.

As is usual with volumes from Munksgaard, the book is well produced. It might, however, have benefited from an author index.

D. A. HALL

### Problems in Mathematical Physics

By N. N. Lebedev, I. P. Skal'skaya and Ya. S. Uflyand. Translated by A. R. M. Robson. Translation edited by J. Reinfelds. (International Series of Monographs in Pure and Applied Mathematics, Vol. 84.) Pp. viii+406. (London and New York: Pergamon Press, Ltd., 1966.) 63s. net.

THIS book consists of a collection of problems on the differential equations arising in the mechanics of continuous media, in the vibrations of mechanical systems, in the theory of heat conduction and in magnetism and electricity. It includes problems on the use of curvilinear co-ordinates, Green's functions, conformal transformations, eigenfunction expansions and integral transforms. There is a short chapter on integral equations. Solutions to about one in seven of the problems are given. There are also hints for the solution of some of the others, and each problem has its answer printed immediately below it. At the beginning of each section there is a brief introduction to the general method involved in the following problems.

The easier parts of the book are of a standard appropriate to a first-year undergraduate course in applied mathematics or physics at a British university, but some of the problems reach a degree of complexity unlikely to be encountered in first degree work. The solutions provided are of adequate clarity, but the translation is very often clumsy and sometimes obscure.

It is difficult to see who will use the book. It cannot be used as a text-book; the introductory sections are too brief, and the number of solutions too small for this. Many of the existing text-books in this field already have adequate selections of problems. No doubt hard-pressed examiners will turn to it from time to time.

The book is well produced, and the price is reasonable.

W. E. PARRY

### Modern Methods of Chemical Analysis

By J. A. Barnard and R. Chayen. Pp. xiii+273. (Maidenhead and New York: McGraw-Hill Publishing Company, Ltd., 1965.) 42s. 6d.

THIS book is primarily for student readership and the subject matter derives from a second year course for chemical engineering undergraduates. There are six chapters—Volumetric Methods, Polarography, Spectroscopic Analysis, Mass Spectrometry, Radiochemical Methods, and Separation Techniques. Included in the text are 56 detailed experiments covering all the chapters with the exception of mass spectrometry. In their preface the authors state that the book will persuade the reader that "...many of the skills needed can be fairly quickly acquired, and the basic theory readily understood". On the experimental side the claim is fully substantiated; the exercises are carefully chosen to illustrate the facets of the techniques described and they are within the scope of a reasonably well equipped college laboratory. This part of the work can be recommended with confidence.

In general the basic principles are less well presented. One would like to have seen rather more emphasis on the scope and limitations of the techniques described to give the reader a true perspective of the factual material. The balance of theoretical material is occasionally uneven; in discussing ion exchange, for example, several pages are devoted to the synthesis of organic resins, but no mention is made of the experimentally important selectivity of the resins for ions of varying charge and size.

Nevertheless the book has much to commend it. The presentation is excellent and the text is carefully pitched at student level throughout. Provided the reader accepts that additional theoretical material from other sources is essential to support its undoubted practical value it will serve as a most useful handbook. The standard of production is good and the book is reasonably priced.

J. K. FOREMAN

### A Dictionary of the Flowering Plants and Ferns

By J. C. Willis. Seventh edition, revised by H. K. Airy Shaw. Pp. xxii+1,214+liii. (London: Cambridge University Press, 1966.) 100s. net; \$18.50.

IN the seventh edition of "Willis" perhaps the original aim of its author is not quite fulfilled. Dr. Willis said, in his original preface, "I have endeavoured to bring together in this book as much information as is required by all but specialists, upon all plants generally met with, and upon all these points—morphology, classification, natural history, economic botany etc.—which do not require the use of a microscope . . .". The great increase in botanical knowledge in the last seventy years has required that some of the information previously included in the dictionary be omitted if the compact, single volume format is to be preserved. This "Willis", therefore, in its 40,000 entries gives only generic and family names; common names have gone, and so have the horticultural notes—possibly a loss to some non-specialists, but such topics are treated in full in other easily accessible works. The information which is given in this largest-ever edition is as full as it could be; the aim has been to include every generic name from 1753, and every published family name from 1789. Some suprafamilial and infrafamilial taxa have been included if they are not based on generic and family names, and alternative generic names and variant spellings have been supplied in many cases where these exist. All this must greatly increase the value of the book to students and others who use it as a reference work of taxonomy.

MARY LINDLEY

### Tables of Physical and Chemical Constants, and Some Mathematical Functions

By T. H. Laby and G. W. C. Kaye. Thirteenth edition. Pp. 249. (London: Longmans, Green and Co., Ltd., 1966.) 35s. net.

PREPARING comprehensive tables of physical and chemical data is a thankless task. The authors must jettison opinions, enthusiasms and prejudices in the pursuit of accuracy; no sooner have they finished one edition than they must start work on the next, like intellectual painters of the Forth Bridge. Kaye and Laby, who fathered the first edition of this book in 1911, had at least the satisfaction of being immortalized by it, but the present editorial board soldier on in comparative anonymity. They are Professor N. Feather, Dr. H. Barrell, Dr. E. A. Coulson and Mr. J. M. C. Scott, and they have produced the thirteenth edition with the help of a large band of contributors.

There has been a seven year gap since the last edition, a long time, as Professor Feather observes, in relation to the growth of knowledge in science. There are five additional tables, covering critical constants and second virial coefficients of gases, bond lengths, energies and angles, force constants, stability constants, and solubility products. The format remains the same; each entry includes a brief résumé containing references, which are invaluable because a book this short could scarcely hope to include everything, and references to further sources of information are certainly needed. The fact that the book has not been allowed to expand unchecked is a considerable editorial triumph, and although the growth of science probably means that it is less comprehensive than it was in 1911, it is no less indispensable. NIGEL HAWKES

## OBITUARIES

### Dr. Alick Isaacs

THE death on January 26, 1967, at the age of 46 of Dr. Alick Isaacs, has robbed this country and the Medical Research Council in particular of a leading virologist with an international reputation. After a brilliant undergraduate career in Glasgow where he was born, Isaacs graduated in 1944 and spent the next three years as McCunn research scholar in the University Department of Bacteriology. He then sought training in virology, and spent first a year in Sheffield in the Department of Medicine and then two years at the Walter and Eliza Hall Institute for Medical Research, Melbourne. Two subjects attracted his interest as early as 1948—virus variation and virus interference, and it was the pursuit of the latter phenomenon which brought him stimulus, achievement and reputation to the end of his life.

First, however, variation of different cultural lines of influenza virus drew Isaacs's interest. In Melbourne he studied it as a genetic phenomenon, utilizing the technique of passage at limiting dilution to isolate pure clones of virus. This experimental work stood him in good stead when he returned to London to direct the World Influenza Centre at the National Institute for Medical Research in 1950. There strains of influenza viruses from all over the world were studied serologically and identified by this and other methods. Isaacs used the antigenic variation of the influenza viruses to pin-point the spread of epidemics and thus to throw light on the transmission of the infection. His subsequent interest in attenuation of influenza strains for possible use in immunization was hampered by his absorption in the subject of virus interference.

This had interested him particularly in Australia, and he continued work on the phenomenon with increasing tempo. Then in 1957, together with Lindermann (a Swiss virologist), he reported the evidence that the phenomenon was a result of the elaboration by the animal cell of a protein with broad antiviral properties named by him "interferon". In the next five years, Isaacs and a series of visiting collaborators at the National Institute studied interferon, its production, mechanism of action and chemical and physical properties. He showed its biological purpose in aiding recovery from virus infection, its potential use as an antiviral prophylactic, and its experimental value as a model for research on antiviral substances. That it never became a practical weapon in the prevention or treatment of virus infections was a disappointment which in no way clouded the scientific importance of his discovery.

Isaacs succeeded Sir Christopher Andrewes in 1961 as head of the Virology Division at the National Institute, but later his health, already precarious, showed the need to conserve his energy. He became head of the Laboratory for Research on Interferon where he had fewer distractions.

In 1962 he received an honorary M.D. at the Catholic University of Louvain, and in 1966 he was elected F.R.S. To many who visited the Institute or worked in collaboration, Isaacs showed a warmth of friendship which was surprising in one so shy in youth. He retained his puckish wit and enjoyment of life to the end, and in this he was aided by his wife, Dr. Susannah Gordon—whom he met in Sheffield. His loss from a second subarachnoid haemorrhage has left a vacant corner in the lives and memories of many who knew Isaacs and admired the simplicity and integrity of all that he undertook.

C. H. STUART-HARRIS

### Dr. T. A. Munro

THOMAS ARTHUR MUNRO died on December 18, 1966, at the age of 61. He had been medical superintendent of the Royal Edinburgh Hospital since 1954. At that time many developments were taking place, and Munro delighted in them, for he was a shrewd and practical man who made constant improvements in the general amenities of the hospital. His post was a demanding one, but he found time for clinical as well as administrative work.

Munro was educated at Edinburgh Academy and the University of Edinburgh, where he graduated M.B., Ch.B. in 1928. He was a resident in the Edinburgh Royal Infirmary and then joined the staff of Sir David Henderson in the Royal Edinburgh Hospital, Morningside. He was awarded a Beit fellowship and went to work with Professor Meyer in Baltimore and with Professor Macfie Campbell in Cambridge, Massachusetts. He returned to England and to a research post with Dr. Turner at the Royal Eastern Counties Institution and here his ability for research was revealed. Working with a large body of clinical material he investigated genetic and biochemical factors in the aetiology of what, for simplicity, can be called mental defect. Finally he was able to clarify the condition of oligophrenic phenylketonuria and its avoidability. This hopeful approach has continued to develop.

When the Second World War began he joined the Emergency Medical Service and later the R.A.M.C., where he became a colonel A.M.S. At the end of the war he was offered the position of chief of the Psychiatric Services in Guy's Hospital. This post was an arduous one; there was much administrative, teaching and clinical work; he had the interesting but difficult task of integrating the work of the Psychiatric Department with that of the hospital generally. A certain amount of private clinical work was inescapable, but he approached it all with enthusiasm until in 1954 he returned to be chief of the hospital in which his career had begun. In 1965 he saw a new department, the Andrew Duncan Clinic, opened.

Munro was a man with a strong sense of humour and he was a good public speaker. He was still, in the sixties, being visited by American friends he had first met thirty years earlier. He was extremely social and it is a happy recollection that one of the last gatherings he organized was to celebrate the eightieth birthday of Sir David Henderson, from whom Munro gained much of the knowledge and experience which he used to the benefit of psychiatry.

W. M. H.

### Professor G. P. Crowden

PROFESSOR G. P. CROWDEN, emeritus professor of applied physiology of the University of London, died at Edgware on November 22, 1966.

He was born in 1894, and had a distinguished academic career at the University of London, graduating B.Sc. with first class honours in physiology in 1921, M.Sc. in 1926, M.R.C.S., L.R.C.P. in 1929, D.Sc. in 1937 and in the same year he was elected M.R.C.P., London. He was appointed lecturer in physiology at University College in 1927 and at the London School of Hygiene in 1929, where he served successively as reader in industrial physiology and professor of applied physiology until his retirement in 1962. He was awarded the O.B.E. in 1944.

Crowden served with the British Expeditionary Force from 1915 to 1918, first as intelligence officer and later for special duties with a gas brigade. He maintained his connexion with the armed forces through the Territorial Army and the O.T.C. medical unit, and served during the Second World War as colonel, R.A.M.C., in London and West Africa. Since then he has been consultant in

applied physiology to the army and a member of the Army Personnel Research Committee.

In 1927 Crowden had spent a year at Harvard School of Public Health and early in his career he saw the need to apply the principles of engineering, physics and chemistry to the solution of problems of preventive medicine. His teaching and research covered an astonishingly wide range of subjects within the field of applied physiology and occupational health. He was a practising ergonomist many years before this word had been coined. Man's physiological environment and the reactions of man as a whole rather than of individual organs were his interests. Although his main research was in the field of muscular work, nutrition, noise and deafness, his interests ranged from comfort in living quarters in ships in tropical heat to insulation against cold in Antarctica. He published work on physiological problems of housing, protective clothing, artificial respiration and pneumatic drills. He did much to popularize the use of aluminium foil for reflecting radiant heat in solar topees and other headgear, fire-fighting suits and insulation of buildings.

He was a lucid and effective teacher, concentrating on the principles of the common ground between the relevant disciplines. Whether he was lecturing to the main classes of medical graduates or nurses or students of tropical architecture he emphasized the need to look for and, as far as possible, rectify any departure from optimum conditions of man at work or in the home. He was a strong advocate of the value of practical work, striving wherever possible to provide individual student participation; for example, every student had an opportunity to measure his own hearing by gramophone audiometer and to assess the components of the thermal environment, summarizing their overall effect on man by the effective temperature scale. There were many demonstrations in the evaluation of lighting, noise, radiant heat, muscular work, ventilation and protective clothing.

I shall long remember the applause from members of the Physiological Society after a communication given by Crowden at a meeting at the National Hospital, Queen Square. The subject was the superiority of nylon as compared with cotton mosquito nets with respect to permeability to air movement and to light. The spontaneous ovation showed that the audience as a whole appreciated the clear exposition of an important practical point, but it also owed much to their esteem and affection for the speaker.

M. L. THOMSON

### A. J. Berry

ARTHUR JOHN BERRY, senior fellow emeritus of Downing College, Cambridge, and formerly university lecturer in chemistry, died on January 15, at the age of 81.

He graduated from the University of Glasgow and then went to Cambridge, where he took his Natural Sciences Tripos. He started to teach chemistry in 1911, and this remained one of his great interests; he became director of studies in chemistry and physics at Downing College soon after his election as fellow in 1913, and continued in this post until his retirement in 1951. For many years Berry had great influence on the teaching of chemistry in Cambridge. His early research interests were in analytical chemistry; he was the author of several books on the subject and on the chemistry of the atmosphere. In his later years he was increasingly concerned with the history of science. Berry wrote a number of useful books on the history of chemistry, including a biography of Cavendish, which was notable for a particularly clear style and the obvious affection of the author for his subject. Although Berry made no original contribution his books will continue to be valuable as introductions for students for a long time.

Berry had served as vice-master of his college and as a senior proctor.

Fig. 1. Amino-acid sequence of ribonuclease<sup>1</sup>.



## X-ray Investigation

This section deals with X-ray data collected since 1964, using the linear X-ray diffractometer<sup>9</sup>. The unit cell dimensions for monoclinic ribonuclease grown from 50 per cent aqueous alcohol are  $a = 30.31 \text{ \AA}$ ,  $b = 38.26 \text{ \AA}$ ,  $c = 52.91 \text{ \AA}$ ,  $\beta = 105^\circ 55'$ , with space group  $P2_1$  and two molecules per unit cell. These crystals correspond to ribonuclease II described by King *et al.*<sup>7</sup>. The X-ray molecular weight measured by Carlisle and Scouloudi<sup>8</sup> is reported as 13,400 and as determined from the amino-acid sequence is 13,683. The phases of the X-ray reflexions from spacings greater than  $5.5 \text{ \AA}$  have been determined by the method of multiple isomorphous replacement which has been used so successfully in the study of myoglobin<sup>9,10</sup>, haemoglobin<sup>11-13</sup>, lysozyme<sup>14</sup> and carboxypeptidase A (ref. 15). Table 1 shows a list of heavy atom derivatives prepared and used in the course of this work.

Table 1

No.	Derivative	Method of preparation
(1)	RNase + sodium <i>p</i> -hydroxy-mercuribenzenesulphonate	Co-crystallized
(2)*	RNase + potassium hexachloroiridate	RNase crystals soaked for 6 months
(3)	RNase + potassium hexachloroiridate	RNase crystals soaked for 6 weeks
(4)	RNase + 2'-cytidylic acid + potassium uranyl pentafluoride	RNase crystallized with nucleotide and then soaked for 6 weeks
(5)	RNase + potassium uranyl pentafluoride	RNase crystals soaked for 6 weeks
(6)*	Homocysteinyl-RNase (2) + sodium <i>p</i> -hydroxymercuribenzoate	Ribonuclease chemically modified and then reacted with sodium <i>p</i> -hydroxymercuribenzoate
(7)	Homocysteinyl-RNase (1) + sodium <i>p</i> -hydroxymercuribenzoate	

\* This derivative was not used in the final phasing of the X-ray reflexions.

One of the difficulties in this work, and which also occurred in an earlier study of ribonuclease<sup>18,22</sup>, has been the appearance of multiple heavy atom sites in metal substituted crystals. With the exception of the chemically modified ribonucleases, numbers 6 and 7 of Table 1, where there is only one heavy atom site for each protein molecule as shown by Patterson difference maps (see Figs. 2a and 2b), all the other substituted crystals yield multiple occupancy whether or not the substitution has been carried out by co-crystallization or diffusion methods. The aim, therefore, was to use those substituted crystals with the least number of effective sites in any substitution and to neglect those sites where occupancy fell below about 10 per cent for the heavy metal concerned. Column 5, Table 2, shows the effective site occupancy for those heavy atom derivatives used in the final phasing of the X-ray reflexions.

The positions of the heavy atoms, in each of the derivatives, were found independently from Patterson difference maps and refined by Rossmann's method<sup>18</sup>. The positions of heavy atoms from different derivatives were then brought to a common origin through Fourier difference maps using approximate phases for the reflexions from the parent protein crystals. In this way, from single isomorphous methods, the phasing of the X-ray reflexions was extended to double isomorphous methods, taking the derivatives in pairs, and then to triple

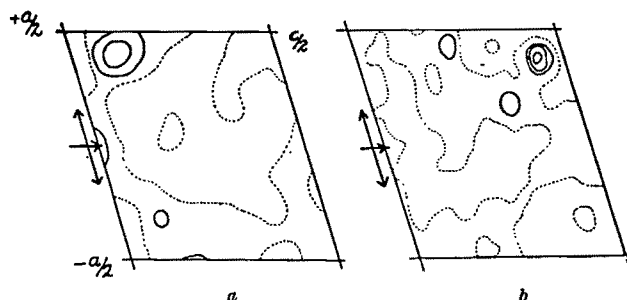


Fig. 2. Section at  $V = 0.5$  of Patterson difference of (a) Hg-homocysteinyl-RNase (1) and (b) Hg-homocysteinyl-RNase (2).

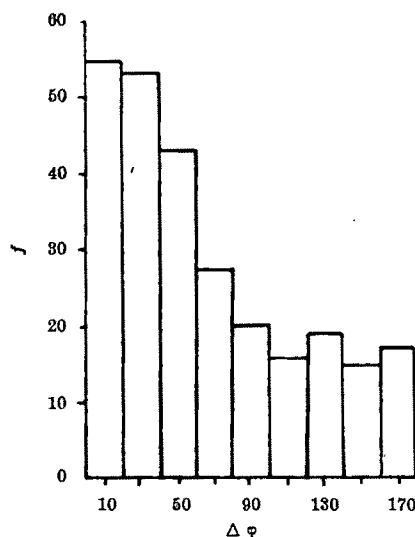


Fig. 3. Histogram showing the agreement between the phases calculated from derivatives 1, 3, 4 and 5, 6, 7.

isomorphous methods. The phase determining procedures used here were those based on the phase probability method described by Cullis *et al.*<sup>12</sup>.

In this way the atomic positions were cross-checked and finally two independent sets of triple isomorphous phases were obtained. The histogram (Fig. 3) shows the number of reflexions plotted against  $20^\circ$  ranges of  $\Delta\phi$ , the phase angle difference between the two sets of data. All the reflexions from spacings greater than  $5.5 \text{ \AA}$  have been used in plotting this curve and one encouraging feature not revealed by it is that nearly all the reflexions with  $\Delta\phi$  greater than  $90^\circ$  either have a low figure of merit in one or other set of data or else have a small protein structure amplitude. The average difference in phase angle as calculated between the two sets of derivatives for reflexions with "figures of merit" greater than 0.7 was  $40^\circ$ . As these independent sets of phases involved more than 60 per cent of the reflexions in the  $5.5 \text{ \AA}$  region, we had some confidence in bringing the two sets together for the final phasing. Here about 80 per cent of the reflexions had figures of merit greater than 0.7.

For the final phasing, however, five derivatives (see Table 2) were used and the refinement of the heavy atom parameters was carried out by a programme written by R. E. Dickerson, R. A. Palmer, D. M. Blow and D. F. Koenig, based on the method described by Dickerson *et al.*<sup>17</sup> in their study of myoglobin. Throughout the work, electron density maps were calculated at intervals  $a/30$ ,  $b/20$ ,  $c/30$  of the unit cell sides and for the final synthesis 389 reflexions were used, using slightly sharpened coefficients with  $\phi$  best phases.

Table 2. HEAVY ATOM PARAMETERS

	$x$	$y$	$z$	$B$	$N$	$E$	$R\%$
(1) Sodium <i>p</i> -chloro-mercuribenzenesulphonate	0.957 0.317 0.922 0.188	0.448 0.472 0.031 0.090	0.950 0.542 0.948 0.089	77.8 61.4 80.1 48.3	39.7 31.5 8.4 21.8	24.1	11.0
(2) Potassium chloroiridate	0.786 0.018 0.929	0.068 0.014 0.359	0.374 0.064 0.009	150.0 153.9 191.8	45.5 25.3 29.8		
(3) 2'-cytidylic acid + potassium uranyl pentafluoride	0.958 0.977 0.254 0.280	-0.008 0.280 0.290 0.442	0.072 0.011 0.521 0.919	137.8 102.3 52.9 57.7	66.5 68.1 18.9 15.5	40.4	17.8
(4) Potassium uranyl pentafluoride	0.971 0.950 0.155	-0.039 0.312 0.228	0.076 0.002 0.415	59.9 50.3 50.4	35.3 37.6 13.2		
(5) Homocysteinyl-RNase (1) + sodium <i>p</i> -hydroxymercuribenzoate	0.769	0.043	0.964	64.4	47.8	32.8	15.1

$B$ , Isotropic temperature factor;  $N$ , occupancy of heavy atom sites in electrons;  $E$ , root mean square "lack of closure" error averaged over all reflexions;  $R$ ,  $\frac{\sum |F_{\text{calc}} - D|}{\sum |F_{\text{calc}}|}$  where  $D$  is the third side of the

phase triangle and  $D$  is calculated for the most probable phase angle.

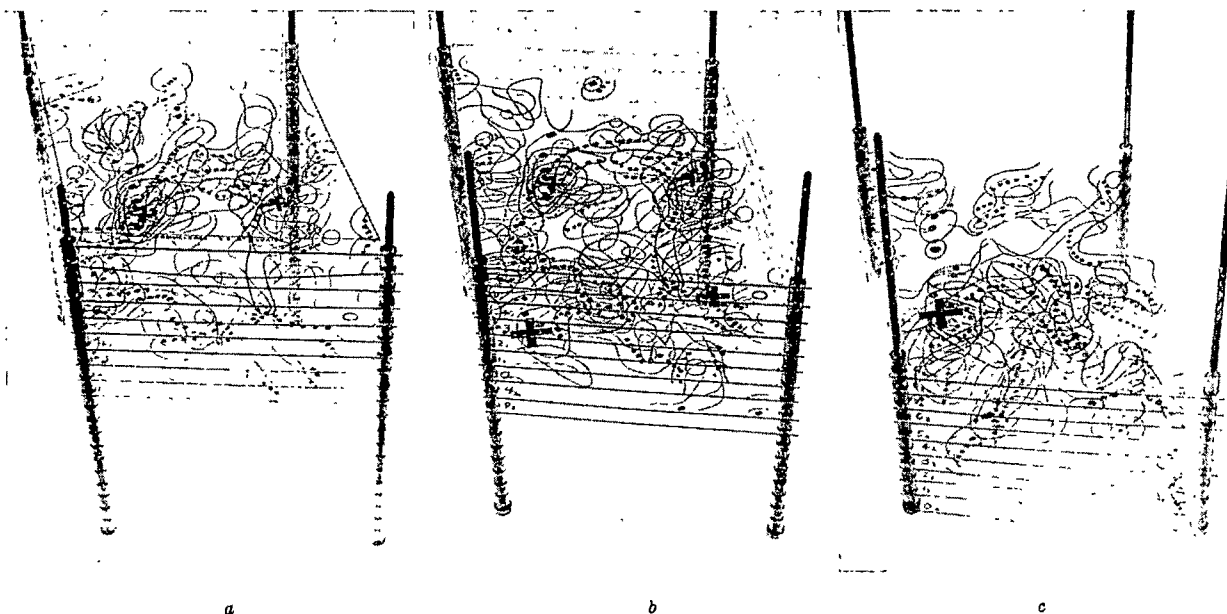


Fig. 4a, b, and c. Photographs of the electron density map of ribonuclease shown in overlapping groups of sections.

As a check that the positions of the heavy atoms of the different derivatives had been correctly determined and that the correct origins had been found, electron density maps were calculated using phases determined by taking the derivatives singly, then combined in pairs and finally in threes. It would be expected that the phases used in each of these calculations were not necessarily as reliable as those where all derivatives were used together. In none of these syntheses was it possible to use all the terms within the 5.5 Å limit and also no two syntheses necessarily contained the same terms. Naturally, such maps must show variations within themselves, but a close scrutiny revealed many consistent features between all of them which gave an indication of what to expect, and to look for, in the final electron density map calculated using phases determined from the five derivatives taken together.

### Electron Density Distribution

Figs. 4a, b and c show sections parallel to (010) of the electron density of the asymmetric unit  $a \times b \times \frac{1}{2}c$  set out on 'Perspex' sheets. For clarity of presentation, the sheets have been photographed in three separate overlapping groups. A noticeable feature is a crude ring of density distributed about a lighter region, and photograph Fig. 1b has been taken in such a way as to emphasize this aspect. It will be shown later that the four cysteine residues of the molecule are to be found in this ring. The axis of this ring of density lies approximately perpendicular to (304), which set of planes give rise to one of the strongest reflexions from the crystal. This particular feature of the electron density distribution was also found in an earlier study of the crystals, using photographic data<sup>18</sup>. Fig. 5 shows the packing of the molecules on (010).

In this respect it is interesting to look at the distribution of the heavy atom sites in the unit cell. Figs. 6a and b show these sites plotted on (010) and (100), respectively. There is a tendency for many of the heavy atoms, especially those with reasonably well occupied sites, to cluster in a region parallel to the  $b$  axis at  $x=0, z=0$ , where the two molecules are well separated from one another. Another noticeable feature is that this cluster of atoms lies near to the active site of the molecule, but it is

clearly not possible to comment on the significance of this feature at this stage of the work.

Fig. 7a shows a photograph of a wooden model of the density distribution. The dashed line indicates a cleft in the molecule in which lie inhibitors such as 2'-cytidylic acid, and the homocysteinyl residue which is probably attached to lysine 41 (see Figs. 7b and c).

### Active Region of the Molecule

Chemically it is known that histidines 12 and 119 and lysine 41 are connected with the activity of the enzyme. The locations of 2'-cytidylic acid and a homocysteinyl residue which are both known to inhibit enzyme activity can therefore be used to assist in delineating the molecule.

Barnard and Ramel<sup>19</sup> showed that 2'-cytidylic acid binds strongly to the enzyme, acting as an inhibitor. Fig. 7b (compare Fig. 7a) shows that the cytidylic acid, located by difference Fourier synthesis, is found in the cleft in the density distribution. Thus in the neighbourhood of this nucleotide there ought to be those portions of the polypeptide chains carrying the two histidines, 12 and 119, and lysine 41.

Shall<sup>20</sup> was able to prepare two homocysteinyl ribonucleases, one of which (homocysteinyl RNase (2)) was inactive. This would indicate that the homocysteinyl group had been attached to lysine 41, and there is other chemical evidence for this<sup>20</sup>. (Hirs *et al.*<sup>21</sup> have shown conclusively that reaction of lysine 41 with 1-fluoro-2,4-dinitrobenzene (FDNB) results in inactivation of the enzyme.) A difference Fourier map showed a column of density indicating the mercury atom and possibly a part of the homocysteinyl group attached to it. This column of density is shown in Fig. 7c. Two points must be noticed: (1) the region of positive density lies in the cleft of the density distribution model; (2) this column of density itself lies in the neighbourhood of a heavy region of the density distribution (see Fig. 7c), which is clearly a connecting region between two polypeptide chains and is most likely to be the region containing cysteine II-VII (see later).

### The Other Cysteine Residues

The location of another cysteine follows reasonably well, given that II-VII has been found. There are in the

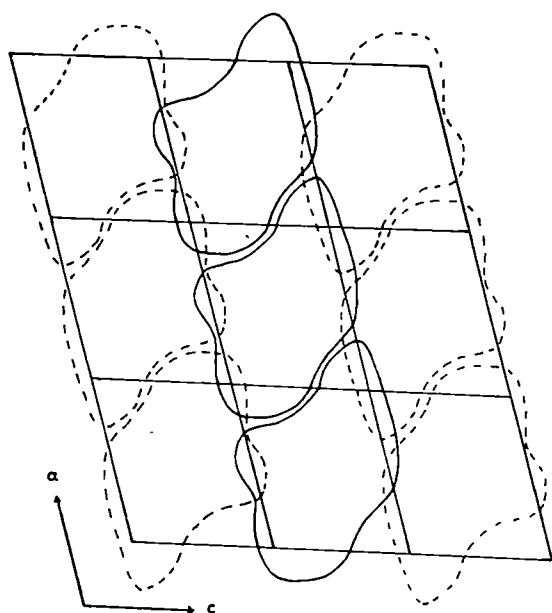


Fig. 5. Packing of the molecules projected along 010.

distribution two sinuous regions of density that start from II-VII and which follow independent paths to a heavy region at the farther end of the cleft (see Fig. 4a and Fig. 4b). From the distribution of sinuous regions of density emerging from it, this second peak provides strong evidence for placing a cystine in this region. If this were a cystine region then it is likely that it would be either III-VIII or I-VI, and the latter, from considerations of chain length, can be eliminated in the following way. Close to what is most likely to be the phosphorous end of the 2'-cytidylic acid peak is a peak which encloses the cleft and could be one end of the chain. This is connected to the cystine region in question by a short region of density. As the amino end of the chain has twenty-five residues before a cystine occurs, it is likely that this peak must be the carboxyl end, which has only fourteen residues (see Fig. 4c). Given that this peak represents a turning back of the carboxyl end of the chain on itself, then the cystine in question can only be III-VIII.

The electron density distribution does not indicate unambiguously the location of the remaining cystines, IV-V and I-VI, but by using the known sequence in conjunction with the electron density map, it is possible to draw some conclusions about their locations. Cystine III-VIII (see Fig. 1) is connected to IV-V by six amino-acids and this, in turn, is connected to I-VI by a single length of chain containing eleven amino-acids. About 15 Å from III-VIII, in a direction along *a*, is a peak which has the appearance of a cystine, which, because it is so close to III-VIII, can only be IV-V (see Fig. 3b). This region lies on the other side of the model and is not visible from the photograph. There remains only one suitable region in the ring of density where I-VI can be placed because there is only one pathway of high density in the ring connecting those two likely positions for the remaining cystine residues. This region is visible in Figs. 4b and c. Given that the four cystines have been located it is possible to envisage a schematic picture of the polypeptide chain and this is discussed in further detail in the following sections.

#### Previous X-ray Studies

Evidence that the cystine positions suggested here are probably correct comes from an earlier completely independent analysis of ribonuclease carried out from a single isomorphous study of this enzyme<sup>18,22</sup>. Here only

the main argument will be reiterated—namely, the location of the cystine residues in the molecule.

In that work three three-dimensional Fourier distributions were calculated, phased from two mercury atoms per protein molecule. These maps were at levels of 6 Å, 3.5 Å and 2.5 Å resolution, respectively, using in each case about 65 per cent of the possible reflexions. It was argued that if the phasing of the X-ray reflexions was approximately correct then there should be evidence from a comparison of the three partial electron density distributions for the presence of the four cystine residues as found by Hirs, Moore and Stein.

Because the X-ray evidence<sup>23</sup> shows that there is little  $\alpha$ -helical conformation in this molecule, and owing to the poor resolution of the higher order maps as a result of partial phasing of the X-ray reflexions, it was argued that pairs of sulphur atoms should be more easily recognized as single heavy peaks than the single sulphur atoms of methionine residues. The 6 Å distribution (which closely resembles the 5.5 Å distribution already discussed) showed five heavy regions, four of which increased in weight in going from the 3.5 to the 2.5 Å maps. Furthermore, a plot was made of the integrated electron density of each heavy region against the local peak height. Integration was performed over a sphere of radius 3 Å, because this would be large enough to contain two sulphur atoms and two  $\beta$ -carbon atoms of a cystine residue. In this way four outstanding regions having both large peak height and

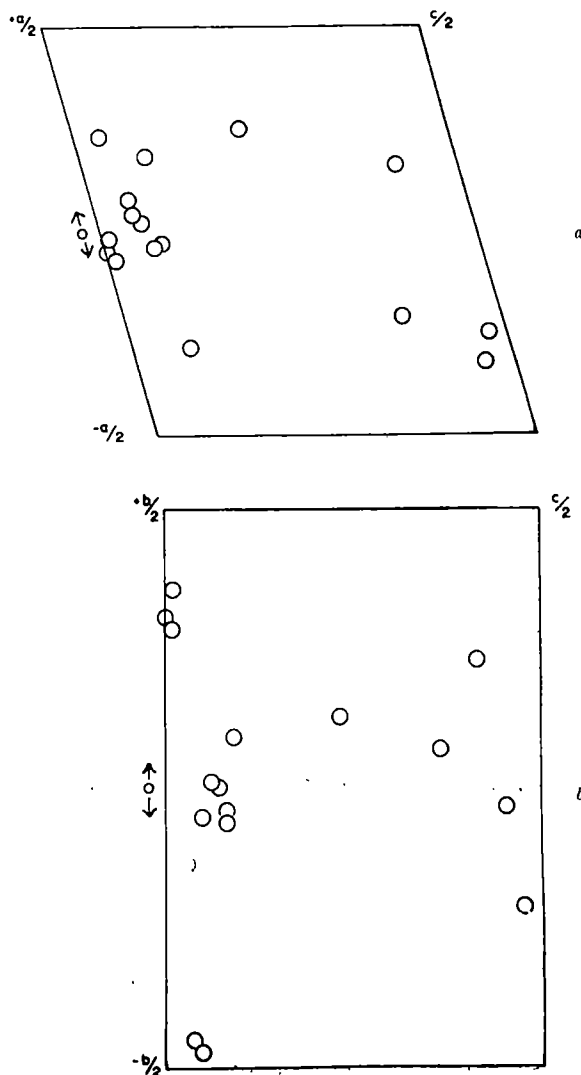


Fig. 6. Projection of the heavy atom positions along (a) 010 and (b) 100.

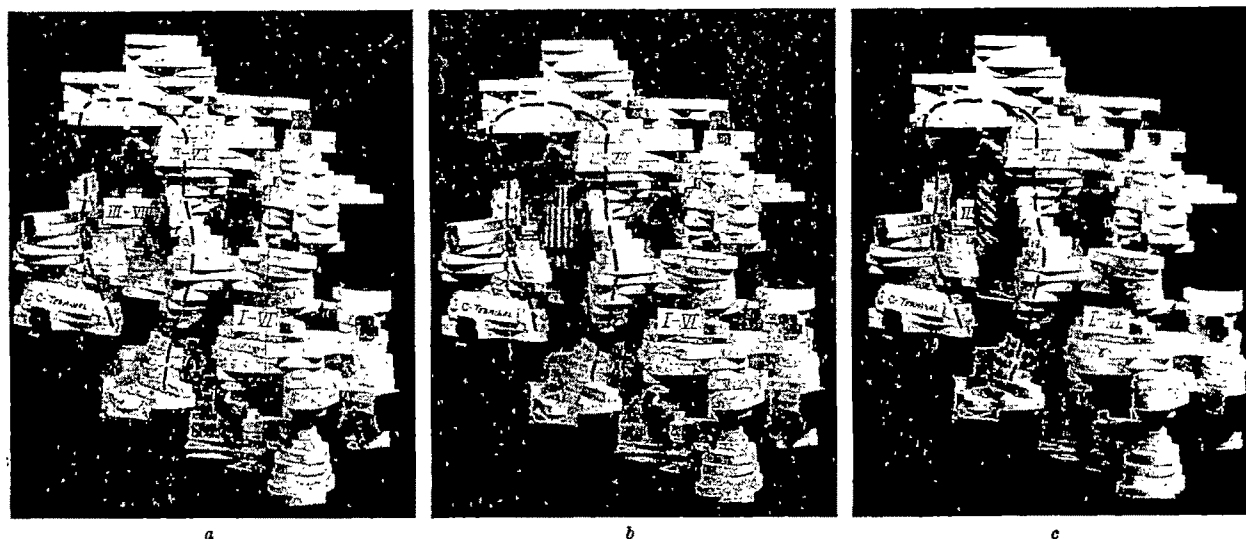


Fig. 7. Photographs of a model of the electron density showing (a) the "cleft"; (b) the cytidylic acid located in the "cleft"; (c) the Hg-homocysteinyl group located in the "cleft".

integrated density were located corresponding to the same four regions which had increased in weight in going from 3.5 Å to 2.5 Å resolution. These four cystine positions lie on or near the positions already indicated from the present investigations. Their locations are indicated by crosses in Figs. 4a, b, c. It is noticeable that they lie in the crude ring of density shown in Fig. 4b.

The approximate co-ordinates of the cystines are as follows:

III-VIII	$x = 0.00, y = 0.75, z = 0.14$
II-VII	$x = 0.22, y = 0.85, z = 0.45$
IV-V	$x = 0.94, y = 0.45, z = 0.46$
I-VI	$x = 0.69, y = 0.45, z = 0.09$

#### Possible Arrangements of the Polypeptide Chain

Because the four cystine cross-links, at that time, had been located and identified with some confidence, an attempt was made to find the arrangement of the polypeptide chain with respect to them. In this study<sup>18</sup>, a possible conformation was found by a comparison of the 6 Å and 2.5 Å electron density maps, and by model building observing stereochemical principles. With the exception of the amino end of the chain, which was thought to be unsatisfactorily placed at the time the model was built (1961), the path of the chain is schematically shown by the string of dots superimposed on the latest 5.5 Å electron density map, Figs. 4a, b and c.

There is an almost one-to-one correspondence between the model of the molecule based on this previous work and the latest electron density distribution. There is a clear indication now from the recent work that the part of the amino end of the chain in the neighbourhood of histidine 12 approaches histidine 119 in the carboxyl end of the chain, at one end of the cleft, and that lysine 41 lies towards the other end of this cleft. If the cystines have been correctly located, then this 5.5 Å Fourier distribution is in agreement with the previous X-ray investigations and reveals the conformation of the polypeptide chain. The photograph of the wooden model is, in fact, a crude representation of the molecule as we now see it, and Fig. 8 is a wire model showing the approximate arrangement of the backbone of the polypeptide chain.

There is some evidence, from an examination of the anomalous X-ray scattering from the uranium atoms, that the wrong enantiomorph may have been chosen.

We thank Dr. E. A. Barnard, of King's College, London, and Dr. H. Witzel, of the University of Marburg, for the

gifts of 2'-cytidylic acid. Co-crystallizations of ribonuclease with this nucleotide were carried out at both Birkbeck and Sussex.

We collectively acknowledge the help we are receiving from the Science Research Council, the Medical Research Council\* and Birkbeck College which is making this work possible, and we thank Professor J. D. Bernal for his advice and encouragement in all aspects of this work.

*Note added in proof by Dr. C. H. Carlisle, January 30, 1967.*

At the recent conference on "Protein Structure" held in Madras, January 18-21, 1967, Dr. Harker, Dr. Kartha and their co-workers of the Roswell Park Memorial Institute, Buffalo, N.Y., have put forward a model for ribo-

\* Dr. Shall is the recipient of a grant from the Medical Research Council.

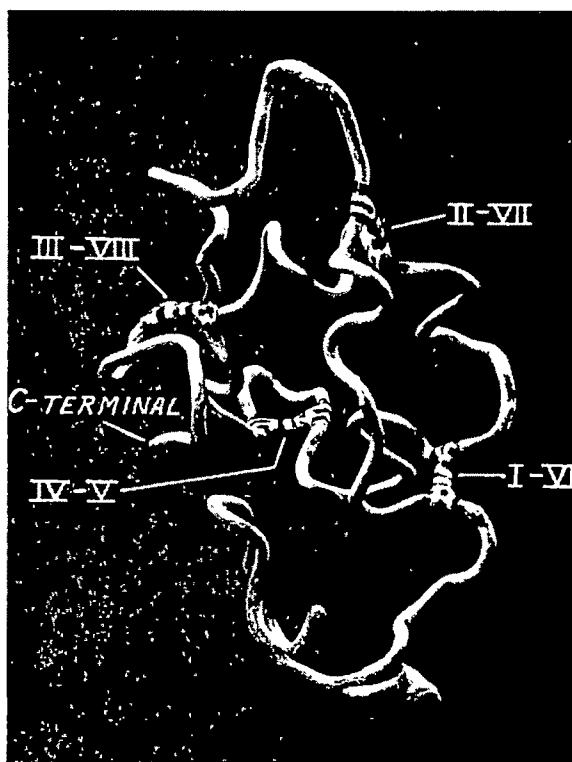


Fig. 8. A wire model showing the path of the polypeptide chain.

nuclease based on an electron density map calculated at 2 Å resolution. This model and that proposed above show significant differences in two respects. First there are differences in the electron density maps themselves, and second there are differences in interpretation of the parts of the maps which are the same. There are two regions common to both which have been interpreted as cystines, but these have been identified differently. The one that we have labelled II-VII, and which was identified by the location of the mercurated homocysteinyl group chemically attached to the neighbouring lysine 41, has been labelled I-VI by Dr. Harker. The remaining two cystines are quite widely different.

The second important difference concerns the active site. Dr. Harker has identified this region by the location of a phosphate and arsenate binding site in the crystal, whereas in our work we used the strongly binding inhibitor cytidine 2'-phosphate. We have additional evidence in that the mercurated homocysteinyl group attached to lysine 41 lies in almost the same position as the nucleotide inhibitor, and this chemical modification of the enzyme is known also to be inactive.

When details are published by Dr. Harker and his co-workers, it will be interesting to assess the differences between their findings and the structure we have described in this communication.

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### Specific Derivatives of Ribonuclease for Crystallographic Determination of the Protein Structure

THE X-ray diffraction studies on bovine pancreatic ribonuclease described in the preceding article required the preparation of several different isomorphous crystals, each of which contained one or a few stable, heavy atoms at defined locations in the crystal. Knowledge of the site of attachment of the heavy atom makes possible the identification of regions of the electron density maps. This article describes the methods which were used to synthesize the specific derivatives.

### Mercurial Mercaptides of Ribonuclease

In the first step (thiolation) of the synthesis of the derivative, a side-chain carrying a reactive thiol group was introduced into the ribonuclease molecule, as a site for heavy atom complexing. The reaction of *N*-acetyl-homocysteine thiolactone (AHTL) with amino groups<sup>1,2</sup> was applied for this purpose. This reaction is normally non-selective<sup>3</sup>, giving mixtures of a large variety of products; here, it was rendered specific by using very low reagent concentrations and by use of silver ion<sup>2</sup> as catalyst.

**Thiolation reaction of ribonuclease.** Ribonuclease A nitrate was prepared from commercial ribonuclease by chromatography<sup>4</sup>, dialysis, deionization and neutralization. It was reacted with DL-AHTL (recrystallized, melting point 111°C) and silver nitrate, on a radiometer pH-stat, at room temperature; the concentrations used and the enzyme inactivations obtained are illustrated in Table 1 for some experiments at pH 7.5. Enzyme inactivation could be obtained with the use of only slightly more than 1 equiv. AHTL/mole of protein (Table 1). More than 1 equiv. silver/mole of AHTL was needed, however, for full inactivation.

Table 1. REACTION OF RIBONUCLEASE A NITRATE WITH AHTL AND SILVER NITRATE

Experiment	Ribonuclease A (mmolar)	AHTL (mmolar)	Ratio*	Silver nitrate	Final activity† (per cent)
(1)	0.31	1.26	4.1	1.24	3
(2)	0.02	3.30	3.3	15.0	<0.1
(3)	0.36	0.95	2.6	4.0	<0.1
(4)	0.38	0.83	2.2	3.28	<0.1
(5)	0.34	0.66	1.9	1.04	4
(6)	0.20	0.27	1.4	2.23	9
(7)	0.64	0.69	1.1	3.27	5

The reaction was conducted at pH 7.5, 20°–23° C, 30 min.

\* Molar ratio of AHTL to ribonuclease.

† Enzyme activity as a percentage of the original activity.

Two protein products were formed, each thiolated at a different site. This was shown by chromatographic separation after alkylation of the introduced homocysteinyl-SH groups with <sup>14</sup>C-iodoacetate. The main products were always two well separated, mono-substituted, homogeneous protein peaks, one inactive and the other with 100 per cent of ribonuclease activity. This latter derivative (termed homocysteinyl-RNase-1) is enzymatically active in the thiol form and in the *S*-carboxymethyl form, but is reversibly inactivated by silver ions.

Both the two main products (Fig. 1) showed, on amino-acid analysis (using the Beckman Spinco analyser and standard methods<sup>5</sup>), 1 mole of *S*-carboxymethylhomocysteine/mole of protein, but no other significant change in any of the amino-acids of ribonuclease A. It was shown that neither of these derivatives involved a change at the disulphide bonds. Thus, *S*-carboxymethylcysteine was absent in the amino-acid analyses. No inactivation occurred when ribonuclease was incubated alone with the separate components of the reaction mixture, or their products. The dependence of the thiolation reaction on the pH value was a further indication that disulphide interchange was not responsible for the enzyme inactivation.

Amino groups are the sole site of reaction between AHTL and proteins<sup>1,2</sup>. That this was also so in the specific reaction with ribonuclease was shown by repeating the reaction with ribonuclease in which lysine ε-amino groups were altered by guanidination. Two specific derivatives, prepared by Glick and Barnard<sup>6</sup>, in which the number of lysine residues blocked by the guanidino group had been shown to be 9 (an enzymatically active derivative) and all the possible 10 (an inactive derivative), respectively, were used. Each was reacted with AHTL as for ribonuclease A, and then carboxymethylated with <sup>14</sup>C-iodoacetate. The active derivative became mono-substituted (as shown by the incorporation of carbon-14) and inactivated. The inactive derivative, on the other



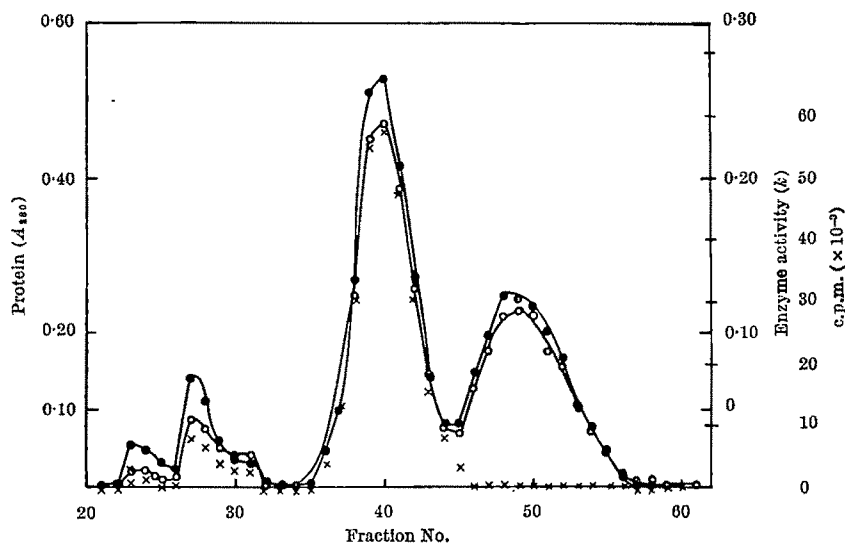


Fig. 1. Chromatography of *S*-carboxymethyl-(*N*-acetyl)-homocysteiny-ribonuclease *A* derivatives. Ribonuclease *A* nitrate (0.34 mmolar) was reacted with AHTL (1.11 mmolar) and silver nitrate (1.71 mmolar) at 22° C and pH 7.5, in a total volume of 10.0 ml. Enzyme activity dropped to 5 per cent of normal. The silver was removed with potassium cyanide and ion exchange and the free thiol groups were alkylated with 2-<sup>14</sup>C-iodoacetate. The overall yield of protein was 95 per cent. The products were separated chromatographically on an 'Amberlite IRC-50' column (1 × 40 cm), using 0.17 molar sodium phosphate at pH 5.95 as eluant. The effluent was collected in 1.45 ml. fractions; on each fraction, protein concentration (O) was determined (by absorbance at 280 mμ), radioactivity (●) in 0.100 ml. aliquots, and enzyme activity<sup>11</sup> (x) on cytidine 2',3'-phosphate.

hand, showed no incorporation. Thus, the one free lysine group in the active guanidinated derivative contains the ε-amino group the acylation of which in ribonuclease leads to enzyme inactivation. The evidence<sup>6</sup> on these guanidinated derivatives indicates (by the occurrence and rate of the specific dinitrophenylation<sup>7</sup> reaction) that this last remaining ε-amino group is at lysine 41.

An active centre character for the silver-catalysed reaction that produces homocysteiny-*RNase-2* is indicated by the great efficiency of that inactivation at about equimolar reagent ratio, and also by a protection exerted by sulphate ion on the reaction site. The sulphate ion is an inhibitor of the enzyme and active centre alkylation reactions of ribonuclease<sup>8,9</sup>. 50 per cent protection of the inactivation at pH 6.0 was obtained with 13 mmolar sulphate, consistent with its affinity for the active centre near that pH (ref. 4). Protection by more tenacious inhibitors could not be tested because they form complexes with silver ions.

### Crystals containing Heavy Atoms

The synthesis of heavy atom-containing crystals was effected by treating ribonuclease *A* with AHTL and silver nitrate as already described at pH 7.5, at room temperature for 30 min. This gave rise to two isomeric *S*-Ag-(*N*-acetyl)-homocysteiny-ribonuclease *A* derivatives. The silver was removed by complexing with 0.1 molar potassium cyanide, followed by dialysis or ion exchange with chloride, and *p*-hydroxymercuribenzoate was then added to form the *S*-mercuric mercaptide of each isomer. The two isomers were separated from one another by chromatography at pH 5.95 in 0.17 molar phosphate buffer. Again, two main components were resolved, one of which was enzymatically 100 per cent active and the other inactive. Each derivative was dialysed and freeze-dried. The derivatives were crystallized at pH 5.6 in 50 per cent (v/v) ethanol. These crystals were used in the X-ray analysis described in the preceding article.

The value of the derivatives described in this article can be assessed from the X-ray crystallographic work described in the preceding article. They have provided isomorphous heavy atom-containing crystals suitable for phase determination. In this respect they have been

used in conjunction with conventional heavy atom-containing crystals; the two methods are complementary. While only one of the two homocysteiny-*RNase* derivatives has been used for phasing, in fact both derivatives would be suitable for this purpose.

Of special interest is the fact that these derivatives contain substituents at defined, known positions. Such information has been of value in the interpretation of the electron density maps. In particular, the *p*-mercuribenzoate mercaptide of the homocysteiny-*RNase-2* has been shown by the evidence presented in this article to have the homocysteine on lysine 41. This identification locates an element associated with the active site (lys-41) and also permits the possible identification of cys-40, and the associated disulphide bond (II-VII). The X-ray observations (preceding article) show that the 2'-cytidylic acid is located in the neighbourhood of the heavy atom in homocysteiny-*RNase-2*. This indicates the proximity of lysine 41 and the 2'-cytidylic acid. Chemical evidence implicates histidine 12, histidine 119, and lysine 41 in the binding of 2'-cytidylic acid<sup>10,11</sup>.

This method could be developed further. Instead of two singly substituted derivatives, it would clearly be worthwhile to synthesize the doubly substituted compound.

The specificity of the reaction which leads to the enzymatically active derivative (homocysteiny-*RNase-1*) probably rests on the reaction conditions which have been devised. The AHTL/Ag<sup>+</sup> reaction, therefore, is probably fairly generally applicable for the preparation of derivatives suitable for X-ray crystallographic investigations.

The specificity of the AHTL thiolation which leads to the inactive derivative, on the other hand, appears to involve an active centre interaction of one silver ion which leads to a specific local aminolysis. Such enzymes as are inhibited by silver ions in cases where this is not by reason of mercaptide formation should be examined by the AHTL/Ag<sup>+</sup> reaction, because this may yield further information about the interaction of lysine and imidazole groups at the active centres of enzymes.

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# Polarography of Proteins containing Cysteine

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Polarographic characterization of tobacco mosaic virus and cytochrome *c* reveals the typical "double wave" phenomenon of proteins. By varying the temperature, pH and composition of the supporting electrolyte the two "catalytic maxima" can be resolved into three. It would seem probable that the three catalytic maxima are caused by the cysteinyl residue present in both TMV and cytochrome *c*.

BRDIČKA was the first to report that proteins containing cysteine and cystine in solutions of hexamminecobalt (III) chloride or cobalt (II) chloride, ammonium chloride and ammonia give a characteristic polarographic effect<sup>1</sup>. The phenomenon has been called the "protein double wave" (for review, see ref. 2). Under "standard conditions", that is, at 0° C and in a supporting electrolyte with a composition of 0.001 molar hexamminecobalt (III) chloride, 0.1 molar ammonium chloride, and 0.1 molar ammonia, tobacco mosaic virus (TMV) and its "native" A-protein give only the first part of this protein "double wave"<sup>3,4</sup>. The effect is manifested by a sharp catalytic maximum the peak of which is situated at about -1.5 V compared with the bottom mercury electrode (henceforth called maximum *B*). After heat denaturation or denaturation by treatment with phenol the TMV protein, under "standard conditions", prevalently shows the second part of the "double wave"—the first part is very low and overlapped by the second one<sup>4</sup>. The latter is characteristic of a sharp catalytic maximum, but its peak is situated at about -1.6 V (henceforth called maximum *C*) instead of at -1.5.

Shortly after we had started to use these "standard conditions" for the polarographic investigations of the TMV protein, we observed a slight inflexion on the positive branch of the maximum *B* (especially at high virus concentrations) at about -1.4 V; it seemed probable that this was caused by a new catalytic wave<sup>5</sup>. In further experiments, which are briefly described here, we identified this slight inflexion as a new catalytic maximum (henceforth called maximum *A*). Under "standard conditions" maximum *A* is almost entirely overlapped by the maximum *B*, but can be visualized by raising the temperature of the polarographed solution. We also found the three catalytic maxima *A*, *B* and *C* with cytochrome *c*. With this compound it was possible to demonstrate the appearance of maximum *A* and its separation from maximum *B* much more easily than with TMV. These findings show that, at least with the two proteins mentioned, the traditional "double wave" is in fact formed of three "waves", or more exactly of three catalytic maxima.

The experimental procedure used during the polarographic determination was essentially the same as that previously described<sup>4</sup>. The polarographic curves were registered from -0.8 V (compared with the bottom mercury electrode) with intervals of 0.2 V on the abscissa, a galvanometer sensitivity of  $2.5 \times 10^{-8}$  amp/mm/m, a mercury flow rate of 4.75 mg/sec. a dropping time of 2.1 sec, using a model LP 55 polarograph. The supporting electrolyte consisted of 0.001 molar hexamminecobalt (III) chloride, 0.1 molar ammonium chloride and 0.01–1.0 molar ammonia. The pH with 0.1 molar ammonia was 9.4. The concentrations of TMV and cytochrome *c* in the polarographed solution are given in the legends to

Figs. 1–5. The solutions under investigation, the constituents of the supporting electrolyte and the capillary were all thermostated before and during the polarographic analysis at the temperatures indicated. The recording of the polarographic curve was commenced 4 min after the virus or cytochrome *c* had been mixed with the supporting electrolyte. For each polarographic analysis a new TMV or cytochrome *c* solution was used. A TMV preparation which had been purified by precipitation with ammonium sulphate combined with repeated differential centrifugation, and a horse heart cytochrome *c* preparation, were used throughout the experiments.

Curve 1 of Fig. 1 represents the polarographic effect of TMV under the "standard conditions". The catalytic maximum *B* appears on the cobalt limiting current at about -1.5 V. The catalytic maximum *C* in this example is very low and therefore difficult to discern. Nevertheless, it rises with temperature in the range 0°–25° C, but then decreases together with maximum *B* (curves 2–4, Fig. 1). With rising temperature over the range 0°–25° C, however, the new catalytic maximum *A* becomes observable at about -1.4 V. At 25° C this maximum is greater than either *B* or *C*, and at 37.5° C it predominates in the polarographic effect of TMV. The simultaneous presence of all three catalytic maxima *A*, *B* and *C* at 25° C (pH 9.4) on one and the same polarographic curve of TMV is demonstrated for various viral concentrations in Fig. 2.

The polarographic effect of cytochrome *c* at 0° C closely resembles that of TMV (compare Figs. 3 and 4). Nevertheless, compared with that of TMV, the catalytic maximum *B* of cytochrome *c* is shifted slightly towards more negative potentials. Consequently, with the former substance the appearance of the catalytic maximum *A* during the temperature rise can be followed more easily than with TMV (Fig. 5). With cytochrome *c* the maximum *C* remains low during the temperature increase and is overlapped by the maximum *B*. But here also the

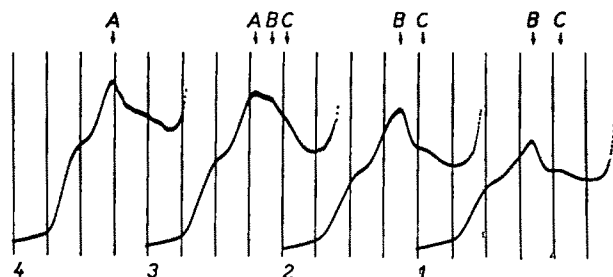


Fig. 1. Variation of the polarographic effect of tobacco mosaic virus (1.8 mg/ml.) with temperature. Curves 1, 2, 3 and 4 were registered at temperatures of 0, 12.5, 25 and 37.5° C, respectively, starting from -0.8 V with intervals of 0.2 V along the abscissa and the galvanometer sensitivity reduced to 1/150. Supporting electrolyte: 0.1 molar ammonia, 0.1 molar ammonium chloride, 0.001 molar hexamminecobalt (III) chloride. The three catalytic maxima are marked *A*, *B* and *C*.

maximum *C* becomes apparent if the concentration of ammonia in the supporting electrolyte is raised to 1.0 moles/l. at 0° C. Under these conditions the maximum *D* is shifted towards more positive potentials, and this is accompanied by a considerable rise of the maximum *C* (Fig. 4). The latter effects are also encountered with TMV (Fig. 3).

A paradoxical phenomenon is apparent when curve 1 in Fig. 4 is compared with curve 4 in Fig. 5: both curves result from cytochrome *c*, and show what seem to be "double waves". The former curve, however, is composed of maxima *B* and *C* and the latter of maxima *A* and *B*. This becomes evident from curve 3 in Fig. 4 and curve 1 in Fig. 5 (both are recorded under identical "standard conditions" and show practically only one catalytic maximum, namely, *B*) and the progressive appearance of maximum *C* in Fig. 4 (curves 3, 2 and 1) and of maximum *A* in Fig. 5 (curves 1–5).

Cytochrome *c* was first studied polarographically by Carruthers<sup>6</sup>, who showed that when it is polarographed in 1.0 molar ammonia, 1.0 molar ammonium chloride and 0.001 molar hexamminecobalt (III) chloride at room temperature it gives a single catalytic maximum the peak of which is situated at about -1.5 V compared with a saturated calomel electrode. Analysing cytochrome *c* under identical conditions we found that maxima *A* and *B* closely approached each other, thus forming a single high peak which almost completely overlapped the much lower maximum *C*.

Maxima *A*, *B* and *C* bear no relationship to the so-called "prenatrium wave" of proteins (see ref. 2) or to the waves III and IV of the octapeptides oxytocine lysine-vasopressin and arginine-vasopressin which appear in solutions of hexamminecobalt (III) chloride, ammonium chloride and ammonia<sup>7</sup>. In contrast to the catalytic maxima *A*, *B* and *C*, these waves show a rapid decrease with increasing pH and ammonia concentration, are

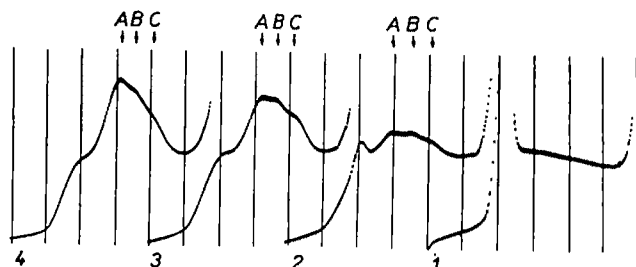


Fig. 2. Variation of the polarographic effect of tobacco mosaic virus at 25° C with the viral concentration. Curves 1, 2, 3 and 4 were registered at TMV concentrations of 0, 0.18, 0.54 and 1.8 mg/ml., respectively, in the supporting electrolyte with the same composition as in Fig. 1, starting from -0.8 V, with intervals of 0.2 V along the abscissa and the galvanometer sensitivity reduced to 1/150. Marks *A*, *B* and *C* are the same as in Fig. 1. The non-catalytic cobalt maximum occurring at about -1.2 V on curve 1 is partly suppressed by TMV on curve 2 and completely on curves 3 and 4. Note the simultaneous presence of all three catalytic maxima *A*, *B* and *C* on curves 2–4.

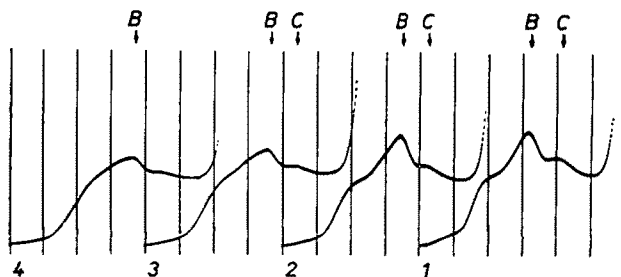


Fig. 3. Variation of the polarographic effect of tobacco mosaic virus (1.8 mg/ml.) at 0° C with the concentration of ammonia in the supporting electrolyte which also contained 0.1 molar ammonium chloride and 0.001 molar hexamminecobalt (III) chloride. Curves 1, 2, 3 and 4 were registered at ammonia concentrations of 0.3, 0.1, 0.03 and 0.01 moles/l., respectively, starting from -0.8 V with intervals of 0.2 V along the abscissa and the galvanometer sensitivity reduced to 1/150.

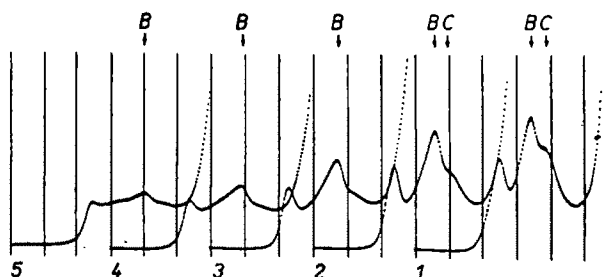


Fig. 4. Variation of the polarographic effect of cytochrome *c* (50 µg/ml.) at 0° C with the concentration of ammonia in the supporting electrolyte which also contained 0.1 molar ammonium chloride and 0.001 molar hexamminecobalt (III) chloride. Curves 1, 2, 3, 4 and 5 were registered at ammonia concentrations of 1, 0.3, 0.1, 0.03, 0.01 moles/l., respectively, starting from -0.8 V at intervals of 0.2 V along the abscissa with the galvanometer sensitivity reduced to 1/200. The maximum appearing at about -1.3 V is the non-catalytic cobalt maximum and has no relation to proteins, except that it is suppressed by them (see Fig. 2). Note the appearance of the catalytic maximum *C* with increasing ammonia concentration.

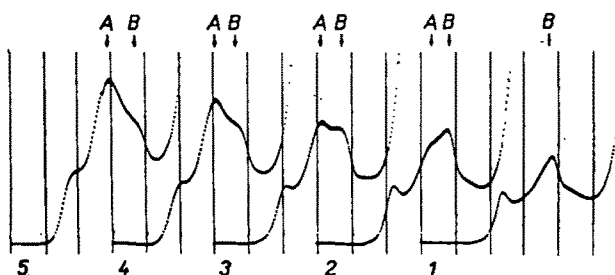


Fig. 5. Variation of the polarographic effect of cytochrome *c* (50 µg/ml.) with temperature. Curves 1, 2, 3, 4 and 5 were registered at temperatures of 0, 12.5, 25, 37.5 and 50° C, respectively, starting from -0.8 V at intervals of 0.2 V along the abscissa with the galvanometer sensitivity reduced to 1/200. The supporting electrolyte had the same composition as in Fig. 1. Note the appearance of the catalytic maximum *A* with increasing temperature.

poorly developed and occur on the steep current intensity rise caused by the discharge of the ammonium ions between -1.8 and -2.0 V compared with a saturated calomel or bottom mercury electrodes.

Of all the amino-acids present in TMV, only cysteine gives a polarographic catalytic maximum comparable with that of a protein<sup>2</sup>. Cucumber virus 4 is biologically closely related, morphologically identical and structurally very similar to TMV, and contains all the amino-acids present in TMV with the exception of cysteine. Besides being polarographically inactive in the native state, cucumber virus 4 is also inactive in its denatured state<sup>4</sup>. Thus cysteine appears to be responsible for the catalytic maxima found in the polarographic effect of TMV. Because the protein of TMV is composed of identical polypeptide sub-units—each containing only a single cysteinyl residue (see ref. 8)—it would seem that one and the same cysteinyl residue is capable of giving rise to all three catalytic maxima *A*, *B* and *C*, or to any one or two of them. (The effect would, of course, depend on its environment or state and on the external conditions such as temperature, pH and the composition of the supporting electrolyte.) It is therefore probable that by suitable variation of the conditions of polarography the three catalytic maxima can also be resolved for other proteins.

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# Membrane Phospholipid Synthesis and the Action of Hormones

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Incorporation of precursors of phospholipids, RNA and protein after treatment with growth and developmental hormones suggests a simultaneous control of the rates of production of cytoplasmic RNA and membranes in the cell.

THE increase in the rate of protein synthesis provoked by growth and developmental hormones is often accompanied by the appearance of additional cytoplasmic ribosomes<sup>1</sup>. In experiments in which thyroid hormones were used to accelerate the growth of thyroidectomized rats<sup>2</sup> or to induce metamorphosis in bullfrog tadpoles<sup>3</sup>, a higher proportion of ribosomes was tightly bound to membranes of the hepatic endoplasmic reticulum after hormone-induced increase in the population of ribosomes (and polysomes). Virtually all protein synthesis *in vivo* may occur on membrane-bound ribosomes<sup>4,5</sup>, and so it was important to know how the "new" ribosomes formed on hormone administration were distributed on membranes. This in turn necessitated the determination of the rate of proliferation of cellular membranes, especially those of the endoplasmic reticulum, before and after the application of the stimulus for growth and protein synthesis.

I wish to summarize here some work on the rate of phospholipid synthesis in hepatic membranes of normal, hypophysectomized, thyroidectomized and castrated rats. Livers of bullfrog tadpoles and unfractionated seminal vesicles of rat were also examined. Growth hormone, thyroid hormone and testosterone all enhanced the rate of formation of microsomal and mitochondrial phospholipids of the livers and of unfractionated seminal vesicles of young rats deprived of the endogenous hormones. The three hormones have sufficiently different lag periods of action in their target tissues, and so it was possible to find in each case that the enhancement in the rate of proliferation of microsomal membranes was temporally co-ordinated with the hormone-induced increase in cytoplasmic RNA and protein synthetic activity *in vivo*.

The overall design of the experiments reported here was based on our earlier work on the sequential stimulation by thyroid and other hormones of the rate of RNA and protein synthesis in target cells<sup>2,8</sup>. Measurement of the rate of synthesis of phospholipids was followed by measurement of the incorporation of phosphate labelled with phosphorus-32 and choline labelled with carbon-14 *in vivo* at different time intervals after a single dose of the hormone. Phospholipids were extracted with chloroform and methanol from cell fractions after precipitation and washing with 0.4 normal perchloric acid or 5 per cent trichloroacetic acid, and their identity was established by thin layer chromatography on silica<sup>9</sup>. Subcellular fractionation of the liver and determination of the rate of synthesis *in vivo* of nuclear and cytoplasmic RNA were carried out as described by us earlier<sup>2</sup>. Microsomes were fractionated into smooth and rough membranes in the ultracentrifuge according to Dallner<sup>10</sup> except that 0.01 molar magnesium chloride replaced caesium chloride in the bottom layer of 1.3 molar sucrose. The rate of protein synthesis *in vivo* was estimated from the incorporation into protein of radioactivity from a mixture of amino-acids labelled with carbon-14 (*Chlorella* protein hydrolysate).

The term "membrane" as used in this article refers to the insoluble phospholipid-rich components of nuclei, mitochondria and microsomes derived by differential centrifugation of liver homogenates. In the case of mito-

chondrial and microsomal membranes, their composition in phospholipids, sedimentation characteristics, morphological appearance and constitutive enzymes correspond to those in preparations obtained by other workers<sup>11-13</sup>.

From preliminary experiments, which will be described in detail elsewhere, time intervals of isotopic labelling with phosphorus-32 and choline labelled with carbon-14 approaching the maximum levels of incorporation into phospholipids were selected for comparison of hormonal effects. These corresponded for mitochondria and microsomes from normal rat liver to those described elsewhere<sup>14-17</sup>. Fig. 1 shows the rather similar pattern of

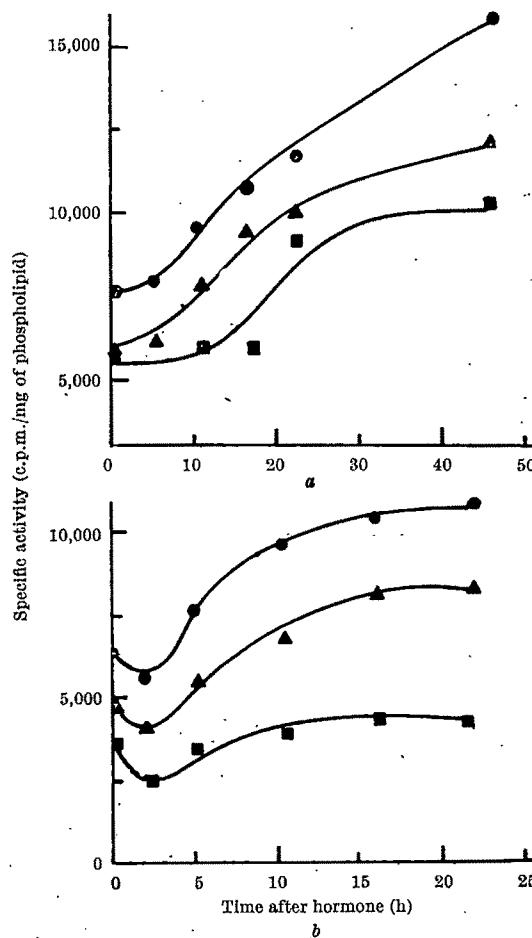


Fig. 1. Incorporation of labelled choline into phospholipids of (■), hepatic nuclei; (▲), mitochondria; and (●) microsomes as a function of time after the injection of (a) 3,5,3'-triiodothyronine (T3) to thyroidectomized rats and (b) human growth hormone (HGH) to hypophysectomized rats. Thyroidectomized rats weighing 140–160 g were injected intraperitoneally with 6  $\mu$ c. of labelled choline chloride (methyl-<sup>14</sup>C, 37.6 mc./mmole) 60 min before they were killed, and hypophysectomized rats weighing 110–130 g received 5.2  $\mu$ c. of the radioisotope 40 min before death. The two hormones were administered subcutaneously. Each point represents the average of four determinations on material pooled from three rat livers.

change produced by a single dose of triiodothyronine or of human growth hormone on the incorporation of labelled choline into the phospholipids of nuclei, mitochondria and microsomes from thyroidectomized and hypophysectomized rats. Growth hormone is usually more effective than triiodothyronine in promoting growth of liver; the latter produces a greater increase in weight when administered in a single dose. Incorporation of labelled choline (and also phosphorus-32) into nuclear phospholipids was less affected, or unaffected, by both hormones than incorporation into mitochondrial and microsomal phospholipids.

The increase in specific radioactivity of phospholipids was followed by a net accumulation of phospholipids, especially in the microsomal fraction (Table 1). Taken together with our earlier work on increases in microsomal and mitochondrial membrane proteins and enzymes<sup>6,7,18,19</sup>, these results suggest a substantial proliferation of membranes after administration of hormone. The effect of thyroid hormones on the proliferation of mitochondrial membrane phospholipids and structural proteins<sup>18,19</sup> is of importance in considering the biogenesis of these organelles<sup>20</sup>.

The rough and not the smooth microsomal membranes are the principal site of protein synthesis, and so we fractionated microsomes obtained at different stages of hormone action before analysing the distribution of phospholipids and RNA. An example of such experiments in which triiodothyronine stimulated the growth of the liver of thyroidectomized rats is summarized in Table 2. There was an increase in specific activity of phospholipids and RNA labelled with phosphorus-32 in both the smooth and the rough membranes as a result of triiodothyronine injection and a shift in the distribution of phospholipid and RNA between the smooth and the rough membranes. A higher proportion of phospholipid and RNA was recovered in the rough membranes from hormone-stimulated animals than from the controls. Newly formed phospholipids in the rough membranes were also more resistant to solubilization with a small amount of detergent (0.18 per cent sodium deoxycholate) than was the total phospholipid of unfractionated microsomes as judged by the differences in specific activity. It is on the synthesis of this phospholipid fraction resistant to detergent that the hormone seems to have the most pronounced effect. When similar experiments were performed in which the animals were killed 10 min after a pulse of labelled amino acids, the greatest protein synthesizing capacity was associated with this 0.18 per cent deoxycholate-resistant component of rough membranes. A shift in the distribu-

Table 1. PHOSPHOLIPID, RNA AND PROTEIN CONTENT OF HEPATIC MICROSOMES DERIVED FROM NORMAL, THYROIDECTOMIZED AND HYPOPHYSECTOMIZED RATS WITH AND WITHOUT TREATMENT WITH 3,5,3'-TRIODO-L-THYRONINE AND HUMAN GROWTH HORMONE

Rats	Treatment	Phospholipid (mg/g of liver)	Phospholipid Protein	RNA (mg/g of liver)
Normal	None	8.70	0.33	5.33
Thyroidectomized	None	6.01	0.24	4.08
	T <sub>3</sub>	7.45	0.27	5.10
Hypophysectomized	None	5.35	0.20	3.69
	HGH	6.30	0.27	4.31

Normal and thyroidectomized rats weighed 130–160 g when killed whereas hypophysectomized rats weighed 120–135 g; 3,5,3'-triiodo-L-thyronine (T<sub>3</sub>) was injected as a single dose of 18  $\mu$ g/rat 48 h before death and 75  $\mu$ g of human growth hormone (HGH) were injected twice, 48 and 18 h before death. Each value is the average derived from four separate determinations on livers pooled from three rats.

Table 2. CHANGES INDUCED IN THE DISTRIBUTION IN MICROSOMAL SUBFRACTIONS OF PHOSPHOLIPIDS AND RNA SYNTHESIZED DURING ACCELERATED GROWTH OF THE LIVERS OF THYROIDECTOMIZED RATS INDUCED BY A SINGLE INJECTION OF TRIIODOTHYRONINE

Fractions	Specific activity of phospholipids (c.p.m./ $\mu$ g of phosphorus)		Relative distribution of phospholipids (per cent)		Specific activity of RNA (c.p.m./ $\mu$ g of phosphorus)		Relative distribution of RNA (per cent)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Microsomes	77.5	185.0	100	100	7.8	15.3	100	100
Smooth membranes	84.9	227.0	40	25	17.5	47.6	6.6	7.9
Rough membranes	79.9	172.0	53	62	6.3	14.9	86.0	74.0
0.18 per cent DOC pellet	95.0	585.0	8	13	3.5	7.9	7.7	18.0

Half of the thyroidectomized rats were treated with 18  $\mu$ g of T<sub>3</sub> for 32h before they were killed; all animals received 75  $\mu$ c. of phosphorus-32 12 h before death. Microsomes were isolated from one aliquot of the supernatant free of mitochondria, and smooth and rough membranes were isolated from another. A part of the rough membrane fraction was suspended in 0.18 per cent sodium deoxycholate (DOC) (20 mg of DOC/3 mg of phospholipid) and the sediment (0.81 per cent DOC pellet) was collected for analysis. Each value is the mean of four determinations made on preparations from three rat livers.

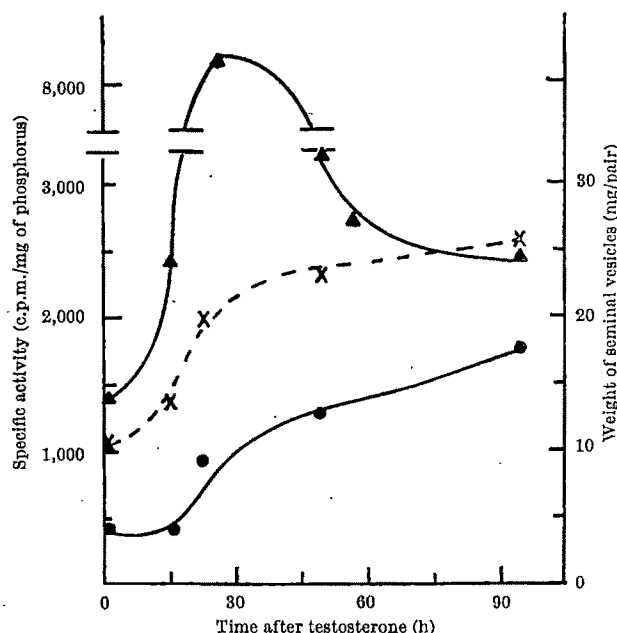


Fig. 2. Incorporation of labelled phosphate into ( $\Delta$ ) RNA and ( $\bullet$ ) phospholipid during the course of growth ( $\times$ ) of seminal vesicles induced by a single injection of testosterone propionate to castrated rats. Male rats weighing 50–60 g were castrated 2 weeks before the experiment in which 50  $\mu$ c. of carrier-free phosphorus-32 was injected intraperitoneally 2–6 h before the death of animals which had received 200  $\mu$ g of testosterone propionate intramuscularly at the different times indicated. Seminal vesicles were weighed, chilled to 0° C, finely chopped and homogenized in 0.15 molar sodium chloride in a Kontes conical all-glass homogenizer before extraction of phospholipids and RNA. Each point is the average value obtained from four determinations in six castrated rats.

tion of microsomal membranes between smooth and rough, with a relative increase in tightly membrane bound ribosomes induced by these hormones, is compatible with sudden acceleration in the rate of growth and protein synthesis. This change is, however, unlike the increase in the smooth relative to rough membrane observed in the liver of rat soon after birth, as recently reported by Dallner *et al.*<sup>16,17</sup>.

As shown in Table 2, a small portion of microsomal RNA is associated with smooth membranes, and we have repeatedly found its specific activity and turnover rate to be greater than that of RNA from rough membranes. A species of rapidly labelled RNA in smooth membranes of rat liver microsomes with sedimentation characteristics and base composition different from all other species of cytoplasmic RNA has been described previously<sup>21–23</sup>. Its role in protein synthesis is still unknown.

In contrast with the mammalian system<sup>2</sup>, there was relatively little accumulation of cytoplasmic RNA after intense labelling of RNA both in the nucleus and cytoplasm at short time intervals after the induction of metamorphosis in bullfrog tadpoles<sup>3</sup>. Table 3 shows that there is, however, a noticeable accumulation of microsomal phospholipid, and also of protein, which accompanies the turnover of microsomal RNA. We have also observed, both by fractionation of microsomal membranes and by electron microscopy, that one of the principal features of induced metamorphosis is a proliferation of rough membranes of endoplasmic reticulum accompanied



Table 3. SYNTHESIS OF HEPATIC MICROSOMAL PHOSPHOLIPIDS, RNA AND PROTEIN FOLLOWING THE PRECOCCIOUS INDUCTION OF METAMORPHOSIS IN *Rana catesbeiana* TADPOLES

Time after induction (days)	Incorporation of phosphorus-32 into		Amount of microsomal (mg/g of liver)		
	Phospholipid (c.p.m./mg P)	RNA (c.p.m./mg of phosphorus)	Phospholipid	Protein	RNA
0	492,000	204,000	2.44	11.0	2.8
1.8	420,000	217,000	2.51	10.1	2.7
3.7	594,000	510,000	2.69	9.6	2.9
6.8	683,000	628,000	3.80	14.2	3.1

Groups of 12 *Rana catesbeiana* tadpoles (with no hind limbs) were injected with 1  $\mu$ g of 3,5,3'-triiodothyronine at the times indicated before they were killed. Labelled phosphate (10  $\mu$ c.) was injected 18.0 h before the animals were killed and the microsomes were prepared as described elsewhere. Each value is the mean of two determinations in duplicate.

by a redeployment of cytoplasmic ribosomes (my own unpublished observations). Mitochondrial phospholipid synthesis was also enhanced concomitantly with an increase in total mitochondrial structures<sup>3</sup>.

Poor recoveries of subcellular fractions from accessory sexual tissues of castrated rats prevented us from fully investigating the formation of phospholipids in microsomal membranes from the prostate and seminal vesicles after growth of the prostate and seminal vesicles had been induced by testosterone. In view of the massive accumulation of RNA and proliferation of membranes in seminal vesicles produced by the administration of the androgen to castrated animals<sup>24</sup>, we investigated the turnover of phospholipids and RNA in unfractionated homogenates of this tissue (Fig. 2). An increased rate of synthesis of total phospholipid was observed at much the same time as the increased rate of bulk RNA synthesis which accompanies the dramatic hypertrophy produced by testosterone in

this organ. It was shown earlier that a single injection of testosterone under similar conditions led to a four- to five-fold increase in bulk RNA/DNA if the tissues were analysed 5 days after treatment with hormone<sup>1</sup>.

Perhaps the most significant point that emerges from these investigations is the timing of the effects of the different hormones on the synthesis of phospholipid in their target cells in relation to the acceleration of the rate of protein synthesis. The magnitude and speed of hypertrophy induced by growth hormone, thyroid hormone and testosterone in the liver of animals in which the rate of growth of the organ was retarded or arrested by deprivation of the hormone followed a distinctive pattern for each hormone. These features are reflected in biosynthetic activities of the target cells, as has already been demonstrated by us in the synthesis of RNA by the nucleus<sup>2,8</sup>.

The data summarized in Fig. 3 show that growth hormone stimulated the protein synthetic capacity of hepatic microsomes *in vivo* several hours earlier than did testosterone or triiodothyronine. This change accompanied the appearance of additional microsomal RNA or ribosomes (see also refs. 1 and 2). The onset of increase in the rate of microsomal phospholipid synthesis, and eventually the proliferation of rough membranes, coincided for each hormone with the foregoing two effects. It is also interesting to compare the magnitude of these effects produced by testosterone in the liver and seminal vesicles of the same castrated rats. The rate of growth of the accessory sexual gland is several times more sensitive to the androgen than is that of the liver (a single injection of 250  $\mu$ g of

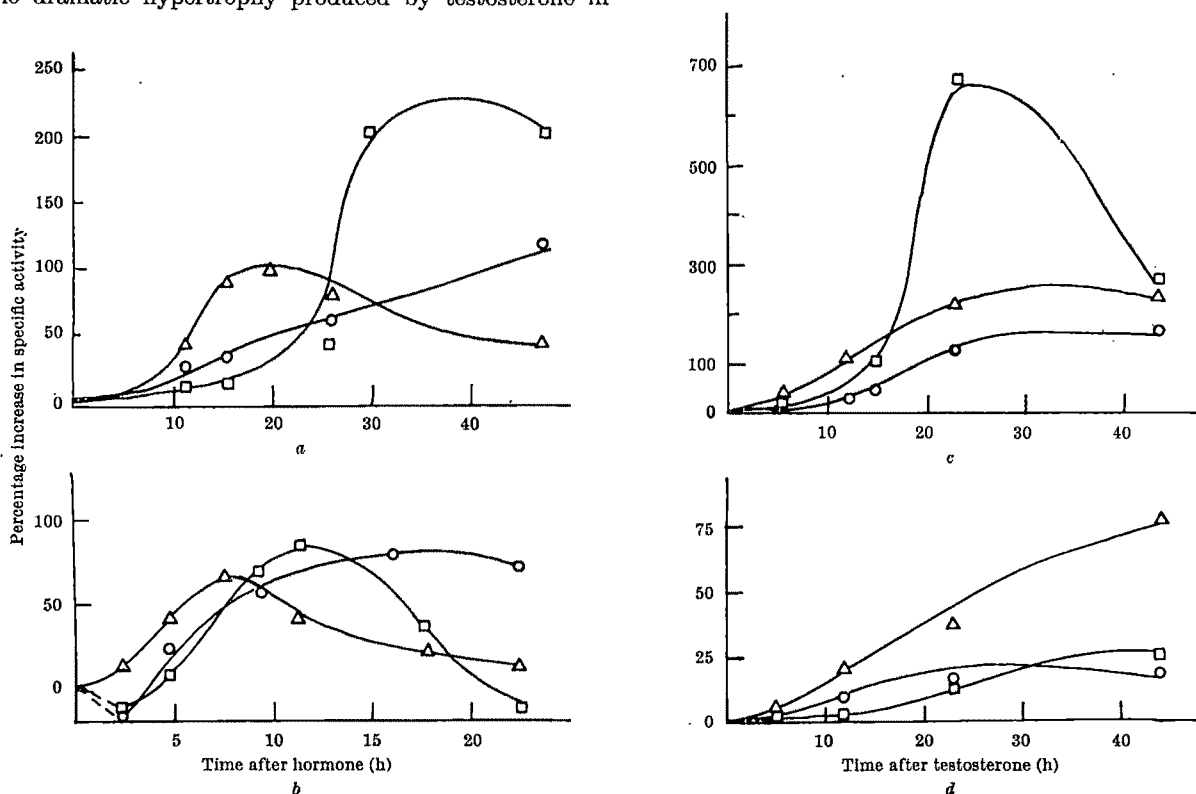


Fig. 3. Co-ordination of the synthesis of microsomal phospholipids with the appearance of labelled RNA in microsomes and their capacity *in vivo* to incorporate amino-acids into protein after hormone-induced growth of liver and seminal vesicles. The rate of microsomal RNA and phospholipid synthesis in the liver and of total RNA and phospholipids in seminal vesicles was determined from the incorporation of radioactivity into the macromolecules 9–11 h after the administration of 60–75  $\mu$ c. of phosphorus-32. The rate of protein synthesis *in vivo* was estimated from the incorporation of radioactivity into proteins after the administration of 4–7  $\mu$ c. of a mixture of amino-acids labelled with carbon-14 (*Chlorella* protein hydrolysate; 600  $\mu$ c./mg) 10 min before death in experiments with liver microsomes and 20 min in the case of seminal vesicles. (a) Stimulation observed in liver microsomes caused by a single injection of triiodothyronine to thyroidectomized rats; (b) changes produced by a single injection of 75  $\mu$ g of GHG in hepatic microsomes from hypophysectomized rats; (c) stimulation of the rates of macromolecular synthesis in unfractionated seminal vesicles of castrated rats at different times after a single injection of 250  $\mu$ g of testosterone propionate; (d) the effect of testosterone administration on hepatic microsomes from castrated rats similar to those used in (c). For incorporation of phosphorus-32 the same rats were used in (c) and (d). An important point to note is the wide differences in the scales of ordinates and abscissae of the four diagrams. The drop in the specific radioactivity of nascent protein at the later time-intervals is mainly a result of the substantial increase in membrane and ribosomal protein content of microsomes. The results are expressed as percentage increase in specific activity of (O), phospholipid; (□), RNA; and (Δ), protein in hormone-treated animals for the values obtained in preparations from untreated controls.

testosterone increased the weight of seminal vesicles of castrated rats by 200 per cent in 4 days, whereas liver weight increased by only 6 per cent–8 per cent). It is therefore interesting to see that responses of these two tissues in terms of rates of synthesis of macromolecules are of comparable order (compare Fig. 3c and d). The time of onset of the stimulatory effect was, however, the same in both tissues.

In conclusion, it seems that the regulation of the rate of protein synthesis by growth and developmental hormones involves a simultaneous control of the rates at which cytoplasmic RNA and membranes are generated in the cell. A concomitant proliferation of membranes of the endoplasmic reticulum and redistribution of ribosomes has also been observed in other situations of growth and maturation or enzyme induction<sup>16,17,27,28</sup>. A co-ordinated proliferation of membranes that is geared to the rate of protein synthesis or adaptive functions may be a common property of cells of higher organisms. It should be realized, before any generalizations are made, that the tissues that have been investigated could represent a special case, because they are largely those that elaborate substantial amounts of proteins for export, a function in which microsomal membranes play an important part<sup>29,30</sup>. Besides the hormones that we have investigated, a very pronounced effect of TSH and oestrogen on synthesis of phospholipid in the thyroid and uterus, respectively, has also been observed to occur at about the same time as the acceleration of synthesis of RNA or the onset of biological effects of the hormone<sup>31,32</sup>. Regulation of the proliferation of the membranes and ribosomes may not, however, be the only way in which all hormones control protein synthesis and indeed hormonal control of translational processes preceding a change in the rate of RNA synthesis has been suggested for the action of ACTH and insulin<sup>33,34</sup>. The results described in this paper represent relatively late effects of the hormones in the chain of events leading to growth and maturation, but the specificity and reversibility of their actions can throw much light on the biogenesis and complex functions of cellular membranes.

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## Effect of Nucleic Acids from Immune Lymphocytes on Rat Sarcomata

by

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Primary rat sarcomata regress, usually temporarily but occasionally completely, after the injection of nucleic acids from lymphocytes of sheep and allogeneic rats which have been immunized with tissues from the tumour to be treated. It is possible that the effect results from the strengthening of an already existing immune reaction and that it is only temporary because it is related to the life span of messenger RNA.

PRIMARY fibrosarcomata in the rat, induced by chemicals, can be caused temporarily to regress by treatment with lymphocytes separated from the lymph of animals immunized with the tumour<sup>1</sup>. The specificity with which the lymphocytes act only against the tumour used for inoculation suggests that the phenomenon is immunological. The fact that our later work<sup>2</sup> showed that heterologous lymphocytes from sheep and goats were also effective suggests that the transfer of a sub-cellular factor may be

involved. A similar interpretation might be placed on the experiments<sup>3</sup> in which the growth of these tumours was retarded when the immune lymphocytes were administered inside intraperitoneally placed 'Millipore' chambers. Work from numerous laboratories (see later) suggests that immune reactions can be induced by specific RNA and this led us to explore the possibility of treating tumour-bearing rats with nucleic acids extracted from the immune lymphocytes. The preparation used contained

all the RNA of the cells but was contaminated with some DNA. The rationale of these experiments was that nucleic acids from immune lymphocytes may confer on the tumour-bearing host an increase in the capacity to react immunologically against the tumour specific antigens of the transplantation type known to be present in these primary sarcomata<sup>4</sup>. Both the aim of the present experiments and the method of applying the nucleic acids are quite different from those studies in which it has been alleged that treating tumour cells *in vitro* with RNA from normal cells causes epigenetic changes in malignant cells such that they lose the capacity to give rise to tumours on being transplanted into suitable hosts<sup>5-7</sup>.

Intravenous injection had proved to be the most effective way of administering the immune lymphocytes<sup>1</sup> to tumour-bearing animals, but this route was not chosen in the present experiments with the nucleic acids because it was thought that the high RNase activity of blood would cause inactivation of the RNA before it could be taken up by the lymphoid cells. The injection of the RNA into the footpads ensured that the RNA reached lymph nodes directly and this route of administration has been successfully used in transferring immunity *in vivo* in another system<sup>8</sup>.

Primary fibrosarcomata were induced with pellets of 3 : 4-benzpyrene as described by Haddow and Alexander<sup>9</sup>. Two rats with comparable tumours having a diameter in the range of 1.5–2.0 cm were selected for each experiment. About half the tumour mass was excised from each rat. One of the tumour masses was used for immunization and the other discarded. The techniques of immunizing rats and of obtaining immune thoracic duct lymphocytes from "donor" rats have already been described<sup>1</sup>. In the experiments with sheep lymphocytes the tumour was injected into the drainage area of the prefemoral lymph nodes, the efferent duct cannulated 3 days later and the lymph was collected daily over the next 3 days<sup>2</sup>. The cells from each collection were washed, re-suspended in Hanks solution and quickly frozen at  $-80^{\circ}\text{C}$ .

The nucleic acids were extracted from the cells (see later) and doses containing 0.5 mg of RNA together with variable amounts of DNA were injected in 0.5 ml. saline into the hind footpads of the tumour-bearing rats. The number of cells that could be obtained from the thoracic duct of immunized rats was sufficient for only one treatment, but sufficient nucleic acids to give repeated doses were obtained from the efferent lymph from the nodes of sheep. The injections were repeated as shown in Table 1 either on successive or on alternate days.

In the 7 days between obtaining the tumour for immunization and the injection of nucleic acids, the tumours grew rapidly and experiments in which the diameter at the beginning of treatment was larger than 2.4 cm have not been reported because such tumours tend not to respond to any type of therapy.

Three days after immunization the proportion of large basophilic cells, which, because they are rapidly dividing, we refer to as lymphoblasts, increases in the efferent lymph of the stimulated nodes and their numbers reach a maximum between 90 and 120 h (ref. 10). The degree of response of the nodes to immunization with tumours is assessed by the percentage of these large cells present in the lymph at the peak period; "poor", less than 7 per cent; "moderate", 7–15 per cent; "good", 15–25 per cent; "excellent", greater than 25 per cent—the highest value was 40 per cent. These lymphoblasts contain about six times as much RNA per cell as the small lymphocytes that are also present, and consequently in most of the experiments the main part of the RNA in the nucleic acid preparation was derived from these large lymphoid cells.

Each gram of frozen cells was gently homogenized with 10 ml. of freshly prepared 6 per cent 4-aminosalicylic acid and mixed with an equal volume of 90 per cent phenol

containing 0.1 per cent 8-hydroxyquinoline<sup>11</sup>. After stirring for 30 min at  $0^{\circ}\text{C}$  in a 35 ml. cellulose nitrate centrifuge tube, the layers were separated by spinning at 5,000 r.p.m. for 20 min. The aqueous supernatant containing the nucleic acids was carefully removed using a pipette with a curved end. This was made 3 per cent with respect to sodium chloride and stirred again with one half volume of phenol containing 0.1 per cent 8-hydroxyquinoline for 15 min at  $0^{\circ}\text{C}$ . The nucleic acids in the aqueous supernatant, separated as before, were precipitated by adding two volumes of 10 per cent *m*-cresol in ethanol and standing for 1 h at  $0^{\circ}\text{C}$ . The pellet was spun out at 60 r.p.m. for 15 min, and washed twice with 75 per cent ethanol containing 1 per cent sodium chloride. After draining, the bulk of the DNA was removed by shaking vigorously with 25 ml. of 3 molar sodium acetate (pH 6), and centrifuged at 30,000 r.p.m. for 15 min to ensure recovery of the fine precipitate of RNA. This was repeated three times and the resulting nucleic acids drained and dissolved in a volume of physiological saline to give a concentration of 0.5 mg/ml. of RNA. Recoveries of 1.6–2.3 mg of RNA/g of wet cells were obtained. The preparation was contaminated with less than 1 per cent protein but always contained a substantial fraction of the cells' DNA. In some experiments the material injected contained as much DNA as RNA.

Table 1. RETARDATION OF GROWTH OF PRIMARY RAT SARCOMATA BY INJECTION OF NUCLEIC ACIDS EXTRACTED FROM THE LYMPHOCYTES OF SHEEP AND ALLOGENEIC RATS IMMUNIZED WITH PIECES OF THE TUMOUR TO BE TREATED (In the sheep experiments both the tumour used for immunization and another tumour which had been biopsied only were treated with the nucleic acid.)

Experiment No.	No. of lymphocytes available for RNA extraction	Degree of response of node to antigenic stimulation	No. of injections of nucleic acid containing 0.5 mg of RNA	Maximum reduction in tumour diameter (percentage of initial size)	Growth rate* (time in days for tumour to increase 1.6 times in diameter)
Efferent lymph from nodes of sheep locally stimulated with rat tumour					
527 (1) Specific	$2.2 \times 10^{10}$	Good	6	40	25
(2) Control			6	None	10
529 (1) Specific	$2.4 \times 10^{10}$	Excellent	8	96	78
(2) Control			3	None	9
			(died before final treatment could be given)		
532 (1) Specific	$5.3 \times 10^9$	Poor	4	None	10
(2) Control			4	None	8
541 (1) Specific	$9.8 \times 10^9$	Poor	8	30	18
(2) Control			8	None	10
549 (1) Specific	$2 \times 10^{10}$	Good	6	85	39
(2) Control			6	None	8
551 (1) Specific	$6.2 \times 10^9$	Good	3	None	23
(2) Control			3	None	17
561 (1) Specific	$1.6 \times 10^{10}$	Excellent	6	60	42
(2) Control			6	None	12
567 (1) Specific	$6 \times 10^9$	Good	5	100	Not recurred
(2) Control			5	0	9
571 (1) Specific	$2 \times 10^9$	Poor	2	0	6
(2) Control			2	0	6
575 (1) Specific	$1.2 \times 10^{10}$	Good	4	20	25
(2) Control			4	0	9
Thoracic duct lymph from rats immunized with allogeneic rat tumour					
510 Specific	From 2 to $9 \times 10^8$ cells		1	50	16
526 (1) Specific	with increased proportion of large lymphocytes		1	25	19
(2) Control†			1	None	9
528 Specific			1	20	24
531 Specific			1	78	27
534 Specific			1	66	30

\* Measurements based on diameter of tumour at the time of first treatment with nucleic acids.

† Lymphocyte donors immunized with a sarcoma other than the one being treated.

The rate of growth of the different primary fibrosarcomata is remarkably uniform<sup>9</sup> and was determined by regular diameter measurements. These tumours, even after the biopsy procedure used for obtaining material for immunization, never regress spontaneously and any decrease in size must be attributed to treatment. A useful measure for assaying the effectiveness of a treatment is the time required for the tumour diameter to increase by a factor of 1.6 (corresponding for spherical tumours to an increase in volume of four times). In more than a hundred controls which received no treatment

other than biopsy this time was on average 11.5 days and never exceeded 20 days (refs. 1, 2 and 9).

From the lower part of Table 1 it can be seen that all five of the primary tumours treated with RNA from thoracic duct lymphocytes of immunized rats showed temporary regression and in three of the five experiments the time for the tumour to increase its volume by a factor of four was substantially extended. Because the number of lymphocytes that were obtained was relatively small there was usually only sufficient RNA to treat one rat—namely, the one that had been used for immunization—and in only one experiment, No. 526, was it possible to inject also a "control" tumour-bearing rat and this showed no response.

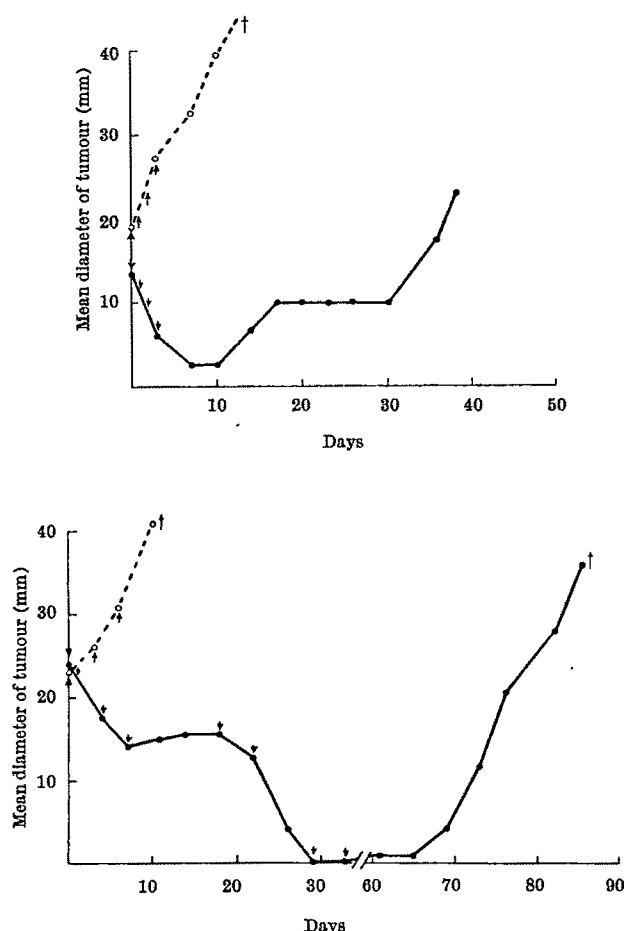


Fig. 1. Growth curves of two pairs of primary rat sarcomata after treatment with nucleic acids extracted from the lymphocytes of sheep immunized with tumour (↓ indicates times of injection of nucleic acids). In each pair the "treated" (●—●) animal provided the tumour for immunization; the "control" (○- -○) also had part of its tumour excised, but this material was not used for immunization.

Nucleic acid from sheep lymphocytes was used in the majority of the experiments and in each case two rats with primary tumours were used and treated with aliquots of the same nucleic acid preparation. Both tumours were biopsied simultaneously, but only one of the tumours was used for immunization; the animal from which this tumour was taken is the "specifically treated" member of the pair while the other is referred to as the control. In none of the seven pairs was the growth of the "control" tumour affected by the RNA (they grew at the same rate as completely untreated tumours) while six of the seven "treated" tumours responded. Fig. 1 shows the growth curves of two pairs of animals in which the "specifically

treated tumour regressed for long periods. The available data suggest, but are not sufficient to establish, a correlation between the response of the draining node in terms of the proportion of basophilic lymphoblasts present and the anti-tumour action of the nucleic acids extracted from the cells in the lymph.

There can be little doubt that nucleic acids derived from lymphocytes issuing from a node stimulated by tumour exert a growth inhibitory effect on the specific primary fibrosarcomata used for immunization although the procedure does not seem to be quite so effective as the intravenous injection of the intact lymphocytes (compare refs. 1 and 2). While direct proof is lacking, the data are consistent with the assumption that the active material is provided by the large lymphocytes rich in RNA which are released from the node as a result of immunization. That this anti-tumour effect of the nucleic acids has an immunological basis involving the tumour specific antigens seems to be indicated by the absence of cross-reaction; the tumour specific antigens of chemically induced tumours have been shown to be unique for each tumour (compare ref. 4).

The very low protein content of the preparation used suggests that a nucleic acid, or possibly a covalently linked nucleic acid-protein adduct, constitutes the active principle. Although the preparation procedure was specifically designed for the isolation of RNA an approximately equal amount of DNA was present. Experiments are now under way with more highly purified materials, but the discussion which follows is based on the assumption that the activity resides in the RNA. If this can be shown to be the case it is still conceivable that the DNA augments the response in a non-specific way by acting as an adjuvant.

In the past 10 years RNA or RNA antigen adducts ("super-antigens") have been shown to be capable of initiating immune responses in a number of systems. The results seem to require that extraneously supplied RNA can act at least at two different points in the sequence of events between the phagocytosis of antigen and the formation of antibody, either free or cell-bound (see Fig. 2). Phagocytic cells in the lymphoid tissues retain antigenic proteins for long periods during which they may be complexed with RNA (refs. 12 and 13). Such RNA complexes are highly immunogenic<sup>14</sup> and may be responsible for the capacity of RNA extracted from macrophages to initiate antibody formation by lymphoid cells *in vitro*<sup>15,16</sup>.

On the other hand, the action of RNA at a different level is indicated: by experiments<sup>17</sup> in which the capacity of lymphoid cells to transfer immunity was temporarily abolished by treatment with RNase, and also by the observation that the capacity to transfer homograft immunity can be bestowed on lymphoid cells by incubating them *in vitro* with RNA extracted from immune cells<sup>18</sup>. Our working hypothesis is that the mechanism by which RNA acts in these latter experiments may be the same as that involved in the anti-tumour action in our experiment. A possible interpretation of the mode of action is based on the concept that the lymphoblasts which are released by the node in response to antigenic stimulation are not principally antibody producers, but are messenger cells<sup>19</sup> that migrate throughout the lymphoid system. In this way an initially local response is propagated throughout the body. Experimental support for this concept was provided by draining off the lymph from the locally stimulated node in sheep. When this was done, no circulating antibody was formed, but on injection of these cells into a chimaeric twin antibody production occurred. The observation<sup>20</sup> that it is the large lymphocytes in the thoracic duct which are responsible for the transfer of delayed hypersensitivity lends further support to this concept.

One can hypothesize (see Fig. 2) that the antigen processed by macrophages and possibly linked to RNA stimulates the node to form the lymphoblasts rich in

RNA. These cells do not propagate the immune response merely as passive vehicles for antigen, and the failure to detect antigen in them strengthens the view that information is passed at a level which does not involve the actual antigen. The simplest hypothesis is that the lymphoblasts proliferate and then differentiate into plasma cells. If that were their only function, then it would be difficult to envisage how they could function on transfer to an allogeneic or even less a heterologous host (that is, sheep basophilic cells in rats)<sup>2</sup>. An alternative mechanism (compare ref. 19) by which the immune response may become generalized is that the lymphoblasts do not survive for any length of time in the lymph nodes to which they have migrated, but that they may convey the immune stimulus by passing a "message" (other than conventional antigen) to the actual antibody forming cells such as the plasma cells.

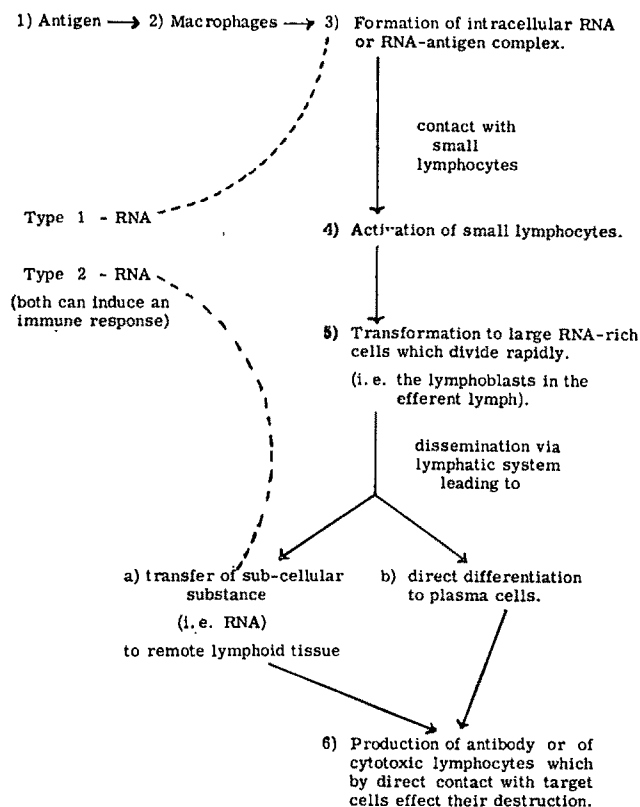


Fig. 2. Diagrammatic representation of a hypothetical sequence of events in the immune response showing stages where externally administered RNA may be active.

The general concepts of molecular biology suggest RNA as a likely candidate for the subcellular message especially as the most characteristic biochemical feature of the lymphoblasts is their high RNA content. These cells are rich in polysomes—which contain the "messenger RNA" clearly identified in protein synthesis—but do not contain the rich endoplasmic reticulum characteristic of cells which produce antibody. Indeed, the use of the term "messenger cell" by Hall *et al.*<sup>19</sup> was indicative of the hypothesis that the message might be the "messenger RNA" of molecular biologists.

The second step in the immune process at which extrinsic RNA can initiate an immune response is, according to this hypothesis, the uptake of RNA by cells capable of producing antibody after receipt of message. This RNA we assume to be different from the RNA of the super

antigen, and in experiments with bacterial antibodies the RNA from the messenger cells, unlike the material isolated by Askonas and Rhodes<sup>14</sup> from macrophages showed no antigenic activity. The tentative explanation of the role of RNA in the present experiment is that under certain conditions the function of the messenger cells may be replaced by administration of RNA from suitably stimulated lymphocytes. This concept is based on the experiments of Hall *et al.*<sup>19</sup> which demonstrate the role of messenger cells in the production or circulating antibody. To apply it to the present experiment it is necessary that an analogous series of steps is involved in the production of cytotoxic lymphocytes because there is no reason to believe that circulating antibody is involved in the anti-tumour action of specifically immunized lymphocytes or their RNA.

It is not necessary to infer from the experiments reported that the injected nucleic acids initiate a primary immune response because there is evidence<sup>4</sup> that the host reacts actively against the specific antigens of the autochthonous tumour even when the response is insufficient to affect significantly the growth of the tumour. The anti-tumour effect of the nucleic acids from the specific lymphocytes—and of the intact cells themselves—may be the result of enhancing an already existing reaction rather than the initiation of a new response. According to this view the production of "message" and not the capacity to carry out instructions received is the factor which limits the extent to which the host is capable of reacting against its own primary tumour. The recurrence of tumour growth after treatment may result from the fact that this reinforced immunity against the tumour specific antigens does not usually persist for long periods and this may be related to the life span of the specifically activated lymphocytes or that of their "messenger" RNA. The procedure can probably cause the total elimination of only small aggregates of tumour cells, but there is no need to emphasize the clinical value of a method which might selectively clear the small numbers of dispersed malignant cells which frequently remain after other forms of therapy. The use of nucleic acids avoids many of the possible complications arising from the use of intact foreign cells—particularly of heterologous origin—and it is difficult to envisage how such a procedure could cause enhancement.

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# Patterns of Selections by Monkeys with Lesions of the Cerebral Cortex

by

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Monkeys with frontal and inferotemporal lesions show different patterns of responses to an array of objects when offered equally rewarded alternatives.

THERE is considerable evidence that monkeys with lesions of the dorsolateral portions of the frontal cortex are defective in delayed response and similar tasks<sup>1</sup>. Various attempts have been made to characterize such animals as being defective in short term memory<sup>1-3</sup>, but such a description fails to account for all the results. For example, in certain circumstances frontal monkeys are superior to controls in learning simple object discriminations<sup>4,5</sup>, and their retention appears to be good when "the test conditions are such that the normally dominant response does not interfere with correct performance"<sup>6</sup>. Accordingly, one is led to examine stages in the processing of information which are logically before storage. The present investigation is concerned with the manner in which frontal monkeys sample stimuli from a large array, with the situation deliberately designed to make storage of information about particular choices unnecessary.

Twelve rhesus monkeys of varying ages and seven young baboons were used as subjects. They all had previous testing experience in other tasks. Five rhesus and three baboons had received bilateral ablations of the dorsolateral frontal region; three rhesus and two baboons had bilateral ablations of the inferior temporal region; and four rhesus and two baboons were unoperated controls. All testing was carried out in a modified Wisconsin testing apparatus. The test board contained eight food-wells, covered by plaques carrying eight different multi-dimensional objects. The objects remained the same throughout the experiment and were always in the same position. Half a peanut was placed under each object, and the wells remained uncovered after the reward had been removed. Before formal testing began, the animals were accustomed to the apparatus and learned to displace the plaques to obtain the reward. The wells remained uncovered after each response, and so further attempts to select the same object were rapidly extinguished.

When the screen was raised the animals were allowed a maximum of 1 min to select all of the eight objects. This constituted a trial. If all the rewards were removed in less than 1 min, the screen was lowered, and the time of completion recorded. Each animal received six trials a day for 9 days. The order in which the objects were selected was observed through a one-way vision screen and recorded. Testing was carried out 22-24 h after feeding. It became apparent, while testing the first few animals, that some broke off from the task and returned to it, while others completed their selection without interruption. Later, all such "breaks" in performance were recorded; a "break" was defined arbitrarily as an occasion when the animal turned away from the test board, either by looking away or moving away from it physically.

The results for all animals showed a tendency for a stereotyped pattern of selection in that there was a strong likelihood of an object being selected which was adjacent to the one just previously chosen. Fig. 1 shows the percentage of successive pairs of all responses as a function of the relative separation between objects displaced by any response and the immediately preceding response. Fig. 1a shows the complete results and ignores "breaks", and

Fig. 1b shows the results between "breaks". It is evident that not only is the stereotypy quite strong, but that frontal animals display it to a lesser extent than control animals. Table 1 lists the probability values (calculated by Mann-Whitney *U* test) of obtaining differences between frontals and non-frontals by chance.

Table 1. PROBABILITY VALUES OF OBTAINING CHANCE DIFFERENCES BETWEEN THE RESULTS OF FRONTALS AND NON-FRONTALS

	Position of second choice in relation to first				
	Adjacent	Two away	Three away	Four away	
Complete analysis (two tail test)	<0.02	<0.05	<0.02	>0.10	Frontals <i>n</i> = 7 Non-frontals <i>n</i> = 11
Between "breaks" analysis	<0.021	<0.047	<0.021	>0.35	Frontals <i>n</i> = 6 Non-frontals <i>n</i> = 6

A more general, but more precise, analysis can be carried out by considering the chance probabilities of obtaining observed entries in a table of digrams for each response and the immediately preceding response. This was done for each animal on each day of testing using an approximation to  $\chi^2$ . The greater the value of  $\chi^2$ , the greater the stereotypy of the results (independent of the exact type of stereotypy). An animal was considered to show stereotypy if the  $\chi^2$  value for the day in question exceeded the 1 per cent level of significance.

When all trials, irrespective of interruptions, were considered, it was found that frontal animals made significantly fewer selections ( $P < 0.025$ , Mann-Whitney) which satisfied the criterion of stereotypy than did animals with inferotemporal lesions or unoperated controls. The inferotemporal group could not be distinguished from the unoperated group ( $P = 0.3$ , Mann-Whitney) and has therefore been combined with the unoperated animals as the

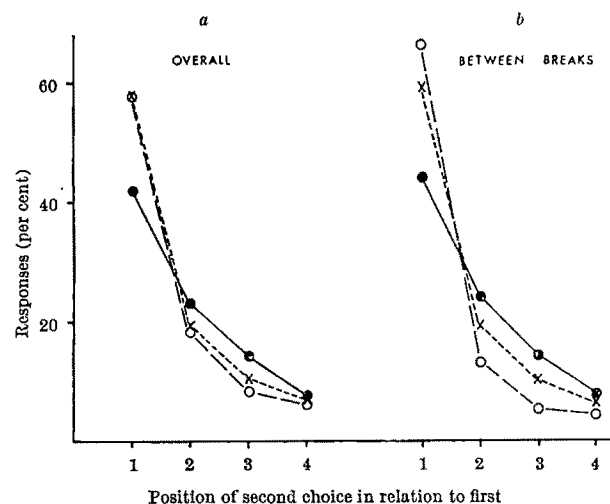


Fig. 1. a, Percentages of successive pairs of responses, ignoring "breaks", expressed as a function of the relative separation between objects. b, The same, for successive pairs of responses between "breaks". ●, Frontals; ×, normals; and ○, temporals.

"non-frontal" group for comparison. The results are shown in Table 2. The frontals showed less stereotypy than the non-frontals in the analysis both of the complete results and of the results between "breaks". The situation also permitted a measure of the rate of object selection. Excluding the selections after a "break", the time for each response ranged from 1.4 to 2.9 sec, with the frontals taking significantly longer to choose ( $P=0.05$ , Mann-Whitney). Finally, it was quite evident during the course of testing that the frontal animals took more "breaks" than non-frontals, and computation shows that they took a mean of 29.7 "breaks" over all trials, while non-frontals took a mean of 8.0 ( $P=0.001$ , Mann-Whitney).

Table 2. MEDIAN PERCENTAGES OF STEREOTYPED SELECTIONS

	Frontals $n=7$	Infero- temporals $n=5$	Normals $n=6$	Non- frontals $n=11$	$P$ Frontals versus non-frontals (1 tail test)
Complete analysis	11	77	55	66	$<0.025$
Between "breaks" analysis	$n=6$ 38.5	$n=2$ 77.5	$n=4$ 55	$n=6$ 60.5	0.03

Our results therefore indicate that frontal animals show less stereotypy (more randomness) in selecting stimuli where no reinforcement contingency is placed on their successive selections. Furthermore, the frontals interrupt their selections much more frequently, but the greater degree of randomness cannot be accounted for in this way. Finally, their rate of stimulus selection is lower than that of controls, but it is not possible in our situation to determine to what extent the decrease in rate is a direct result of the more random pattern of selection (for this involves more successive choices of widely separated stimuli).

A number of previous investigations have demonstrated that patterns of response by frontal monkeys are abnormal, but all of these required that the animal store information from trial to trial in order to maximize reinforcement<sup>6-8</sup>, and therefore it is difficult to disentangle the possible effects of alterations in memory mechanisms from such response patterns. Our results clearly reinforce the conclusion that frontals are less systematic in making successive responses<sup>9</sup>, even when no specific reinforcement contingency exists, but the suggestion that such increased randomness is due to frontal "hyperreactivity to environmental fluctuations" is not supported by our analysis, which shows that the frontal pattern was the

same whether "breaks" were considered or not. On the other hand, our results are in conflict with the hypothesis that frontals are more prone to stereotypy than control animals<sup>6</sup>.

There seems to be increasing evidence that frontal animals sample stimuli quantitatively and qualitatively differently from control animals. Thus, frontal monkeys have been shown to take briefer "glimpses" in a Butler Box situation<sup>10</sup>. Frontals make more errors, relative to controls, as the number of stimuli is increased in a non-delayed matching from sample situation<sup>11</sup>. Novel stimuli are given priority by frontals<sup>6,7,11</sup>. The characterization of the frontal defect in terms of an alteration in "flexible noticing order" would appear to encompass much the same type of attentional change as we indicate here<sup>7</sup>. The question which remains is whether such an attentional defect could account for the classical delayed response impairment. Some evidence that it could do so is provided by the observation that human delayed alternation performance is significantly impaired directly as a function of the number of irrelevant stimulus inputs during the delay period<sup>12</sup>, as well as the finding that delayed response performance of frontal monkeys is much improved by a drug which appears to interfere with perceptual performance in normal monkeys<sup>13</sup>.

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## Earth Oscillations and the Earth's Interior

by

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Using data on spheroidal and torsional Earth oscillations, and taking account of the revised value of the Earth's moment of inertia, the variation of density and elasticity in the Earth has been re-examined. The most important among several new conclusions are that the radius of the core needs increasing by about 15 km and that a much reduced density gradient is required in the upper mantle.

THIS article reports some preliminary results on an extended revision we have been carrying out on values of the density  $\rho$ , incompressibility  $k$  and rigidity  $\mu$  as functions of depth in the Earth's interior. The revision takes account of the recently revised value<sup>1</sup>, from 0.3335 to 0.3309, of the coefficient  $\gamma$ , where  $\gamma = I/Ma^2$  and  $I$ ,  $M$  and  $a$  are the moment of inertia, mass and radius of the Earth respectively. It also takes account of observations of spheroidal and torsional Earth oscillations and evidence similar to that used in constructing Earth models of the types *A* and *B* (ref. 2).

The work has reached the stage where we can state some new conclusions. One of the conclusions (on the reduced density gradient in the outermost 400 km of the Earth) appears to be roughly in accord with a conclusion of Pekeris<sup>3</sup> whose paper reached us while we were preparing this article. Our approach differs from his, however, in significant respects and has led us to further conclusions not in his paper. (See also Toksöz and Anderson<sup>4</sup>.) Sections of an analysis by Landisman, Satō and Nafe<sup>5</sup> have proved useful in our investigation, though our main conclusions differ considerably from theirs.

We have sought to fit the oscillation data by starting from the Earth Model  $A'$  (ref. 2), corrected to fit the revised value of  $y$ , and have introduced a limited number of adjustable parameters which we have allowed to vary within what we would regard as plausible ranges. We have assumed that the Williamson-Adams equation<sup>2</sup> sets an approximate lower bound to the density gradient below, but not above, 1,000 km depth. Our procedures will be discussed in detail in a later publication.

After trial and error, we confined the parameters to the following three: the radius  $R$  of the core and the densities  $\rho_2$  and  $\rho_3$  just above and just below the boundary (at 413 km depth) between the regions  $B$  and  $C$  of model  $A'$ . In model  $A'$ ,  $R=3,473$  km and  $\rho_2=\rho_3=3.64$  g/cm<sup>3</sup>. Varying  $\rho_2$  meant changing the density gradient (which we kept constant) and so departing from the Williamson-Adams equation, in the region  $B$ . In all other respects, including initially the use of the Jeffreys values<sup>6</sup> of the  $P$  and  $S$  seismic velocities  $\alpha$  and  $\beta$ , we adhered to the procedures originally used in constructing model  $A'$ .

The parameter  $R$  was included for two reasons. First, a preliminary inspection had indicated that increasing  $R$  would act towards bringing the calculated periods for the lower-mode spheroidal observations into line with the observations. Secondly, evidence conveyed to us in 1964 by Sacks<sup>7</sup> added some physical plausibility to the notion of a larger core. Independent seismic evidence which points the same way has been presented by some other authors, for example, Kogan, Carder<sup>8</sup>. After our work had begun, Dorman, Ewing and Alsop<sup>9</sup> published details of an Earth model with  $R > 3,473$  km, but their oscillation calculations assumed the old value of  $y$ . Our procedure also differs from theirs in further respects.

Our selection of  $\rho_2$  and  $\rho_3$  as parameters was in part conventional. Early results from our calculations showed that the mean density in the region  $B$  of model  $A'$  has to be reduced to fit the oscillation data. Reducing the model  $A'$  value, 3.32 g/cm<sup>3</sup>, of the density  $\rho_1$  just below the crust would, however, run counter to current evidence<sup>10,11</sup>. Thus our preference was for taking  $\rho_2$  (or, equivalently, the density gradient in the region  $B$ ) rather than  $\rho_1$  as a parameter. The parameter  $\rho_3$  was introduced to provide further flexibility in the simplest way. We are not suggesting that there is necessarily a sharp change of property near 413 km depth or a first-order discontinuity in density anywhere inside the Earth's upper mantle. Our parametric representation for changes in the upper mantle is taken solely for convenience in a first approximation to corrections to model  $A'$ . It transpires, incidentally, that  $\rho_2=\rho_3$  in one of our better models, details of which are given below.

We have computed oscillation periods  $T$  for a considerable number of models with different  $R$ ,  $\rho_2$  and  $\rho_3$ . So far, we have concentrated mainly on fitting the observed  $T$  for fundamental spheroidal oscillations of order  $n$  up to 24, for fundamental torsional oscillations for  $n$  up to 18, and for a number of overtones. Our best models to date give nearly complete agreement with the observed  $T$  for these oscillations. For spheroidal oscillations with  $n > 16$ , the agreement is significantly better than in the results of Landisman *et al.*<sup>5</sup>, but can be slightly improved for  $n$  between 24 and 48 (the highest  $n$  for which we have computed values of  $T$ ). We expect to improve this agreement in a second approximation where further parametric adjustments will be made in the upper mantle.

A selection of the conclusions which seem to us fairly well established and of special interest to the broad problems of the Earth's interior are now reviewed.

If it is agreed to reject the zero density gradient in the lower mantle of Landisman *et al.*<sup>5</sup> as implausible, then there seems no easy escape from the necessity for a larger core radius. Our calculations favour an increase of 10–15 km. Any smaller increase would seem to us to entail greater departures from the Jeffreys (or Gutenberg)

velocities than seem likely. It seems possible that the oscillation data for the Earth will enable fairly close bounds to be set to the core radius independently of methods used in the past. Our work indicates that it is improbable that  $R$  exceeds (about) 3,495 km. The density gradient for a considerable distance below the crust is appreciably less than would be consistent with an adiabatic temperature gradient. Our present best models give density gradients in the region  $B$  only 0.2–0.3 times that given by the Williamson-Adams<sup>2</sup> equation (compare Pekeris<sup>3</sup>). Whereas Landisman *et al.*<sup>5</sup> reduced the density gradient in the lower mantle, we have reduced it in a part of the Earth where a reduction seems much more plausible.

The essential formal calculations of Landisman *et al.* have incidentally been verified in our work. If the value 3,471 km assumed by them for  $R$  is retained, our calculations indicate that a large reduction in the density gradient in the lower mantle is required. We would stress that our success in fitting the oscillation data, along with the revised  $y$ , comes about as a conjunction of two key features in our results: the increased core radius and the reduced density gradient in the region  $B$ . The reduced density gradient indicated in the upper mantle supplies important evidence on temperature gradients (compare Pekeris<sup>3</sup>).

Contrary to what a number of authors had regarded as well established, we find that the spheroidal data do not favour the Gutenberg velocities<sup>12</sup> in the upper mantle rather than the Jeffreys. When the optimum agreement with the spheroidal oscillations for  $n \leq 24$  is achieved using the Jeffreys velocities, we find, however, discrepancies (a little greater than the standard deviations) with the lower-mode torsional observations, discrepancies which cannot be fully removed by any adjustment in density that we would regard as plausible. The overall observational evidence (spheroidal and torsional) thus supports the conclusion of others that the values of  $\beta$  (but not  $\alpha$ ) are smaller than the Jeffreys values in the region  $B$ . We have yet to ascertain whether our approach will lead to definitive evidence on a "low velocity layer" for  $S$  in the upper mantle. The indicated reductions in  $\beta$ , and thus also  $\mu$ , inside the upper mantle are consistent with our other conclusions. They agree with the reduced density gradient in implying a comparatively high temperature gradient below the crust. They also add some plausibility to the adoption of a larger core radius, the previous estimate of which rests considerably on seismic  $ScS$  observations and so depends on assumed  $S$  velocities in the upper mantle.

The following values of  $\rho$ ,  $k$  and  $\mu$  apply to one of our best models (Table 1). Values in brackets give the changes in density from the original model  $A'$  values.

Table 1

Depth (km)	Density $\rho$ (g/cm <sup>3</sup> )	Incompressibility $k$ (10 <sup>12</sup> dyn/cm <sup>2</sup> )	Rigidity $\mu$ (10 <sup>12</sup> dyn/cm <sup>2</sup> )
33	3.32 (+0.00)	1.14 (−0.02)	0.62 (−0.01)
413	3.42 (−0.22)	1.61 (−0.12)	0.83 (−0.07)
1,000	4.65 (+0.00)	3.53 (−0.01)	1.86 (−0.02)
2,883	5.68 (+0.02)	6.48 (+0.01)	3.01 (+0.01)
2,900	9.80 (+0.1)	6.53 (+0.1)	0.0
4,983	11.90 (+0.0)	13.09 (0.0)	0.0
6,371	12.22 (+0.0)	(a = 11.36 km/sec)	

The density values of this model differ remarkably little from those in the original model  $A'$ , the only sizable changes being in the neighbourhood of the depth 413 km. So far as  $\rho$  is concerned, agreement with the oscillation data and the revised  $y$  is largely achieved by a simple transfer of mass from the upper mantle to the core. It would appear that the corrections needed to the original model  $A'$  are much less drastic than those inferred by Landisman *et al.*, and are largely confined to the upper mantle and the vicinity of the mantle-core boundary.

The above model is not final, and we hope to be able to refine it by taking further account of higher-mode oscillations, as well as of Rayleigh and Love wave observations; but we think that the conclusions listed here will survive our further calculations.

The behaviour of  $k$  at the mantle-core boundary of our model is interesting. Whereas the change  $\Delta k$  in  $k$  at the boundary was negative in the original  $A$ -type models,  $\Delta k$  is now positive, thus agreeing better with the indications of finite-strain theory. The change  $\Delta k$  in the model is also very small (less than 1 per cent), and gives remarkably close agreement with earlier assumptions<sup>13</sup> on compressibility in the Earth's deep interior.

In the second approximation, we propose to take account of additional detail in the upper mantle, evidence relating to the region  $D''$  (ref. 2), and recent work on the lower core<sup>14</sup>.

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## Gravity Shock Waves and Stratification in the Upper Atmosphere

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Rocket measurements have produced direct evidence of small-scale fluctuations of wind speed and ionization, and observations show that these fluctuations are related. The fluctuations in wind speed are accounted for by the propagation of a novel form of shock wave in the atmosphere, and fluctuations of ionization such as sporadic E phenomena are then accounted for by the presence of metallic ions.

It has been known for many years that in middle latitudes the electron density does not in general vary smoothly with height between 90 and 125 km. Profiles of electron density measured with the help of electrostatic probes<sup>1</sup> and radio transmitters<sup>2,3</sup> mounted on rockets show narrow steep-sided peaks and sharp ledges. The vertical logarithmic gradient of electron density ( $d \log N/dz$ ) at the edge of a thin overdense layer sometimes exceeds  $100 \text{ m}^{-1}$ . Simple layers are typically about 1 km thick; thicker layers usually have two peaks. The enhancement of electron density in a thin layer relative to the background varies from a few per cent to an increase of three-fold or more during the day and ten or more at night. The most dense layers and those with the steepest gradients give rise to various sporadic E phenomena at very high frequency, such as long distance propagation of low-band television.

A recent study of winds in the upper atmosphere at latitude  $38^\circ \text{ N}$ . (Bedinger, J. F., Knaflitz, H., Manring, E., and Layzer, D., to be published) has shown that between 85 and 135 km the horizontal wind profile is invariably characterized by a well defined small scale structure. A typical hodograph of the horizontal wind in this height range is shown in Fig. 1. It consists of smooth arcs joined at sharp corners (which mark narrow layers, often less than 100 m in width) where the vertical gradient of the horizontal wind—but not the horizontal wind itself—changes abruptly.

On several occasions during the past 4 years horizontal winds and electron densities have been measured along similar rocket trajectories at nearly the same time<sup>4,5</sup>. An examination of a few early records suggested that narrow ionization peaks usually coincided with corners of the hodograph of horizontal winds<sup>6</sup>. This conclusion was confirmed by a subsequent study of a larger body of data

which included measurements of horizontal winds and ionization density along the same rocket trajectory<sup>4</sup>. The present communication suggests a physical interpretation

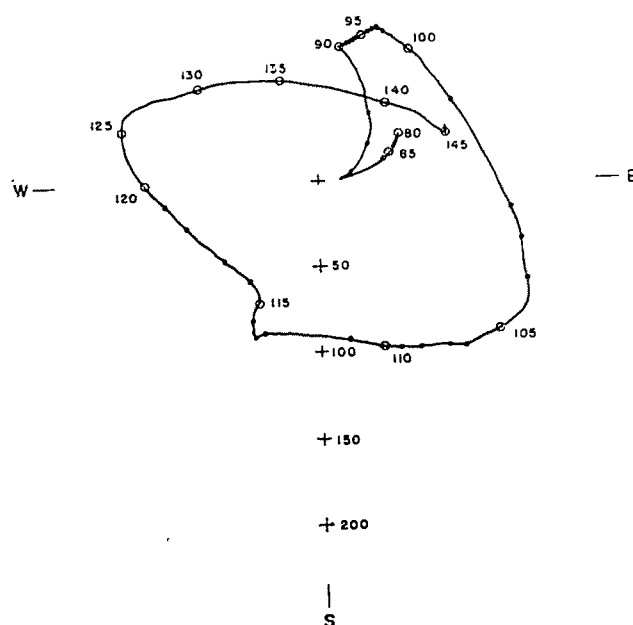


Fig. 1. Hodograph of the horizontal wind between 80 and 145 km, derived from multi-station photography of a sodium vapour trail on September 16, 1961. The horizontal wind is represented by a vector drawn from the centre of the figure (marked by a +). Between 80 and 120 km, 1-km height intervals are indicated; above 120 km, 5-km height intervals. The velocity scale, in m/sec, is indicated along the north-south axis.

of the small scale structure of the horizontal wind profile which accounts qualitatively for the accompanying small scale structure of the ionization profile.

To a first approximation, we may regard the narrow layers represented by corners in the hodograph of horizontal winds as surfaces of discontinuity. Such surfaces are of two types: those at which one or more components of the velocity are discontinuous (strong discontinuities); and those at which the velocity itself is continuous but one of its derivatives is discontinuous (weak or gradient discontinuities). Unlike strong discontinuities, weak discontinuities cannot arise spontaneously, but must be produced by some kind of singularity in the initial or boundary conditions of the flow. Because the surfaces under consideration do not appear to be associated with singularities in the boundary or initial conditions, they are presumably strong discontinuities—that is, shocks or contact discontinuities. The observation that the horizontal component of the velocity is continuous rules out contact discontinuities, which, moreover, are known to be unstable. Thus by elimination we arrived at the conclusion that the surfaces must be shock fronts, that is, surfaces at which the normal component of the velocity changes discontinuously.

The shocks cannot be ordinary compression shocks, however, as: (a) they move much too slowly; and (b) the observational evidence indicates that the winds in this region can only be interpreted as highly non-linear oscillations in the gravitational (rather than the compressional) mode. The gravitational mode is characterized by predominantly horizontal winds, small pressure fluctuations, and opposite vertical directions of phase and energy propagation. The surfaces of discontinuity under discussion are presumably related to gravity waves in the same way that compression shocks are related to compression (acoustic) waves. Observational evidence suggests that wave energy propagating upward is partially reflected at these surfaces.

A proper theory of gravity shocks (as they will be called here) would describe the growth of a surface of discontinuity in an initially linear gravity wave as it propagates upward. Such a theory has not yet been given. We shall adopt the working hypothesis that a discontinuity does develop. From the laws of conservation of mass, momentum and energy we can then derive a simple generalization of the Rankine-Hugoniot relations that describes gravity shocks as well as compression shocks.

Let  $\tilde{p}$  denote the dynamic contribution to the pressure

$$\tilde{p} = p - \int_z^\infty g \rho dz \quad (1)$$

where  $g$  denotes the acceleration of gravity and  $\rho$  the density. For simplicity we use the relation  $p = (\gamma - 1)\rho\varepsilon$ , valid for a perfect gas, where  $\gamma$  is the ratio of specific heats and  $\varepsilon$  is the density of the internal energy. We further neglect the accession of heat by gas moving through the front, and assume that the front is stationary with respect to the surface of the Earth. Finally, we assume that the thickness of the front,  $h$ , is sufficiently small that time derivatives (including the Coriolis contribution) can be neglected in comparison with vertical gradients. Then integration of the conservation relations across the front yields the three jump conditions

$$[\rho u_z]_1^2 = 0 \quad (2)$$

$$[\rho u_z^2 + \tilde{p}]_1^2 = 0 \quad (3)$$

$$\left[ \frac{1}{2} u^2 + \frac{\gamma}{\gamma - 1} \frac{p}{\rho} + gz \right]_1^2 = 0 \quad (4)$$

where  $u^2 = u_x^2 + u_y^2 + u_z^2$  and the indices 1 and 2

designate levels immediately below and above the front. From these equations and the continuity of the horizontal velocity components (which makes it possible to replace  $u^2$  by  $u_z^2$  in equation (4)), we can derive the formulae

$$\frac{u_{iz}^2}{c_i^2} = \frac{1 \mp \frac{gh\bar{\rho}}{p_i} \pm \frac{\gamma+1}{2\gamma} \frac{\Delta\tilde{p}}{p_i}}{1 + \gamma\eta \left( 1 - \frac{\gamma-1}{\gamma} \frac{\rho_i}{\bar{\rho}} \right)} \quad (5)$$

where the upper sign is to be used with  $i = 1$ , the lower sign with  $i = 2$ . In these equations

$$\Delta\tilde{p} \equiv \tilde{p}_2 - \tilde{p}_1, \int_{z_1}^{z_2} \rho dz \equiv \bar{\rho}h, c_i^2 \equiv \frac{\gamma p_i}{\bar{\rho}},$$

$$\eta \equiv -gh\bar{\rho}/\Delta\tilde{p} \quad (6)$$

The dimensionless number  $\eta$  is the ratio between the static and the dynamic contributions to the pressure jump. When  $|\eta| \ll 1$ , equation (5) describes a compression shock the structure of which is slightly modified by the effects of buoyancy. If  $|\eta| \gg 1$ , it follows from equation (5) that  $\eta$  must be positive, and hence  $\Delta\tilde{p}$  must be negative. If  $\eta \gg 1$  the equations describe a gravity shock. In this case they reduce to

$$\frac{u_{iz}^2}{c_i^2} \simeq \eta^{-1} \frac{1 \mp gh\bar{\rho}/p_i}{\gamma - (\gamma-1)\rho_i/\bar{\rho}} + O(\eta^{-2}) \quad (7)$$

Thus  $|u_z| \ll c$ , in agreement with observation. Measurements of vertical winds suggest that  $1 \lesssim |u_z| \lesssim 3 \text{ msec}^{-1}$ , so that  $10^4 \lesssim \eta \lesssim 10^5$ .

It follows from equation (2) that  $u_{1z}$  and  $u_{2z}$  have the same sign, and from equation (3) and from the inequality  $\Delta\tilde{p} < 0$  that  $|u_{2z}| > |u_{1z}|$ . Thus from equation (1)  $\rho_2 < \rho_1$ . From equations (2), (3) and (7) we obtain the relations

$$\frac{\Delta u_z}{u_z} = -\frac{\Delta\tilde{p}}{p_2} = \frac{gh\left(\bar{\rho} - \frac{\gamma-1}{\gamma}\rho_1\right)}{p_2} \equiv \frac{h}{H_2} \quad (8)$$

where  $H_2$  is a length closely comparable to the scale height. For a layer 1 km thick,  $\Delta\rho/\rho \simeq 0.1$ .

Next we consider the ionization profile. The equation of continuity for the electrons in a frame of reference which moves with the surface of discontinuity is

$$\frac{\partial N}{\partial t} + \frac{\partial(wN)}{\partial z} = q - r + \nabla \cdot (D\nabla N) \quad (9)$$

where  $N$  is the electron density,  $w$  is the vertical drift speed of neutral ionization,  $q$  is the rate of electron production,  $r$  is the rate of electron loss, and  $D$  is the electron diffusion coefficient. In middle latitudes and in the height range 90–125 km,  $w$  is approximately given by<sup>8-10</sup>

$$w = a_x(u_x - u_{0x}) + a_y(u_y - u_{0y}) + a_z u_z \quad (10)$$

where

$$u_{0x} = -E_y^{\text{pol}}/B_z, u_{0y} = E_x^{\text{pol}}/B_z \quad (11)$$

If the ionized component consists of electrons and positive ions,

$$a_x = -\frac{r^2}{r^2 + 1} \cos \chi \sin \chi, a_y = -\frac{r}{r^2 + 1} \cos \chi \quad (12)$$

Here  $E^{\text{pol}}$  denotes the electric polarization field, a slowly varying but in general unknown function of height;  $B_z$



denotes the vertical component of the Earth's magnetic field,  $\chi$  denotes the magnetic dip angle, and  $r$  denotes the ratio  $\omega_i/v_i$  between the gyro-magnetic and collision frequencies of the ions. The ratio  $r$  varies with height approximately like  $\rho^{-1}$ . At 100 km,  $r \simeq 1/10$ , while at 120 km  $r \simeq 1$ . Because in middle latitudes  $\sin \chi \simeq 1$ ,  $a_x$  is an order of magnitude smaller than  $a_y$  at 100 km but is comparable with  $a_y$  at 120 km.

In a layer where  $u_z$  and  $\rho$  change abruptly,  $w$  also changes abruptly. For appropriate values of the effective horizontal velocity components  $(u_x - u_{0x})$  and  $(u_y - u_{0y})$ , it can happen that  $w_1 > 0$  and  $w_2 < 0$ , so that ionization is drifting into the layer both from above and from below. In these circumstances, a dense layer can be built up. In less favourable circumstances,  $w_1$  and  $w_2$  have the same sign, so that only a small change in the ionization profile can occur. Thus the present theory predicts that conspicuous peaks in the ionization profile will coincide with some, but not all, layers marked by corners of the horizontal wind hodograph. It also predicts that the width of a simple (that is, single-peaked) ionization layer should not depend strongly on the density of the layer. Both predictions agree with experiment. The occasional occurrence of ionization peaks at levels that do not coincide with corners of the hodographs of the horizontal wind (three cases out of seventeen in the material studied in ref. 5) could be explained in either of two ways: (a) the vertical gradient of the horizontal wind could conceivably undergo a small jump at a surface where the vertical wind undergoes a large jump; (b) dense layers of ionization may be expected to persist for a certain period after the wind configurations that gave rise to them have disappeared.

On many of the hodographs of horizontal wind there occur pairs of sharp corners with vertical separations of only 2 or 3 km. If ionization is drifting into the upper layer of such a pair from above and into the lower layer from below, a double-peaked feature will result. If  $w \simeq 0$  at the two inner surfaces, the two peaks will be well separated; otherwise the region between them will be more or less filled in. Because the vertical drift velocity can be written in the form  $w = A\rho^{-1} + B\rho^{-2}$ , where  $A$ ,  $B$  are slowly varying functions of height, and as  $\Delta\rho < 0$  in each layer, a pair of closely spaced layers always constitutes a more effective ion trap than a single layer. The observational evidence does, in fact, indicate that thick layers usually have a well defined double peaked structure. Occasionally they resolve into a pair of distinct layers with sharp inner edges.

If the net flux of ionization into a layer is positive, ionization will accumulate in it until the inward flux is balanced by recombination losses. In the region of the atmosphere under consideration the principal ionized constituents are probably oxygen and nitric oxide. During the day the recombination times of these ions are of the order of  $10^3$  sec. Such times are too short to allow substantial local increases in the electron density to occur because  $\Delta w$  is of the order of 1 m/sec at most. Moreover, the fact that dense daytime layers of ionization are not associated with a marked reduction of the background electron density indicates that redistribution of the principal ionized constituents is not the process by which dense layers are formed<sup>5</sup>. These considerations suggest that the ions responsible for narrow peaks in the ionization profile make a relatively minor contribution to the total number of ions in a column of unit cross-section—at least during the day—but have long lifetimes. Atomic metallic ions, such as sodium, magnesium and iron, the lifetimes of which are measured in days, satisfy these conditions. Because sharp corners in the hodographs of horizontal wind appear to persist for many hours, the long-lived metallic ions may be expected to collect in a few thin layers. Only the densest of these would be conspicuous during the day, because of the high background density of ionization. But at night, with the gradual subsidence

of the background associated with short-lived molecular ions, the underlying spiny structure of thin layers of metallic ions would gradually emerge. This picture is in qualitative agreement with observation. Independent evidence in support of the view that dense layers are made up largely of atomic metallic ions comes from recent mass-spectrometer measurements (ref. 11, and Johnson, C. Y., personal communication).

A conspicuous feature of sporadic  $E$  layers is their patchy structure. A typical sporadic  $E$  layer is a few hundred kilometres in diameter, but its vertical critical frequency  $fE_s$  (the maximum frequency observed to be reflected at vertical incidence) varies considerably over the surface. The horizontal scale of the variation is a few tens of kilometres. The present hypothesis for the formation of dense layers does not lead one to expect substantial local variations of the total number of electrons in a dense layer. On the other hand, the vertical gradient of electron density at the lower edge of a layer may be expected to exhibit large local fluctuations, because it is an extremely sensitive function of the drift speed  $w_1$  when  $w_1$  is close to 0 (in the absence of diffusion,  $dN/dz \rightarrow \infty$  as  $w_1 \rightarrow 0$ ). Because the radio wavelengths in question are comparable with observed values of  $(d \log N/dz)^{-1}$  at the edges of dense layers, small local variations in the winds could give rise to substantial changes in reflectivity at these wavelengths.

The present hypothesis for the formation of narrow, dense layers of ionization may be compared with the wind-shear theory<sup>12-14</sup>, which is also based on equations (9) and (10). In the wind-shear theory  $a_x$ ,  $a_y$  and  $u_z$  in equation (10) are regarded as slowly varying functions of height, and the formation of narrow, dense layers of ionization is attributed to large vertical gradients of the effective components of horizontal wind  $(u_x - u_{0x})$  and  $(u_y - u_{0y})$ . Calculations based on observed profiles of horizontal wind, however, indicate that vertical gradients of the horizontal wind are responsible for comparatively broad and shallow features of the ionization profile, and not—except, perhaps, in isolated instances—for narrow layers of dense ionization<sup>5</sup>.

The possible importance of long-lived ions was pointed out, in the context of the wind-shear theory, by Whitehead<sup>15</sup> and Axford<sup>16</sup> in reply to a criticism of the original, single-ion form of this theory<sup>5</sup>, and also by Cuchet<sup>17</sup>. The possibility that sporadic  $E$  layers might be made up of meteoric ions was explicitly discussed at a seminar on sporadic  $E$  held at Estes Park, Colorado, in the summer of 1965.

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## LETTERS TO THE EDITOR

## ASTRONOMY

Hydrogen Recombination Lines 126 $\alpha$  and 166 $\alpha$  observed in Galactic HII Regions

Using the Parkes radiotelescope, we have searched the Southern Milky Way for the 126 $\alpha$  hydrogen recombination line of rest frequency 3248.713 Mc/s. (The notation  $n\alpha$  indicates a transition from the level of quantum number  $(n+1)$  to level  $n$ ;  $n\beta$  indicates a transition from  $(n+2)$  to  $n$ .) Nineteen sources were inspected and the line was detected in fifteen, all of which were well known HII regions. The nebulae<sup>1</sup> were

Orion Nebula	RCW 57	NGC 6334
IC 434	RCW 74	NGC 6357
RCW 38	1808-51	M 17
$\eta$ Carinae (two positions)	1617-50	W 49
	1618-49	W 51

Four neighbouring points were checked in the Orion Nebula and M 17 to establish that the maximum line intensity corresponded to the maximum continuum temperature.

A probable detection was made in the galactic centre source at  $l_{II} = 40'$ ,  $b_{II} = -2'$ . No detections were made in the two non-thermal sources, Sagittarius A and the Crab Nebula. The extra-galactic source, 30 Doradus in the Large Magellanic Cloud, gave a negative result, possibly because the sensitivity was not great enough.

The three most intense HII regions, Orion Nebula, M 17 and RCW 38, were later observed for the 166 $\alpha$  line, with a rest frequency 1424.736 Mc/s.

Table 1 shows details relating to the three sources of: (i)  $T_L$ , the peak line intensity in terms of aerial temperature; (ii) the ratio  $T_L/T_C$ , where  $T_C$  is the corresponding aerial temperature in the continuum; (iii)  $\Delta\nu$ , the half-intensity width of the line, corrected for broadening with 37 kc/s filters; and (iv) the central line radial velocities (referred to the local standard of rest).

Table 1. SUMMARY OF OBSERVATIONS OF HYDROGEN RECOMBINATION LINES 126 $\alpha$ , 166 $\alpha$

Source	Line	$T_L$ (° K)	$T_L/T_C$ (per cent)	$\Delta\nu$ (kc/s)	Radial velocity (km/s)
Orion Nebula	126 $\alpha$	$6.4 \pm 0.1$	3.5	$430 \pm 10$	-2
	166 $\alpha$	$1.9 \pm 0.1$	0.8	$215 \pm 10$	0
M 17	126 $\alpha$	$5.7 \pm 0.1$	3.1	$388 \pm 10$	+20
	166 $\alpha$	$3.0 \pm 0.1$	1.05	$203 \pm 20$	+20
RCW 38	126 $\alpha$	$2.4 \pm 0.1$	2.5	$398 \pm 15$	+4
	166 $\alpha$	$1.2 \pm 0.1$	1.05	$156 \pm 20$	+8

Palmer and Zuckerman<sup>2</sup> have detected the 166 $\alpha$  line in only M 17. Their values of  $T_L/T_C$  and  $\Delta\nu$  are  $0.9 \pm 0.2$  per cent and  $183 \pm 60$  kc/s when corrections are made for their 80 kc/s bandwidth.

Recently, we have considered<sup>3</sup> the ratio of the peak intensities of the 126 $\alpha$  line to the  $\beta$  lines, 159 $\beta$  and 158 $\beta$ , at nearby frequencies, 3211.245 and 3272.219 Mc/s, respectively. In the source M 17, the ratio  $I(158\beta)/I(126\alpha)$  of  $0.22 \pm 0.04$  was close to the theoretical value 0.224 derived from formulae set down by Kardashev<sup>4</sup>. In the Orion Nebula, however, the ratio was considerably lower:  $0.13 \pm 0.03$ . Without observations at lower frequency to give bandwidths near 158 $\alpha$  it was impossible to infer whether this resulted from departures from local thermodynamic equilibrium of the populations or to appreciable broadening of the energy levels involved in the 158 $\beta$ , 159 $\beta$  transitions (and the corresponding  $\alpha$  transitions) in the Orion Nebula. As the Bohr radius is proportional to the square of the principal quantum number, the 158, 159 levels will be more affected by electron and ion densities than levels near 126. It is well

known that the density is considerably higher in Orion than in M 17.

In Fig. 1,  $T_L/T_C$  and  $\Delta\nu$  have been plotted against frequency over the range of the present observations so that values for the 158 $\alpha$  line may be obtained by interpolation. The results for the 126 and 158  $\alpha$ -lines for the Orion Nebula may be compared with those for M 17. The 126 and 158  $\alpha$ -lines have been observed under similar conditions except for the increase in beamwidth from 6' arc to 14' arc.  $I(158\alpha)/I(126\alpha)$  is 0.31 for the Orion Nebula and 0.43 for M 17; that is, the Orion value is 0.73 of the M 17 value. The ratio  $I(158\beta)/I(126\alpha)$  for the two sources was 0.59. The small difference could be accommodated within the experimental errors.

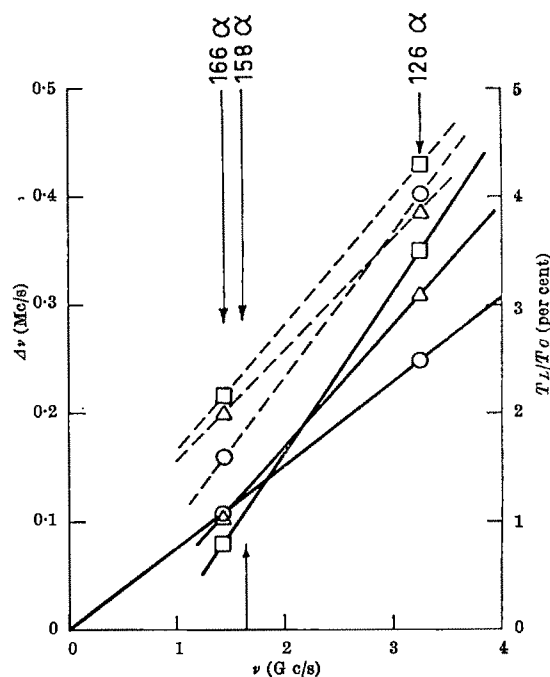


Fig. 1. Recombination line half-width,  $\Delta\nu$  (broken lines), and the ratio of line intensity to continuum intensity,  $T_L/T_C$  (full lines), are plotted against frequency (in G c/s) over the range of the 166 $\alpha$  and 126 $\alpha$  lines for the HII nebulae.  $\square$ , Orion Nebula;  $\Delta$ , M 17;  $\circ$ , RCW 38.

On the other hand, the corresponding ratio of  $\Delta\nu$  for the two lines is effectively the same (0.96) for both sources, and thus broadening is inadequate to account for the intensity ratios.

We infer from the results that the populations at least for Orion are not in agreement with a Boltzmann distribution. The likelihood of such a situation has been discussed by Goldberg<sup>5</sup>.

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### Variation of the Positional Angle of the Polarization Plane of Radiosources with Time

THE expansion of the volume in which relativistic particles, magnetic fields and ionized gases are contained must be accompanied by both a decrease of the flux density of the synchrotron radio-emission<sup>1</sup> and a change of its polarization parameters. If the radio-emission of such a source is linearly polarized and if the plane of polarization undergoes Faraday rotation  $\varphi_s$  within the source itself and Faraday rotation  $\varphi_g$  in the galactic interstellar medium<sup>2-4</sup>, then with a sufficiently rapid expansion of this source, the rate of change of the positional angle will be

$$\dot{\phi} = \dot{\varphi}_s + \dot{\varphi}_g \simeq 8.1 \times 10^5 \lambda^2 \frac{d}{dt} \int_0^R n_e H_r dr \quad (1)$$

But  $\varphi_s \gg \varphi_g$  in equation (1) so that the rotation at a distance of one radius of the source is assumed to be the characteristic Faraday rotation with the source. In the simplest model, the source will be transparent at wavelength  $\lambda$  and the non-uniform magnetic field will consist of quasi-uniform regions differing only in the direction of the field and growing with the source without change in relative size. Then if  $n_e \propto R^{-3}$  and  $H \propto R^{-2}$ , we obtain

$$\varphi_s = 8.1 \times 10^5 \lambda^2 \left(\frac{R_0}{R}\right)^6 \int_0^R (n_e H_r)_0 dr = \varphi_0 \left(\frac{R_0}{R}\right)^4; \quad (2a)$$

$$\frac{\dot{\phi}}{\varphi_s} = -4 \frac{\dot{R}}{R} \quad (2b)$$

If  $\dot{\phi}$ ,  $\varphi$  and  $\dot{R}/R$  are measured (the last by optical means), the rotation in the source and in the Galaxy can be individually determined. The equalities (2) can be considered only to be approximate, because they do not take into account differences in the physical parameters of the elements, their relative movements and the expansion anisotropy.

Let us compare (2b) with the rate of secular decrease of the flux density during the expansion. According to Shklovsky<sup>1</sup>  $\dot{S}/S = -2\gamma \dot{R}/R$ , where  $S \propto \lambda^\alpha$ ,  $\gamma = 2\alpha + 1$ . Here the absence of the injection of relativistic particles is assumed. Substituting from (2b), this can be written

$$\frac{\dot{\phi}/\varphi_s}{\dot{S}/S} = \frac{2}{\gamma} \quad (3)$$

The accuracy of (3) is limited by the previously mentioned assumptions except for the expansion anisotropy.

The approximations of  $\dot{\phi}$  from equation (3) indicate that this effect can probably be discovered in the course of several years. For Cassiopeia A,  $\dot{S}/S \simeq -1.1$  per cent/yr<sup>5-7</sup> at  $\alpha = 0.8$  (ref. 8). Boland *et al.*<sup>9</sup> have measured a small linear polarization of Cassiopeia A at a wavelength of 2.07 cm. Thus at the wavelength at which  $\varphi_s = 1$  rad it is probable that the variation of the positional angle will be  $\sim 0.5^\circ$ /yr. Contrary to the flux density, measurements made on the Earth's surfaces of the permanent positional angle are not subjected to atmospheric extinction at centimetre wavelengths. Because, for the Crab Nebula,  $\dot{R}/R = 10^{-3}$ /yr,  $\varphi \simeq 1$  rad at a wavelength of 21 cm<sup>10</sup> and  $\alpha = 0.25$  (ref. 8) we find that  $\dot{\phi} 0.25^\circ$ /yr and  $\dot{S}/S \leq 0.3$  per cent/yr. Such an estimate does not take into account the extent of the Faraday rotation or the expansion anisotropy of the Galaxy. Because of the presence of the regular magnetic field and the possible injection of the relativistic particles in the Crab Nebula the value for  $\dot{\phi}$  seems to be more reliable than that for  $\dot{S}/S$ .

Of particular interest are measurements of  $\dot{\phi}$  for radio-variable quasars. Given these values it would be possible to establish whether the linearly polarized radio-emission comes from the variable central nucleus of the quasar and, if so, to draw some conclusions about the mechanism involved in the generation of variable radio-emission.

If the mechanism were to be of the synchrotron type and if the radio variability were to be caused by the expansion of the region in which the radio-emission is generated (refs. 11-13), then in some cases the effect would be sufficiently large to be observed over a period of years. For example, if the Faraday rotation of the Galaxy, the opacity of the sources due to the synchrotron reabsorption and relativistic effects resulting from very fast expansion are not taken into account, the decrease of the flux density of 3C 345 during 1964-65 for 20 per cent/yr at a wavelength of 10.6 cm<sup>14</sup> corresponds to  $\leq 5^\circ$ /yr. Here I have taken values for the rotation of about 20 rad/m<sup>2</sup> (ref. 15) and  $\alpha = 0$ . Similarly, values for 3C 279 are  $\sim 28$  rads/m<sup>2</sup> for rotation (ref. 15) and  $\alpha = 0$  (ref. 16). Thus the increase of flux density for 6 per cent/yr during 1963-65 at a wavelength of 21.2 cm could be accompanied by the change of the positional angle  $\dot{\phi} \leq 9^\circ$ /yr.

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## PLANETARY SCIENCE

### Equal Areas of Gondwana and Laurasia

IN a recent communication, Dietz and Sproll<sup>1</sup> have taken the contact between Gondwana and Laurasia as a line along the axis of the Persian Gulf continued through Iraq and Syria to meet the axis of the Mediterranean Sea. Geological evidence does not support this generalization.

Stoeklin (ref. 2, and in a paper presented at the International Colloquium on the Tectonics of the Alpine folded regions of Europe and Asia Minor, held in 1965 at Tiflis, U.S.S.R.) gave reasons for thinking that Arabia and Iran throughout the Palaeozoic era formed part of one stable platform created by Pre-Cambrian orogenies. In the Middle East it is not possible to identify margins for Gondwana and Laurasia in Palaeozoic times. Throughout the Mesozoic and Tertiary eras, however, the well marked Zagros thrust belt, which continues north-west into northern Iraq and Syria, is the geological choice for the northern margin of the Arabian shield. This is a mobile belt of eugeosynclinal type which has suffered two or more orogenies since Jurassic times. The rocks which outcrop in it are different in lithological type from those deposited in the Persian Gulf geosyncline, which subsided gradually during Mesozoic and Lower Tertiary times accumulating some 20,000 ft. of dominantly calcareous sediments which are essentially miogeosynclinal, folded by the Pliocene orogeny, which was most intense in the Zagros thrust belt. It is therefore necessary to place the contact between the shields at least as far north as



the inner Zagros thrust belt; the interpretation farther north-east is still geologically obscure.

Dietz and Sproll do not say where they have taken the contact of Gondwana and Laurasia farther east between the Persian Gulf and West Pakistan. There is clearly insufficient evidence to identify a northern margin to Gondwana in this area now, because it has either foundered beneath the sea or suffered a Cary spenochasm. But in Upper Cretaceous times we do know that the Zagros eugeosynclinal belt bent around into Oman to run south and south-east towards the Arabian Sea. It is logical to assume on geological grounds that this orogenic belt was connected in some way with the belt of Upper Cretaceous folding which runs north from Karachi: that this belt connects under the Arabian Sea with the Murray Ridge is unlikely to be pure coincidence.

The necessary change in the position of the Middle Eastern Gondwana and Laurasia contact adds about 700,000 km<sup>2</sup> to the former supercontinent at the expense of the latter, confirming that the apparent close similarity of the areas of the two supercontinents measured by Dietz and Sproll must be fortuitous, as they acknowledge.

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### Particles in Volcanic Fume

THE relatively quiet outpouring of lava during many volcanic eruptions, such as those in Hawaii and Iceland, is accompanied by the emission of large quantities of fume. Almost nothing is known about the particles in such fume although numerous studies have been made of the gaseous composition<sup>1,2</sup>. At times in the Earth's history, for example during the eruptions that produced the Columbia Plateau in the United States, tremendous amounts of such particles must have been emitted into the atmosphere.

During 1965, Halemaumau (the main vent of Kilauea volcano in Hawaii) emitted large amounts of fume (Fig. 1). This is unusual for Kilauea except during an actual eruption and suggests that magma was very close to the surface. The odour of the fume was similar to that of sulphur dioxide. Scattered light from the fume in the crater was bluish, but as the fume rose it became white, suggesting that the particles were absorbing water vapour and enlarging as they rose.

Samples of the particles in this fume were collected in August, September and December of 1965. Both a 'Unico' multistage impactor and a filter composed of polystyrene fibres less than a micron in diameter were used. Fume was drawn through the impactor and filter with a 'Gelman' battery driven pump or a 'Uni-Jet' air sampler. Particles collected at the crater's edge with the impactor were entirely on the third and fourth stages, which at the flow-rates used collected particles which had mass median diameters of about 5 $\mu$  and 3 $\mu$  respectively, assuming that the particles had a density of 1 g/cm<sup>3</sup>. The particles were undoubtedly much smaller when first



Fig. 1. The appearance of Halemaumau in September 1965. The fuming crater is about one half mile across.

emitted from the vents. Almost no particles were collected on the impactor slides away from the crater.

Microscope examination of the impactor samples showed that they were agglomerated droplets that remained liquid at a relative humidity of 30 per cent, and consisted of supersaturated aqueous solutions of a crystalline solid. Tests with Nessler's reagent and with an aqueous solution of barium chloride showed that the solute contained ammonium and sulphate ions, respectively. The crystalline solute was tinged yellow but probably did not contain elemental sulphur since it was soluble in water and insoluble in carbon disulphide. Possibly some ferric ions were present. Testing with acidified aqueous silver nitrate solution failed to reveal the presence of any chlorine ions. The droplets absorbed water and grew in size when the impactor slide was placed on a moist chamber on the microscope stage.

Using the electron diffraction feature of an electron microscope, electron diffraction patterns were obtained for about thirty crystals of the solute from the impactor slides. All corresponded to ammonium sulphate; no sodium chloride or sulphur particles were found.

Particles collected on the fibre filter were examined with the electron microscope using a replica technique. Many of the particles (which, of course, were evaporated droplets) were in the size range 1–10 $\mu$  diameter. There were also large numbers of smaller particles.

Ammonia has not previously been reported in the fume from Kilauea although it is known to be emitted by other volcanoes<sup>3,4</sup>. Although these samples were handled with great care, the possibility that they were contaminated with atmospheric ammonia cannot be dismissed. Additional investigations of this kind, especially of the fume above molten lava, are needed to establish whether ammonium sulphate is indeed an important component of the fume of erupting volcanoes. If so, enormous amounts of ammonium sulphate must at times be emitted into the atmosphere by volcanic eruptions.

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**Physical and Chemical Properties of Volcanic Glass Shards from Pozzuolana Ash, Thera Island, and from Upper and Lower Ash Layers in Eastern Mediterranean Deep Sea Sediments**

Two volcanic ash layers have been correlated in deep sea piston cores from the eastern Mediterranean<sup>1,2</sup>. The lower ash layer ( $n = 1.521$ ) occurs between late Würm carbonate sediment and originated in an eruption more than 25,000 years ago. The upper ash layer ( $n = 1.509$ ) occurs in postglacial carbonate sediment less than 5,000 yr old. Patterns of distribution indicate that both beds of volcanic ash originated in eruptions of Santorini (Aegean Sea).

During the Würm eruption of Santorini (Fig. 1), the bottom of much of the Eastern Mediterranean was covered by a layer of ash a few centimetres thick<sup>2</sup>. The lower ash ( $n = 1.521$ ) is spread out in an elongate pattern extending to the west and to the south-east. A further big eruption occurred during postglacial time (late Minoan I). It formed the present caldera of Santorini (83 km<sup>2</sup> in area) and deposited the pozzuolana ash which is 30 m thick. The ash covers the islands of Thera, Therasia and Aspronisi, which are remnants of the former volcanic cone. The upper ash layer ( $n = 1.509$ ) in deep sea sediments is distributed between the Aegean Sea and Egypt, decreasing in thickness toward the south-east<sup>2</sup>.

In the present study the physical and chemical properties of pozzuolana from Thera island and the upper and lower ash from deep sea sediments have been examined. This investigation indicates that the upper ash ( $n = 1.509$ ) from deep sea sediments and the pozzuolana from Thera island are identical in composition.

The sample of pozzuolana analysed was obtained to the south of Phira on Thera island and the samples of

upper and lower ash in deep sea sediments chemically analysed are from core V10-58 (Figs. 1 and 2)

The pozzuolana from Thera<sup>3</sup> and the lower and upper ash from deep sea sediments<sup>1,2</sup> are composed of about 97 per cent volcanic glass and 3 per cent minerals. The sample of pozzuolana examined in this study, however, was contaminated with a large amount of residual sand. The three samples were first boiled in a 20 per cent solution of hydrogen peroxide and sieved. The fraction in the range of size 0.177–0.062 mm was further examined. A solution of bromoform and alcohol (specific gravity = 2.65) was used for the separation of volcanic glass shards from heavy minerals. The light fraction was treated with a 15 per cent solution of hydrochloric acid to eliminate the possible presence of calcium carbonate or iron oxide, and was then passed through the Franz isodynamic separator. Maximum current obtained by the instrument was 1.5 amps. The remaining portion of heavy minerals as well as the glass shards which contained inclusions of minerals was separated at less than 0.8 amps. Plagioclase and quartz were separated at more than 1.4 amps. Thus, practically the total amount of pure volcanic glass shards from the original samples in the size fraction 0.177–0.062 mm was concentrated between about 0.8 and 1.4 amps. The magnetic properties of the volcanic glass in the three samples are shown in Fig. 3, and the pozzuolana and the upper ash (core V10-58) are nearly identical. There is a similarity between these two ash beds with regard to other physical properties (Table 1).

Mellis<sup>1</sup> pointed out that in all the *Albatross* cores in which the ash layers were found, the lower layer ( $n = 1.52$ ) was brownish grey and the upper layer ( $n = 1.51$ ) was light grey. Parker<sup>4</sup>, however, has constructed climatic curves based on the percentage of warm and cold foraminiferids for each *Albatross* core. She found that the

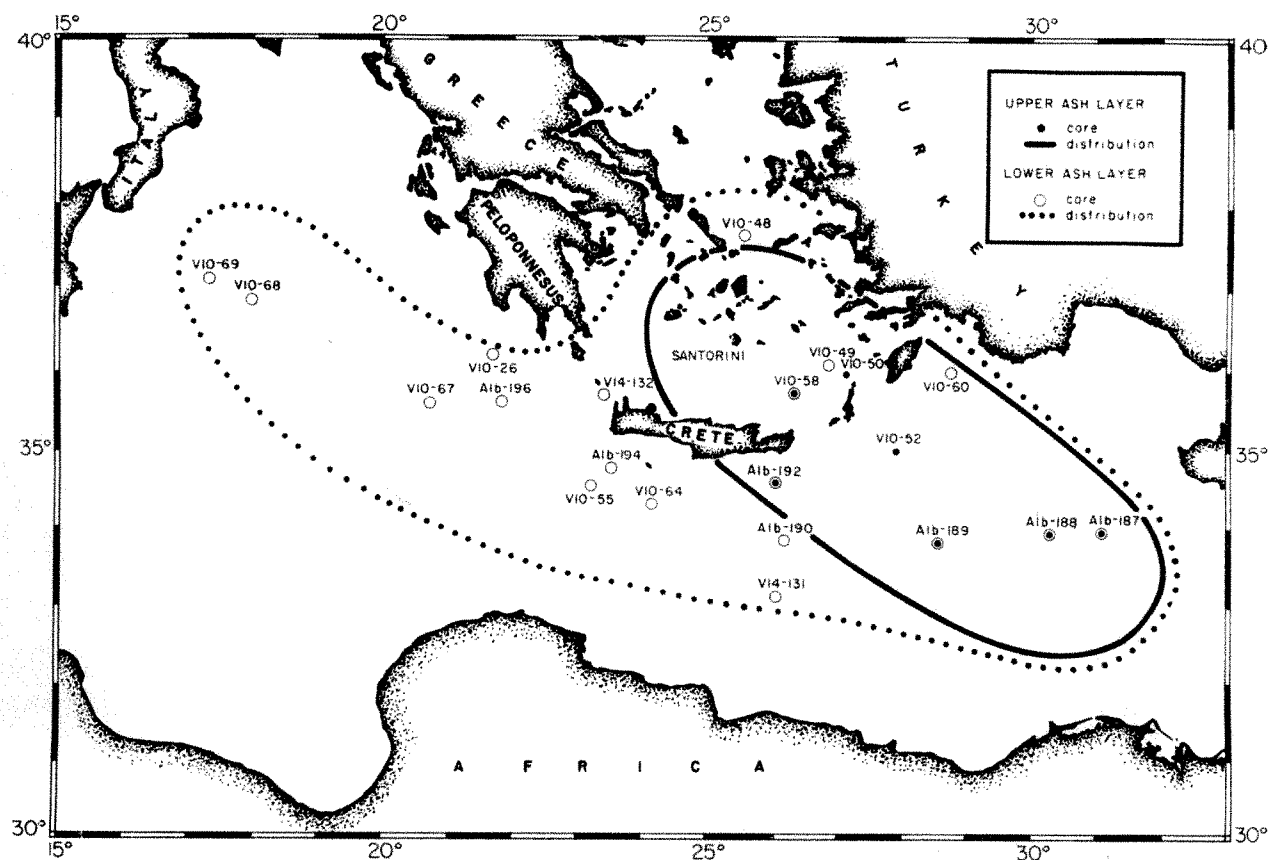


Fig. 1. Distribution of volcanic ash in eastern Mediterranean deep sea cores.



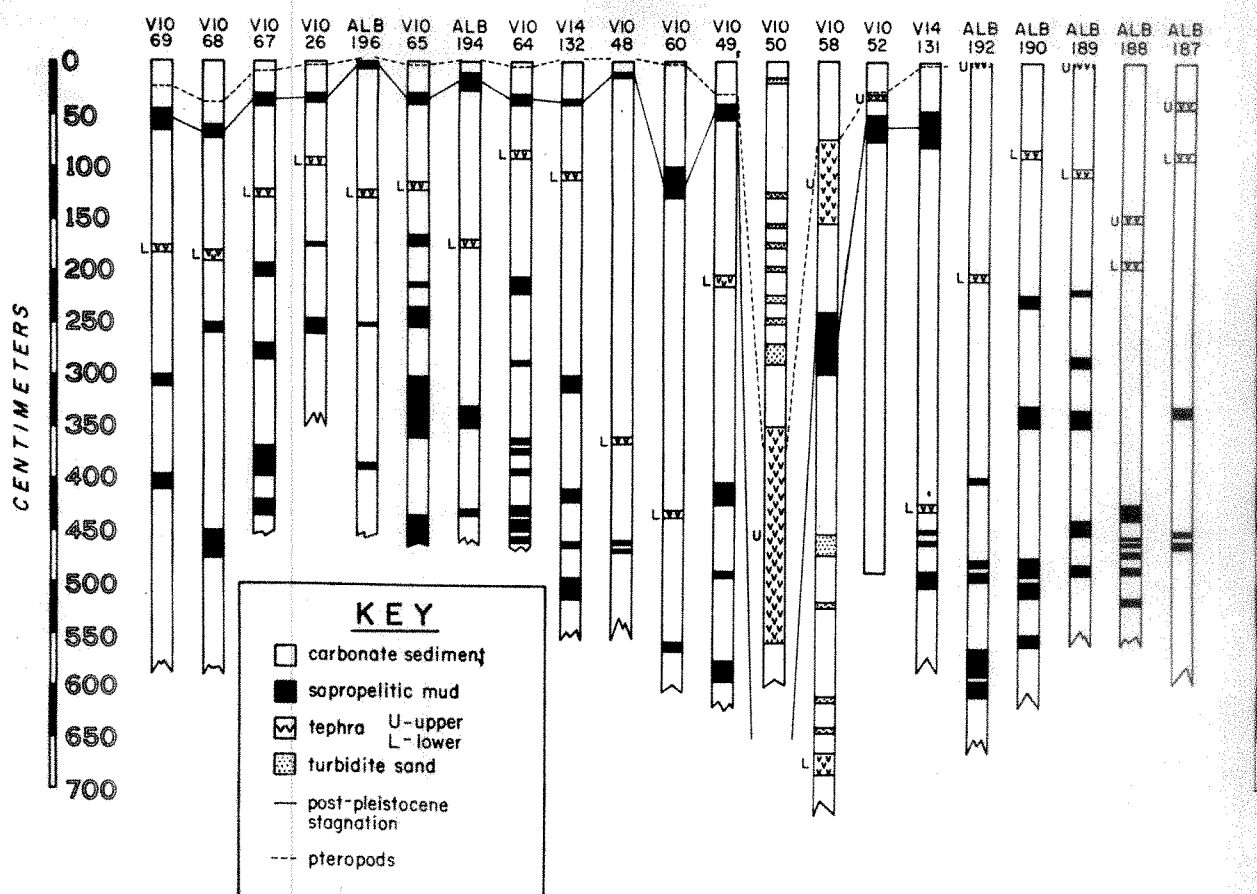


Fig. 2. Graphic logs of eastern Mediterranean deep sea cores containing volcanic ash layers.

lower ash layer occurred in the late Würm and the upper ash layer in postglacial sediments. This corresponds to the sequence found in *Vema* cores<sup>2</sup>. The only exception found by both Parker<sup>4</sup> and Olausson<sup>5</sup> represents cores *Alb* 187 and 188, where both lower and upper ash layers are interbedded with cold, presumably late Würm, foraminifera. We have compared samples of the upper and lower ash in cores *Alb* 187 and 188 provided by Dr. Mellis with ash layers in *Vema* cores. On the basis of their physical properties the upper and lower ash layers in these two cores correlate with the upper and lower ash

layers, respectively, from the *Vema* cores. This suggests some mixing or contamination in the foraminiferal records of these two cores. Fig. 1 shows that the pattern of the distribution of the upper ash layer originated in the post-glacial (Minoan) eruption of Santorini can be extended farther to the south-east if cores *Alb* 187 and 188 are included. During the late Würm eruption of Santorini, however, the prevailing high altitude winds were both north-westerly and easterly.

Pure volcanic glass from pozzuolana was first analysed chemically by Fouqué<sup>3</sup> (sample 1, Table 2). Washington<sup>6</sup> analysed a sample of pozzuolana which contained about 97 per cent volcanic glass and 3 per cent minerals (sample 2). Three samples of pure volcanic glass the physical properties of which were examined in this study were recently analysed by S. Imai of the Analytical Chemistry Research Institute, Tokyo.

Volcanic glass from pozzuolana (sample *Th*) and from the upper ash in core *VIO*-58 (sample *U*) show nearly identical chemical compositions. The volcanic glass from the Würm eruption (sample *L*) differs from the Minoan glass primarily in the ratio silica to alumina and in the percentage of potassium oxide.

The *Vema* sediment cores were collected by Professor Maurice Ewing. Professor O. Mellis provided samples of ash from the *Albatross* cores. The pozzuolana samples

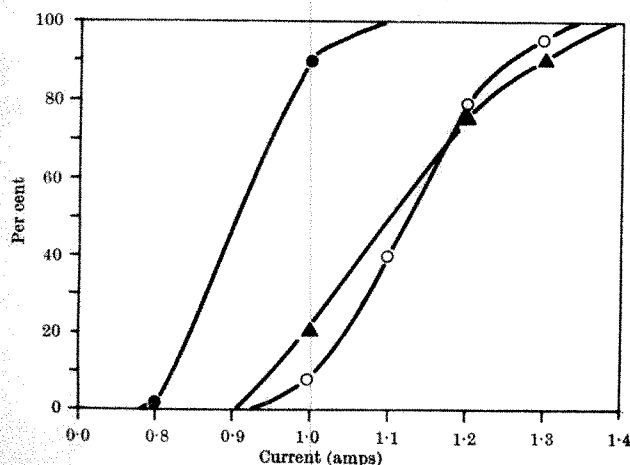


Fig. 3. Isodynamic separation of volcanic ash.

- ▲, Pozzuolana, Thera island.
- , Upper layer
- , Lower layer

VIO-58

Table 1. PHYSICAL PROPERTIES OF VOLCANIC GLASS SHARDS IN TEPHRA FROM THERA ISLAND AND AEGEAN DEEP SEA CORE VIO-58

	Colour <sup>7</sup> 177-62μ Powder	Magnet- ism (amps)	Gaseous and liquid inclusions	Refractive index
Thera island	Light grey N7	Very light grey N8	0.9-1.4	Abundant 1.509±3
Core VIO-58	Upper tephra N7	Light grey Very light grey N8	0.9-1.4	Abundant 1.509±3
	Lower tephra	Light olive Yellowish grey 5F6/1 grey 5F8/1	0.8-1.1	Infrequent 1.521±3

Table 2. CHEMICAL ANALYSIS OF POZZUOLANA FROM THERA ISLAND AND UPPER AND LOWER ASH IN AEGEAN DEEP-SEA CORE V10-58

	Th	U	L	1	2
SiO <sub>2</sub>	68.25	68.96	58.41	71.0	64.88
Al <sub>2</sub> O <sub>3</sub>	12.59	13.13	17.24	16.8	12.98
TiO <sub>2</sub>	0.34	0.30	0.34	0.5	1.53
Fe <sub>2</sub> O <sub>3</sub>	1.12	1.06	1.97	0.8	3.83
FeO	1.51	1.57	1.97	n.d.	2.30
MnO	0.05	0.05	0.15	n.d.	0.05
MgO	0.37	0.43	0.37	0.7	1.06
CaO	1.58	1.78	2.02	0.8	2.95
Na <sub>2</sub> O	5.11	5.17	5.92	7.4	3.16
K <sub>2</sub> O	3.22	3.32	7.10	2.0	2.04
H <sub>2</sub> O +	4.88	3.96	3.85	n.d.	4.58
H <sub>2</sub> O -	0.88	0.57	0.44	n.d.	0.37
P <sub>2</sub> O <sub>5</sub>	0.02	0.02	0.13	n.d.	0.22
Total	99.92	100.30	99.91	100.0	99.95

Th—Pure volcanic glass shards from pozzuolana ash, Thera island.

U—Pure volcanic glass shards from upper ash layer ( $n = 1.509$ ), core V10-58.L—Pure volcanic glass shards from lower ash layer ( $n = 1.521$ ), core V10-58.1. "Amorphous part of pumice" from Thera Island<sup>2</sup>.2. Pozzuolana with about 97 per cent volcanic glass shards and 3 per cent minerals, Thera island<sup>2</sup>.

were collected on Thera by Mrs. C. C. Heezen. The study was supported by the U.S. National Science Foundation.

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## PHYSICS

### Interfering VLF Radio Signals observed on GBR-16.0 Kc/s Transmissions during November and December 1965

In May and June 1962, Rohan *et al.* observed unexplained sudden amplitude and phase changes on GBR signals at Salisbury, South Australia<sup>1</sup>. Allan reported similar observations made simultaneously at Lower Hutt, New Zealand<sup>2</sup>. He suspected interfering signals from a powerful transmitter in the vicinity of Australia and operating near 16.0 Kc/s\*. In April 1963, Allan recorded anomalies on the 18.0 Kc/s very low frequency transmissions emitted by NBA<sup>3</sup> and in 1964 Isted proposed an explanation of these "peculiar phenomena" in terms of antipodal signal interference and changes in propagation conditions over one or both great circle routes<sup>4</sup>.

Our NBA records of April 1963, taken at Deal (New Jersey), also show phase anomalies with times of observation which agree with the times given by Allen. The anomalies of April 12, 13 and 14 were almost unnoticeable, but the anomaly of April 16 was quite strong. If it was caused by an interfering signal, its frequency had an offset of approximately  $7 \times 10^{-7}$  and its amplitude at Deal could have been as large as 6 dB below that of the signal from NBA which is located only 3,500 km south of Deal.

This simultaneous observation of an anomaly at Lower Hutt and Deal strongly reduces the probability of an antipodal signal interference<sup>4</sup> because an antipodal NBA signal to Deal crosses 4,500 km of Antarctic and Greenland ice and has a path length ten times greater than that of the short-path signal.

Malfunction of the transmitter control circuit as a cause of the anomalies was dismissed by Allan because the British Post Office had allegedly found no evidence of an

\* Nominal value, actually emitted on UT-2 time scale.

anomaly on its May/June 1962 GBR records; also the U.S. National Bureau of Standards at Boulder found no NBA phase anomalies in April 1963 (ref. 3). The discrepancy between the Boulder and Deal records can be readily resolved by the fact that the Deal tracking station had a time constant which was probably five times shorter than that of the Boulder equipment—an important consideration with fast phase variations.

Simultaneous observation of numerous phase and amplitude anomalies on the 16.0 Kc/s GBR signal with practically identical equipment (servo time constant 50 sec) at eight widely separated locations in November and December 1965 confirms beyond doubt that the anomalies were caused by an interfering transmitter. Fig. 1 shows two typical interference records. Table 1 lists pertinent data. In all cases the anomalies were strongest at Tokyo, followed in general by those observed at Brisbane; Oslo II (a temporary aurora research site in the Arctic); Kiruna, Sweden; Tananarive, Malagasy; Beirut, Lebanon; Deal; and C. Rivadavia, Argentina. The interference always saturated the amplitude channels at Tokyo and (except in December 1965) at Brisbane, indicating an interference signal level of between 20 dB and more than 35 dB above the local GBR signal strength at Tokyo. The interferences, however, were often barely detectable at Deal and C. Rivadavia. All stations received GBR with tuned loop antennae.

The frequency of the interfering signal was on average either approximately  $10^{-7}$  parts above or  $10^{-7}$  parts below the highly stable GBR carrier frequency, and often changed by several parts in  $10^8$  during the few minutes of interference.

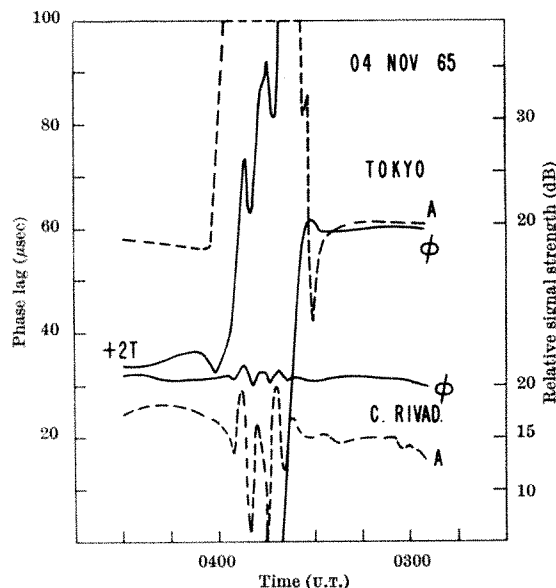


Fig. 1. Typical example of recorded GBR phase and amplitude interference during November 1965.

The possibilities of interference by signals with frequencies (a) equal to the image frequency of the involved receivers (2 Kc/s), and (b) corresponding to harmonics of the local synthesizer frequency, or (c) differing by the amount of the intermediate frequency value of the receiver (1 Kc/s), can all be discarded because the receiver systems involved have a rejection level of well above 50 dB for such signals (as verified by laboratory experiments).

The interfering signals during the 1965 period in question must consequently have originated from a transmitter located somewhere in East Asia. It could have been a transmitter with a frequency assignment close to

Table 1. DATES, TIMES, AND MEASURED FREQUENCY OFFSETS OF 1965 INTERFERENCE SIGNALS ON GBR FREQUENCY

Date	U.T.	$\Delta f/f \times 10^7$	Date	U.T.	$\Delta f/f \times 10^7$
November 4	0330-0358 ?	1.5	November 18	0857-0915	1.8?
10	0739-0743	*†	18	1032-1045 ?	1.3
12	0818-0855 ?	-0.8	18	1140-1145	0.9
13	0200-0210	*†	18	2315-2326	0.9
13	0220-0307 ?	1.3†	19	0016-0045 ?	-1.1†
13	0645-0720	-0.9 ?	19	0823-0830	1.0†
13	2035-2053	-1.0	19	1009-1100 ?	1.0†
14	0155-0204	-1.0	25	0738-0750 ?	1.0†§
14	0215-0224	-1.2	22	1632-1658	*§¶
17	0910-0925	*†	24	0355-0415	*§¶
17	1121-1137	-1.0	27	0200-0212	*§¶
17	2327-2335	*†			
18	0020-0045 ?	-0.6 ?†			
18	0348-0357	*			

\* Too unstable in phase for measurement.

† Strong interference only at Tokyo and Brisbane.

‡ Stronger than usual at Oslo II.

§ No Oslo II record.

¶ Intermittent, mostly noticeable at Tokyo only.

16.0 Kc/s<sup>5</sup> which inadvertently strayed off frequency during testing. Alternatively it could have been any transmitter there which for some reason experimented near 16.0 Kc/s<sup>5</sup>.

The interference always lasted only a few minutes and, as no one realized its true nature at the time, no effort was made at the time to obtain bearings on the interfering transmitter. Oslo II, however, monitored GBR from November 17 to 19, 1965, with two receivers, one fed from a bidirectional loop oriented 25° west of geographic north, the other from an omnidirectional whip. A comparison between the phase and amplitude patterns of the two receivers points to a location of the interfering transmitter somewhere between 100° E. and 140° E.

We computed for all eight receiving sites the field strengths,  $E$ , of GBR and the unknown interfering transmitter,  $X$ . We took the radiated power of both transmitters as 40 kW and selected fourteen locations for  $X$ , distributed between 75° E. and 165° E. longitude. Some locations coincided with listed very low frequency transmitters<sup>5</sup>, some were arbitrarily assumed.

For each of the fourteen locations of  $X$  and each of the eight receiving sites we calculated the difference  $\bar{D} = (E_{GBR} - E_X)_{CALCUL.} - \Delta E$ , averaged over five different epochs, between November 12 and 19, 1965.  $\Delta E$  is the difference of GBR and  $X$  field strengths derived from recorded interference phase patterns. We then calculated the standard deviations of the  $8\bar{D}$  values pertaining to each of the fourteen assumed  $X$  locations. These standard deviations had a minimum value for  $X$  locations within the area bounded by 32° N. and 55° N. latitudes, and 105° E. and 140° E. longitudes.

Very low frequency field strength calculations for such different paths are rather unreliable. The agreement between the locations of  $X$ , however, which were obtained by (a) the method of calculating standard deviations of  $\bar{D}$  values, (b) crude comparison of degrees of interference measured at the eight separate receiving stations, and (c) calculation of the bearing to  $X$  from one receiving site, is none the less rather startling.

The choice of the standard deviation of the  $\bar{D}$  values as a measure for the likelihood that  $X$  lies at a certain location eliminates to some degree the need to know the radiated power of  $X$  for calculating  $E_X$ . The average  $D$  value of the most probable  $X$  location should, however, be close to zero. Because under the given assumptions it turned out to be approximately 8 dB, it is necessary to assume a lower radiated power of GBR and/or a larger radiated power of  $X$ . These conditions could easily be met by reasoning that GBR was actually on keyed transmission (duty cycle less than 1), whereas  $X$  was probably on key-down operation (duty cycle equals 1) according to the nature of the interference.

Fig. 2 illustrates the distribution of observed interferences as a function of time of day. The ordinate is the total number of minutes per associated hour interval during which interferences were observed in November

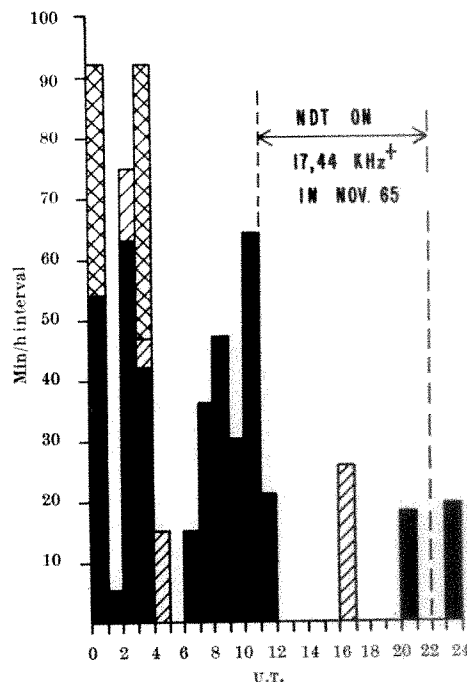


Fig. 2. Min/h interval during which interference was observed on GBR frequency. Solid block: November 1965. Hatched: December 1965. Cross-hatched: May/June 1962.

and December 1965. The cross-hatched blocks represent the 1962 observations made by Rohan and Allan.

It is interesting to note that there were few, if any, interferences during the average time interval in November 1965 for which NDT (at 35° N., 137° E.) reported that it was on its regular 17.44 Kc/s operation. On the other hand, NDT is not phase stabilized, which makes it doubtful that an interference of NDT with GBR could have been recorded with narrow-band phase tracking receivers. We believe therefore that the most probable location of the unknown interfering transmitter cannot be determined any closer after the fact, than by the boundaries given before.

We believe further that the GBR interferences observed by Rohan *et al.* and Allan in 1962 probably came from the same transmitter we observed in 1965, because of the similarity of the frequency offsets and times of interference.

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## New Determination of the Magnetic Moment of the Proton in Terms of the Nuclear Magnetron

THE ratio of  $f_s$ , the spin precession frequency of the proton, to  $f_c$ , the cyclotron frequency, in the same magnetic field is equal to the ratio of the magnetic moment of the proton  $\mu_p$  to the nuclear magneton<sup>1</sup>  $\mu_n$ .

$$f_s/f_c = \mu_p/\mu_n$$

The ratio is used at present in combination with other experimentally measured constants to derive the best values for the atomic constants<sup>2</sup>. The uncertainties of current estimates of the Faraday constant, ratio of proton to electron mass, electronic charge, nuclidic mass unit and many other important constants are effectively determined by the uncertainty of  $\mu_p/\mu_n$ .

Our measurements were made at 0.47 tesla (4.7 k gauss). The spin precession frequency was measured with an amplitude bridge<sup>3</sup> and the cyclotron frequency using the omegatron method<sup>4</sup>. The omegatron was of the quadrupole type<sup>5</sup> and had split plates to improve the uniformity of the radio frequency field. Ratios of the masses of ions are known with great precision and may be regarded as auxiliary constants<sup>2</sup>. The cyclotron frequencies of hydrogen ions ( $H_2^+$ ), deuterated hydrogen ion ( $HD^+$ ) and deuterium ions ( $D_2^+$ ) were measured, and this enabled the shifts in the cyclotron frequencies caused by electrostatic fields to be eliminated.

The results of forty-three sets of three ion resonances, with standard deviations (including both systematic and random errors), are

$$\begin{aligned}\mu_p/\mu_n &= 2.792\,74 \pm 0.000\,05 \text{ without and} \\ \mu_p/\mu_n &= 2.792\,82 \pm 0.000\,05 \text{ (19 p.p.m.) with}\end{aligned}$$

the correction for the diamagnetism of the water sample<sup>6,7</sup>, respectively. This lies within 1.1 standard deviations of the present best value and midway between the values which were included<sup>4,8,9</sup> in the 1965 evaluation and those which were rejected<sup>10</sup> or too late<sup>11</sup> to include. It is hoped to increase the precision of the method by a factor of ten by working at higher magnetic fields.

This work forms part of the research programme of the National Physical Laboratory.

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## Air Flow with Arc Present

THE behaviour of a flowing gas when disturbed by the presence of a high current arc has always been of great interest to circuit-breaker engineers and physicists.

The intense light of the arc under investigation is generally so much greater than that of the conventional background light source of the interferometer or Schlieren system used that it is extremely difficult, and in most cases quite impossible, to photograph the gas movement. This difficulty has been overcome by using a laser in place of the conventional light source of the interferometer system.

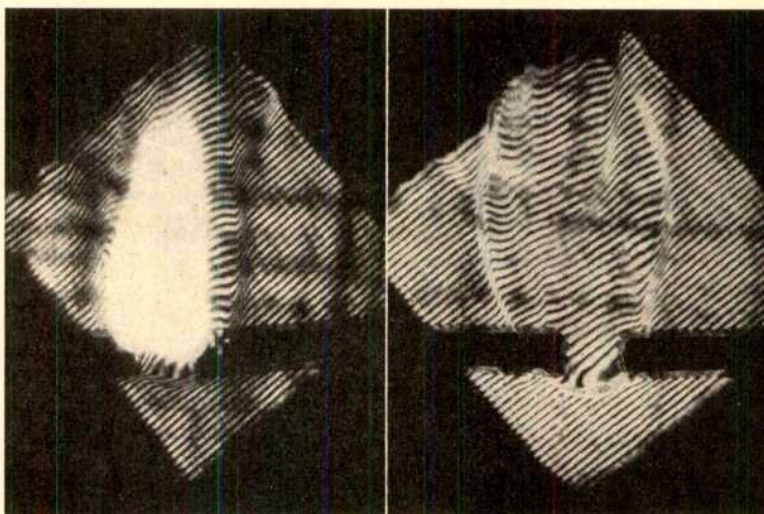


Fig. 1.

Fig. 2.

Fig. 1 is an interferogram of an arc between two horizontal electrodes; the intensity of the laser light source of the optical system is in this case comparable with that of the outer boundary of the arc.

The property of the laser to emit most of its light in a single intense spectral line allows a marked improvement to be made. Fig. 2 was taken with a laser light source, using a very narrow band interference filter between the arc and the camera which allows the light from the laser to reach the film, but cuts off practically all the light from the arc itself, except the relatively small continuum emitted at the laser wavelength. This interferogram shows no obliteration at all, and the fringes can be followed not only in the vicinity of the arc, but also right into the centre of the arc itself.

The records were obtained using a Schlieren polarization interferometer with one Wollaston prism and an analyser. The principle of operation of such an arrangement with a conventional light source has been given by Merzkirch<sup>1</sup>. It applies equally well to the laser light source, which has the additional advantage of simplified "setting up" procedure and very considerable improved quality of the fringes.

This work was a preliminary investigation to prove the principle of the idea, and the technique will now be applied to the study of gas-flow behaviour in the presence of high-power arcs.

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## Operation of the Isobaric Spin Selection Rule in the $^{10}\text{B}(^4\text{He}, ^4\text{He}')^{10}\text{B}^*$ Reaction

THE second excited state of boron-10 at 1.74 MeV is thought to be the  $T=1$  analogue of the ground states of the neighbouring isobars beryllium-10 and carbon-10. If isobaric spin is conserved in the reaction  $^{10}\text{B}(^4\text{He}, ^4\text{He}')^{10}\text{B}^*$ , it should not be possible to populate a pure  $T=1$  excited state in boron-10 because both helium-4 and the boron-10 ground state have isobaric spin  $T=0$ . The conditions under which isobaric spin is expected to be conserved have been outlined previously by Lane and Thomas<sup>1</sup>.

Earlier experimenters<sup>2,3</sup> have searched for the 1.74 MeV state through the  $^{10}\text{B}(\text{d}, \text{d}')^{10}\text{B}^*$  reaction, but see no evidence for it. They have obtained an upper limit of 0.001 on the relative cross section for deuteron scattering to the  $T=1$  state compared with the average of the  $T=0$  states. For the reaction allowed by isobaric spin  $^{10}\text{B}(\text{p}, \text{p}')^{10}\text{B}^*$  the same experimenters observe scattering to both the  $T=1$  and  $T=0$  states. Similarly, in an earlier experiment, we have observed peaks corresponding to the lowest three excited states with the  $^{10}\text{B}(^3\text{He}, ^3\text{He}')^{10}\text{B}^*$  reaction, which, because  $T = \frac{1}{2}$  for helium-3, is the isobaric spin allowed for both  $T=1$  and  $T=0$  final states.

In the course of other experiments an opportunity arose to test the isobaric spin selection rule for the reaction  $^{10}\text{B}(^4\text{He}, ^4\text{He}')^{10}\text{B}^*$ . We have measured the relative cross sections for helium-4 scattering to the ground state and lowest three excited states of boron-10 at a laboratory angle of  $40^\circ$ . In the experiment reported here a 0.2  $\mu\text{amp}$  beam of helium-4 particles with an energy of 10 MeV, obtained from the Australian National University tandem accelerator, was incident on a target consisting of 20–30  $\mu\text{g}/\text{cm}^2$  of enriched boron-10 on a 5  $\mu\text{g}/\text{cm}^2$  carbon backing. The total beam passing through the target was measured by collection in a Faraday cup located at  $0^\circ$ .

The scattered  $^4\text{He}^{++}$  particles were momentum analysed with a 24-in. double-focusing spectrometer and detected with a solid state counter in the focal plane of the spectrometer. The energy spectrum from the detector was recorded in 200 channels of an 'RIDL' pulse-height analyser. A 0.0004 in. thick aluminium foil, placed in front of the detector, separated protons and  $^4\text{He}^{++}$  particles of the same magnetic rigidity and energy. A square aperture, with sides 0.5 in., placed immediately in front of the detector, defined the momentum interval accepted for counting. As this interval was less than the widths of the peaks, it was necessary to step across individual peaks with the spectrometer in order to obtain the total number of counts in them. The length of the steps corresponded, within experimental error, to the width of the defining aperture.

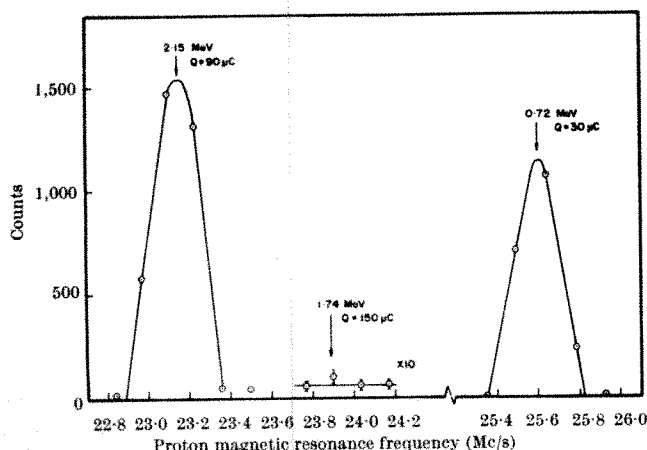


Fig. 1. The inelastically scattered particle spectrum of helium-4 corresponding to the three lowest excited states of boron-10. The values shown for  $Q$  near to each peak indicate the total charge collected in the Faraday cup for each data point in that peak.

The observed spectrum for inelastic helium-4 scattering to the lowest three excited states of boron-10 is shown in Fig. 1. The widths of the peaks are primarily caused by target thickness, kinematic broadening and detector resolution associated with the 0.5 in. defining aperture. The relative cross sections for scattering from the ground and lowest three excited states of boron-10 can be derived and are listed in Table 1. The estimated error in the relative cross sections is  $\pm 10$  per cent and is mainly caused by statistical errors and uncertainties associated with the lengths of the steps across the peaks as compared with the momentum interval accepted by the 0.5 in. defining aperture.

Table 1

Energy level (MeV)	$J^\pi, T$	Relative scattering cross section
0	$3^-, 0$	100
0.717	$1^-, 0$	12.6
1.74	$0^-, 1$	< 0.01
2.15	$1^-, 0$	6.8

The experiment provides no evidence for the existence of a group of inelastically scattered helium-4 particles which correspond to excitation of the 1.74 MeV  $T=1$  state and indicates that the isobaric selection rule is obeyed.

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## Extension of the Sub-regular Solution Model for Binary Alloys

HARDY<sup>1</sup> applied his sub-regular solution model to the interpretation of activity/composition curves and to the calculation of solubilities in binary systems. The model provides an equation for the integral heat of mixing ( $\Delta H^M$ ) of the form

$$\Delta H^M = A_1 X_1^2 (1 - X_1) + A_2 X_1 (1 - X_1)^2 \quad (1)$$

which can be applied to systems which do not conform to the regular solution model where  $A_1 = A_2$ .

If  $X_1$  is taken as the atom fraction of component 1, then in equation 1,  $A_1$  can be shown to be  $\Delta H_{2,\infty}$  and  $A_2$  to be  $\Delta H_{1,\infty}$ , where  $\Delta H_{1,\infty}$  is the limiting value of the partial heat of mixing of component 1 as the concentration of 1 tends towards zero. Equation 1 can thus be rewritten in the form

$$\Delta H^M = \Delta H_{1,\infty} X_1 + (\Delta H_{2,\infty} - 2\Delta H_{1,\infty}) X_1^2 + (\Delta H_{1,\infty} - \Delta H_{2,\infty}) X_1^3 \quad (2)$$

By changing the ratio  $\Delta H_{1,\infty}/\Delta H_{2,\infty}$  from unity for the regular solution case to different values, integral heats of mixing curves of increasing asymmetry are derived.

Using the standard relation between the integral and partial quantities in a binary system<sup>2</sup>

$$\Delta H_1 = \Delta H^M + (1 - X_1) \frac{d\Delta H^M}{dX_1} \quad (3)$$

the equations for the partial heats of mixing can be derived from equation 2 and have the form

$$\Delta H_1 = \Delta H_{1,\infty} + 2(\Delta H_{2,\infty} - 2\Delta H_{1,\infty}) X_1 + (5\Delta H_{1,\infty} - 4\Delta H_{2,\infty}) X_1^2 - 2(\Delta H_{1,\infty} - \Delta H_{2,\infty}) X_1^3 \quad (4)$$

From equation 4 it can be seen that the composition dependence of a partial heat of mixing, even in the dilute solution range, depends not only on the value of the



partial heat of mixing at infinite dilution of the component under consideration ( $\Delta\bar{H}_1^\infty$ ) but also on the value of the partial heat of mixing of the other component at infinite dilution ( $\Delta\bar{H}_2^\infty$ ).

For a regular solution the partial heat of mixing always tends towards zero as the concentration of the component in the solution increases. For the sub-regular case, however, it can easily be shown using equation 4 that when  $\Delta\bar{H}_2^\infty = 2\Delta\bar{H}_1^\infty$  the composition dependence of  $\Delta\bar{H}_1$  is initially zero, and when  $\Delta\bar{H}_2^\infty > 2\Delta\bar{H}_1^\infty$ , initially the value of  $\Delta\bar{H}_1$  can move towards larger values as  $X_1$  increases.

This form of the sub-regular solution model provides a satisfactory description of the behaviour of the partial heat of mixing of gold in liquid tin which has previously<sup>3</sup> been found difficult to interpret. More recent work<sup>4</sup> has confirmed that the dependence on composition of the partial heat of mixing of gold in tin is zero at some temperatures in tin-rich solutions, but this work also showed that the value of  $\Delta\bar{H}_{\text{Sn}}^\infty$  was approximately twice that of  $\Delta\bar{H}_{\text{Au}}^\infty$  so that the sub-regular solution model would predict a very small composition dependence of  $\Delta\bar{H}_{\text{Au}}$ . The calorimetric results showed that at 450° C  $\Delta\bar{H}_{\text{Au}}^\infty = -7.85$  kcal/g atom and  $\Delta\bar{H}_{\text{Sn}}^\infty = -16.4$  kcal/g atom.

The asymmetry of the integral heat of mixing in the gold-platinum binary system<sup>5</sup> gives rise to partial heats of mixing, the composition dependence of which lead to the binary being considered in two parts<sup>6</sup>. This composition dependence has the form predicted by the present model using equations 2 and 4 if  $\Delta\bar{H}_{\text{Au}}^\infty/\Delta\bar{H}_{\text{Pt}}^\infty$  is substantially greater than 2. The values derived by Wagner are  $\Delta\bar{H}_{\text{Au}}^\infty = 5.8$  kcal/g atom  $\Delta\bar{H}_{\text{Pt}}^\infty = 1.9$  kcal/g atom.

From this it is clear that in order to understand the composition dependence of the partial heat of mixing of one component it is necessary to know the limiting values of the partial heats of mixing of both components of a binary system.

The regular solution theory<sup>7</sup> provides no explanation of why  $\Delta\bar{H}_1^\infty \neq \Delta\bar{H}_2^\infty$ . Kleppa<sup>8</sup>, however, has shown that increasing asymmetry of the integral heats of mixing curves is associated with differences in the valency of the two components. The values of the partial heats of mixing at present available are almost all derived from integral heat of mixing data. Before quantitative correlations can be shown between the ratios of the limiting values of the partial heats of mixing of the components and such factors as the valency and size differences, it will be necessary to obtain more accurate values of the partial heats of mixing by direct measurement. This has been done in the case of the gold-tin system<sup>4</sup> and other systems are being investigated.

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### Interstellar Vehicle propelled by Terrestrial Laser Beam

IN a recent article Marx<sup>1</sup> examined the possibility that interstellar travel may be accomplished in the lifetime of an astronaut. He pointed out that the attainment of relativistic velocities by a rocket-propelled vehicle is hampered by the low efficiency, which is a result of the energy acquired by the ejected mass. Marx suggested

that if an interstellar vehicle were driven by the pressure exerted on it by a light beam transmitted from the Earth, then, because the recoil momentum and energy would now be acquired by an extremely massive body—the Earth—the energy wasted would be negligible.

Marx studies a model in which a plane wave of light of constant intensity  $I$  is reflected by a mirror of area  $f$  (the vehicle). The intensity of the reflected light is  $I'$ . Marx states that the momentum gained by the mirror in a time  $\delta t$  is

$$\delta p = f(I + I') \delta t/c = \frac{d}{dt} \left( \frac{M\beta c}{\sqrt{1-\beta^2}} \right) \delta t$$

and that the energy gained is

$$\delta E = f(I - I') \delta t = \frac{d}{dt} \left( \frac{Mc^2}{\sqrt{1-\beta^2}} \right) \delta t$$

The mirror is a perfect reflector, so the energy gained appears as kinetic energy (the rest mass  $M$  is unchanged). These equations are constructed with reference to a terrestrial frame, and are incorrect.

The correct equations for  $\delta E$  and  $\delta p$  can be found by equating the energy radiated by the transmitter in time  $\delta t$  to the gain in energy of the mirror plus the increase in the incident and reflected radiant energy, allowing for the fact that in the time  $\delta t$  the volume of space occupied by the radiation has increased on account of the motion of the mirror.

One then finds that

$$\delta E = f\{(1-\beta)I - (1+\beta)I'\}\delta t \quad (1)$$

and

$$c\delta p = f\{(1-\beta)I + (1+\beta)I'\}\delta t \quad (2)$$

From these equations and the relativistic expressions for  $E$  and  $P$  one can show that

$$I'(1+\beta)^2 = I(1-\beta)^2 \quad (3)$$

It is clear from (1), (2) and (3) that the rate of transfer of energy and momentum to the vehicle approaches zero as  $\beta$  approaches unity. The "instantaneous mechanical efficiency" defined as  $(dE/dt)/(If)$  does not rise to 100 per cent as claimed by Marx, but is found to be equal to  $2\beta(1-\beta)/(1+\beta)$ , which has a maximum value of 0.34 when  $\beta = 0.414$ .

The reason for the decrease in the efficiency is to be found in the fact that the intensity of the radiation measured in the rest frame of the vehicle decreases as the velocity increases.

The fact that the efficiency does not approach 100 per cent does not necessarily condemn this mode of propulsion. The question is whether or not interstellar distances could be covered in a time (in the rest frame) short compared with the lifetime of the astronaut, with a reasonable transmitter power. Thus one needs a relation between distance travelled ( $x$ ), time elapsed ( $t_0$ ) in the rest frame and transmitter power.

By putting  $\beta_0 = (dx/dt_0)/c$ ,  $\tau = (Mc^2)/(2fI)$ , eliminating  $I'$  from equations (1) and (2) and using the relativistic expressions for  $E$  and  $p$ , one can show that

$$\tau(dx_0/dt_0) = \sqrt{1+\beta_0^2} (\sqrt{1+\beta_0^2} - \beta_0)^2 \quad (4)$$

The solution to this equation is

$$x = c\sqrt{1+2t_0/\tau} (t_0 - \tau)/3 + c\tau/3 \quad (5)$$

If  $x$  is 100 light yr and  $t_0$  is 10 yr, then from (5) one finds  $\tau$  to be 0.022 yr. Choosing the superficial density ( $= M/f$ ) of the vehicle to be 0.01 gm<sup>-2</sup> (highly unlikely but extremely favourable), one finds that  $I$  is of the order of 1 MW cm<sup>-2</sup>.

Because we have been considering a plane wave, the area of the transmitter is the same as that of the mirror. Hence the radiate power is  $If$ . The lightest conceivable interstellar vehicle in which a man might exist for 10 yr would have a mass of 10 metric tons. Thus we find that  $f$  is of the order of  $10^9 \text{ cm}^2$  and the power radiated is of the order of  $10^9 \text{ MW}$ . This is so great that one does not need to consider the difficulties of arranging suitable deceleration and landing facilities.

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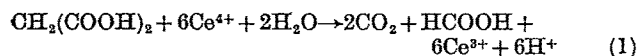
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## CHEMISTRY

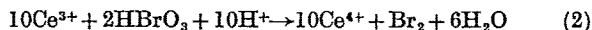
### Effect of Bromine Derivatives of Malonic Acid on the Oscillating Reaction of Malonic Acid, Cerium Ions and Bromate

WHEN malonic acid, ceric sulphate and potassium bromate are dissolved in dilute sulphuric acid there occurs an oscillating chemical reaction which manifests itself by an oscillation of the light absorption which is caused by ceric ions (317  $m\mu$ ) and an oscillation of the rate of evolution of carbon dioxide. An oscillation of the potential of a platinum electrode dipped into the solution can also be measured<sup>1</sup>. Fig. 1 shows the light absorption at 317  $m\mu$  as a function of time in a solution of the above mentioned substances. The curve divides itself into four distinct sections: (a) disappearance of ceric ions; (b) reappearance of ceric ions; (c) stationary concentration of ceric ions; (d) oscillation. The cessation of the oscillation (not shown in Fig. 1) is usually abrupt, and the ceric ion concentration persists at the minimal value attained in the oscillation.

The disappearance of ceric ions in section (a) is caused by the oxidation of malonic acid by ceric ions. According to Sengupta and Aditya<sup>2</sup> this is a first order reaction, when there is an excess of malonic acid, and the oxidation products are carbon dioxide and formic acid. The stoichiometry of the reaction is supposed to be



The reappearance of ceric ions in section (b) is caused by the oxidation of cerous ions by bromate.



According to Zhabotinskii<sup>3</sup> this reaction is autocatalytic and requires an induction period before it starts. This was confirmed by the present author in experiments where no malonic acid was present.

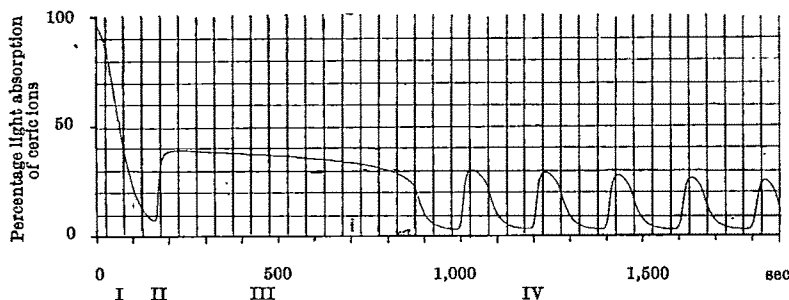


Fig. 1. Light absorption of ceric ions (317  $m\mu$ ) as a function of time in a mixture of the following composition: 0.12 mmolar ceric sulphate, 0.60 mmolar potassium bromate, 48 mmolar malonic acid and 3 normal sulphuric acid at 60° C.

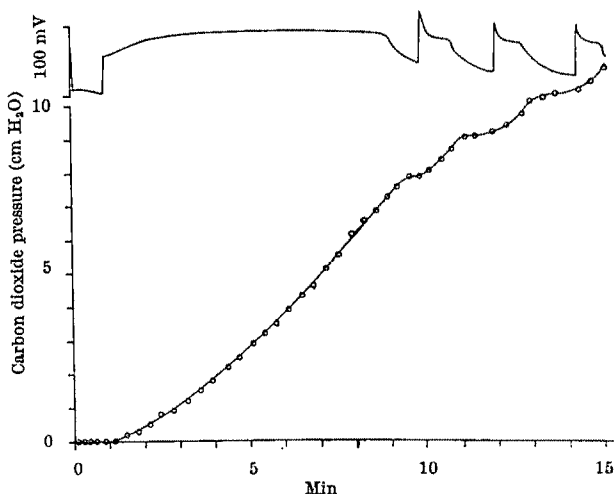


Fig. 2. Simultaneous measurement of evolution of carbon dioxide and potential difference of platinum and platinized platinum electrodes. Composition of reaction mixture: 0.83 mmolar ceric sulphate, 44 mmolar sodium bromate, 32 mmolar malonic acid and 3 normal sulphuric acid at 25° C.

The constancy of the ceric ion concentration in section (c) is supposedly the result of the competition between the ceric ion-consuming reaction (1) and the ceric ion-producing reaction (2). If this assumption is correct there should be a fast rate of evolution of carbon dioxide during this phase of the reaction. This was checked by an experiment in which the carbon dioxide evolution was measured in a Warburg apparatus. This apparatus does not allow a simultaneous spectrophotometric measurement on the reaction mixture, and so the reaction vessel was furnished with two small platinum wire electrodes one of which was platinized. By measuring the potential difference between these electrodes independent information about the progress of the reaction was obtained. Fig. 2 shows the result of simultaneous measurements of the carbon dioxide pressure and the potential difference between the platinum and platinized platinum electrodes as a function of time. It is observed that the different phases of the reaction which may be distinguished in the light absorption-time curve are easily identified in the curve showing the potential difference as a function of time. The rapid evolution of carbon dioxide during section (c) shows that reaction (1) proceeds rapidly during this phase of the reaction and, because the ceric ion concentration is nearly constant, reaction (2) must proceed with the same high rate.

The oscillation in section (d) is initiated by a fall in ceric ion concentration. This is supposedly caused by an inhibition of the autocatalytic reaction (2). The inhibitor is a substance which accumulates during section (c), and the inhibition is probably caused by the formation of a complex compound of the inhibitor and cerous ions, as suggested by Zhabotinskii. According to equation (2) bromine should be formed in this reaction, but no bromine is found. This is easily explained, because bromine and malonic acid are known to react very rapidly in acid solution to form bromomalonic acid and dibromomalonic acid<sup>3</sup>. Dibromomalonic acid decarboxylates to dibromoacetic acid which may be further brominated to tribromoacetic acid<sup>4</sup>. It was suspected that the inhibitor of reaction (2) was to be found among these four bromoacids.

The effect of adding an inhibitor of reaction (2) to the reaction mixture would be a shortening of the stationary phase of the reaction. The effect of adding the four

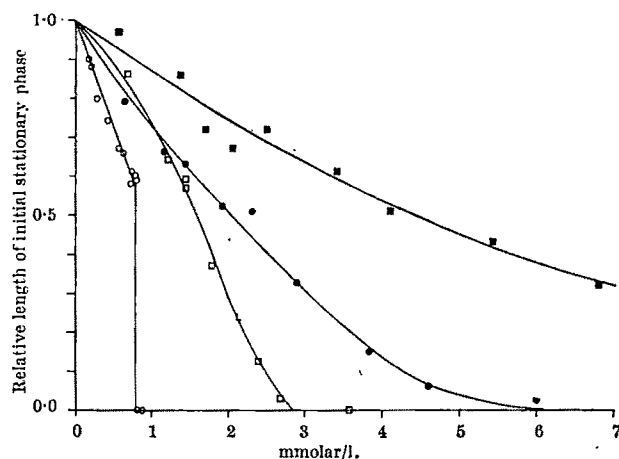


Fig. 3. Shortening of initial non-oscillatory phase of reaction caused by addition of bromomalonic acid, dibromomalonic acid, dibromoacetic acid and tribromoacetic acid to reaction mixture of the following composition: 9.78 mmolar ceric sulphate, 52 mmolar sodium bromate, 75 mmolar malonic acid and 3 normal sulphuric acid at 40° C. ■, Bromomalonic acid; □, dibromomalonic acid; ●, dibromoacetic acid; ○, tribromoacetic acid.

bromoacids already mentioned to the reaction mixture was examined by an experiment where the length of section (c) was measured at different concentrations of added bromoacid. The result is shown in Fig. 3 where the relative length of section (c) is plotted against the concentration of added bromoacid. It appears from Fig. 3 that all the four substances cause a shortening of section (c), but the inhibitory power of the substances does not increase in the chronological order of their formation, because dibromomalonic acid is a stronger inhibitor than dibromoacetic acid.

On the basis of this finding the oscillation may be explained in the following way. During the stationary phase of the reaction the bromine formed by reaction (2) attacks malonic acid to form bromomalonic and dibromomalonic acid. When a critical amount of dibromomalonic acid has accumulated, the gain factor (branching factor) of the autocatalytic reaction (2) turns negative and the reaction rate falls to practically zero. Reaction (1) is undisturbed and so the concentration of ceric ions falls. Simultaneously the concentration of dibromomalonic acid falls as a result of decarboxylation. The product of the decarboxylation, dibromoacetic acid, is a weaker inhibitor than dibromomalonic acid, and so the gain factor of the autocatalytic reaction again turns positive and, after an induction period, this reaction starts again. Now the concentration of dibromomalonic acid increases again and the cycle is repeated.

Fig. 3 shows that there is a remarkably sharp limit of concentration of tribromoacetic acid beyond which section (c) of the graph in Fig. 1 disappears. Furthermore, it was observed also that the oscillation disappeared when the concentration of tribromoacetic acid exceeded this limit. In the present experiment the critical concentration of tribromoacetic acid (0.81 mmolar) causing complete inhibition of reaction (2) is only a little larger than the total concentration of cerium (0.78 mmolar) and about one-hundredth of the initial concentration of malonic acid (75 mmolar). The aforementioned abrupt cessation of the oscillation at a low concentration of ceric ions is probably the result of a definitive inhibition of reaction (2) when this critical concentration of tribromoacetic acid has accumulated.

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## Synthesis of Formaldehyde from Methane in Electrical Discharges

FORMALDEHYDE has been reported to be the chief product of the slow oxidation of methane initiated by electrical discharges<sup>1-4</sup>. The low energy yields so far obtained prevent this method from competing with the catalytic oxidation of methanol in the commercial production of formaldehyde.

We have investigated the effect of the presence of an aqueous phase on the synthesis of formaldehyde from methane-water vapour and methane-air mixtures. The formaldehyde is absorbed almost as soon as it is formed by a stream of water droplets falling in the discharge space. The partial pressure of formaldehyde in the gas phase and its decomposition by the electrical discharge are thereby minimized.

This technique has previously been described in the synthesis of hydrazine from ammonia<sup>5</sup>, and recent work has confirmed increased yields when, under similar conditions, the hydrazine is removed by a spray of ethylene glycol (Charlton, W., unpublished results).

The reactor used in the present investigations was a quartz tube, 20 mm in diameter, in which a high frequency (2.6 Mc/s) thermally cool discharge was established between an axial aluminium rod and an outer metallic conductor 5 mm wide. The water stream was atomized by passing it through a capillary tube entry nozzle together with the methane. A series of runs was conducted in which the rate of flow of water was varied from 2.7 to 56 g/min. All other variables were kept constant—the pressure was 80 mm mercury, the rate of flow of the gas was 360 c.c./min at N.T.P. and the power input in the reaction zone 30 W. The results are plotted in Fig. 1, curve 1, as the energy yield of formaldehyde in g/kWh against the rate of flow of liquid phase in g/min.

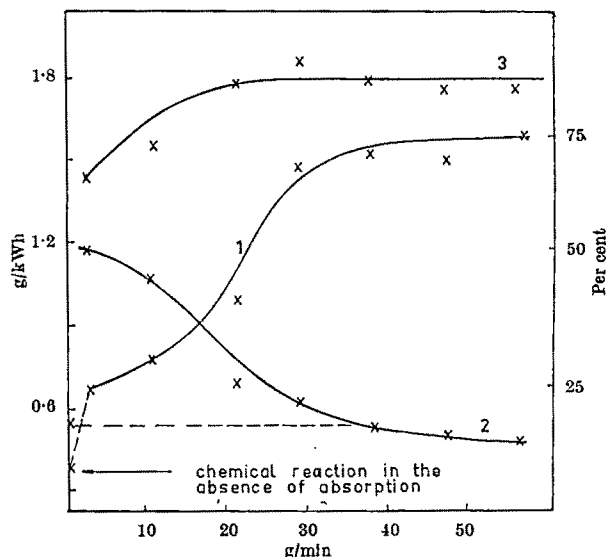


Fig. 1. Influence of liquid absorption in the synthesis of formaldehyde from methane and water vapour: (1) energy yield plotted against the rate of flow of the liquid; (2) percentage of formaldehyde absorbed; (3) percentage of water vapour in reacting mixture.

The temperature of the gas, approximately 30° C a few centimetres after the discharge zone, changed from run to run and decreased with increasing rate of flow of the liquid. Consequently, the percentage of water vapour in the reacting mixture also changed, as can be seen from curve 2 in Fig. 1, and was approximately constant for rates of flow of water in the range 30–56 g/min.

Curve 3 in Fig. 1 shows the percentage of the total amount of formaldehyde formed that is absorbed by the water droplets in or just after the discharge zone. This increases with the rate of flow of water until the concentration of formaldehyde in the gas phase approaches its

vapour pressure over water. Any further increase in the rate of flow of water has no noticeable effect on the rate of absorption beyond this point.

Curve 1 in Fig. 1 shows that the energy yield of formaldehyde increased from 0.68 to 1.5 g/kWh. The shape of this curve is dependent on the efficiency of absorption and the percentage of water vapour present in the gas phase—both of which are functions of the rate of flow of the liquid.

Further experiments were undertaken without the use of the liquid spray, and in these a mixture of methane and water vapour of the same composition as that obtained with a rate of flow of water of 30–56 mg/min was subjected to the discharge under similar conditions. Air cooling of the discharge zone was necessary in order to keep the temperature of the gas mixture at approximately 30°C. The energy yield of formaldehyde increased from 0.38 g/kWh without liquid absorbant to 1.5 g/kWh at a rate of flow of water of 30–56 g/min.

A parallel set of experiments was carried out with a gas mixture containing 37.5 per cent air. The energy yield of formaldehyde increased from 0.86 to 2.31 g/kWh at a rate of flow of the liquid of 20–60 g/min (Fig. 2, curve 4). With this system the amount of carbon

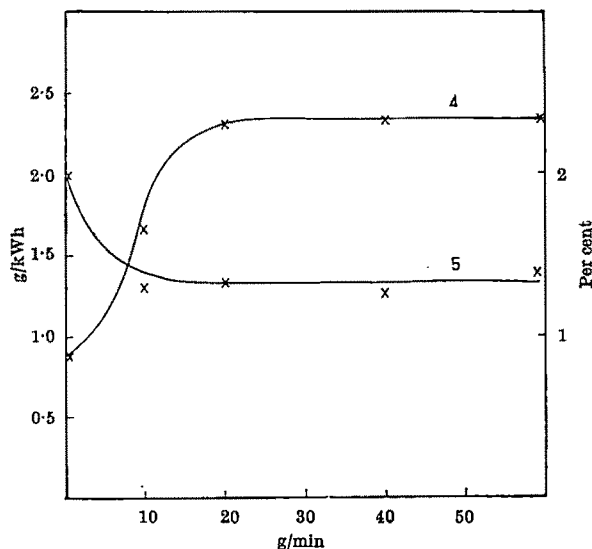


Fig. 2. Influence of liquid absorption in the synthesis of formaldehyde from methane and air: (4) energy yield plotted against the rate of flow of the liquid; (5) percentage of carbon monoxide in outlet gases.

monoxide formed was considerably greater than that previously noted. Curve 5 in Fig. 2 shows a sharp decrease in the percentage of carbon monoxide in the outlet gases as the decomposition of formaldehyde is quenched by its removal from the gas phase.

Although the yields of formaldehyde noted in the present work were still very low, these results show that a considerable improvement can be obtained by the use of liquid absorbents and that this technique may be decisive in systems where the energy efficiency is not far from being commercially attractive.

The two phase gas-liquid reactor has the distinctive advantage of reducing the residence time of the soluble products in the discharge without affecting the rate of input of energy/mole of reactant. In this way the yields of energy can be increased without reducing the conversion per pass. There is also the possibility that by a suitable choice of absorbent discharge induced reactions may be made more selective.

A further feature of the gas-liquid reactor is the ease with which the reactant and product temperatures can

be controlled over reasonably wide limits by virtue of the presence of liquid absorbent<sup>6</sup>.

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### Simultaneous Determination of Several Charge-transfer Complex Association Constants using Nuclear Magnetic Resonance

A METHOD for determining the association constants of organic charge-transfer complexes in solution using nuclear magnetic resonance has recently been described<sup>1,2</sup>. For a series of solutions containing an electron donor species (*D*) and an electron acceptor species (*A*) which interact by a very fast reversible process to form a 1:1 charge-transfer complex (*DA*), if one of the reactants, say *D*, is in large excess over the second reactant, then<sup>3</sup>

$$\Delta/[D]_0 = -K_c\Delta + K_c\Delta_0 \quad (1)$$

where  $[D]_0$  is the total (free and complexed) concentration of the donor,  $\Delta$  is the observed chemical shift of a nucleus in *A* in the complexing medium relative to the shift of this nucleus in uncomplexed *A*, and  $\Delta_0$  is the shift of this nucleus in the acceptor moiety of the pure complex, again relative to the shift in uncomplexed *A*.  $K_c$  is the association constant defined by  $K_c = [DA]/[D][A]$ , where the concentrations represent the equilibrium values for the various species. A plot of  $\Delta/[D]_0$  against  $\Delta$  should yield a straight line, the gradient of which is equal to  $-K_c$  and the intercept with the ordinate equal to  $K_c\Delta_0$ .

Provided the condition  $[D]_0 \gg [A]_0$  (where  $[A]_0$  represents the total, free and complexed, concentration of *A*) the relationship between  $K_c$  and  $\Delta$  does not involve  $[A]_0$ . Consequently, if a series of solutions is made up, containing a common donor, *D*, and a series of acceptors  $A^1, A^2, \dots A^i \dots A^n$ , then providing the sum of the total (free and complexed) concentration of each acceptor,  $\Sigma[A]_0$ , is very much less than  $[D]_0$ , a series of relationships corresponding to equation (1) will hold for each equilibrium simultaneously.

It is therefore possible to determine the association constants of a set of complexes between a series of acceptors and a common donor (or vice versa) from measurements on solutions in which all the complexes coexist. Experimentally it requires that the various chemical shifts of the observed nucleus shall not overlap with one another in any given solution. In practice this is often not a severe restriction because the lines are narrow and they always move in the same direction when the concentration of the excess component is increased. Apart from the economy, the method also provides a means of comparing the complexing ability of a series of acceptors (or donors) in a common experimental environment.

The association constants ( $K_c$ ) for the complexes of hexamethylbenzene with the acceptors: ( $A^1$ ) 1,3,5-trinitrobenzene; ( $A^2$ ) 2,5-dichloro-*p*-benzoquinone; ( $A^3$ ) 1,4-dinitrobenzene; and ( $A^4$ ) *p*-benzoquinone in carbon tetrachloride have been determined simultaneously, and also in pairs. The values of  $K_c$  are compared with the values obtained when each complex occurs singly<sup>3</sup>. The results are summarized in Table 1.

Table 1. VALUES OF  $K_c$  (IN KG/MOLE) AND  $\Delta_c$  (IN C./SEC) FOR COMPLEXES OF HEXAMETHYLBENZENE WITH: ( $A^1$ ) 1,3,5-TRINITROBENZENE; ( $A^2$ ) *p*-BENZO-2,5-DICHLORO-*p*-BENZOQUINONE; ( $A^3$ ) 1,4-DINITROBENZENE; ( $A^4$ ) QUINONE IN CARBON TETRACHLORIDE AT 33.5° C

Acceptor→	$A^1$	$A^2$	$A^3$	$A^4$
↓ Combination	$K_c$ $\Delta_c$	$K_c$ $\Delta_c$	$K_c$ $\Delta_c$	$K_c$ $\Delta_c$
Pairs $A^1, A^2$ ; $A^1, A^4$	5.2 65	2.1 87	0.9 96	0.7 81
$A^1, A^2, A^3, A^4$ together	4.8 66	2.1 86	0.9 97	0.6 90
Each singly	5.1* 65*	1.9† 91†	1.0* 91*	0.7† 89†

\* Ref. 2. † Ref. 3.

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### Oxygen-Peroxide Couple on Platinum

On the basis of experience with carbon electrodes, the behaviour of platinum electrodes in the presence of oxygen-saturated electrolytes has been explained<sup>1-7</sup> in terms of the formation of peroxide. The experimental verification of the standard potential of the oxygen-peroxide couple on platinum in alkaline solution and its Nernst-dependence on the activities of the oxygen and peroxide has not, however, been published, although Bornemann<sup>8</sup> did study the couple in acid solution. As a part of this laboratory's investigation of fuel cell electrodes at simulated deep sea pressures, the oxygen-peroxide couple has been investigated on bright platinum<sup>9</sup>.

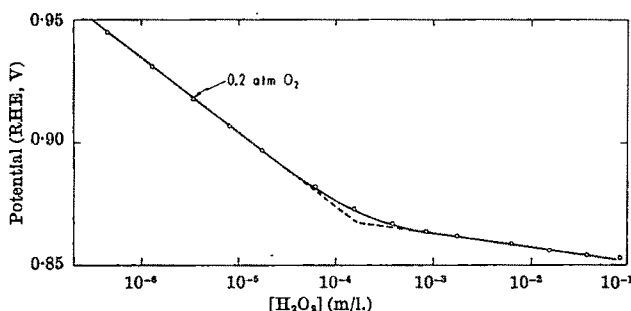
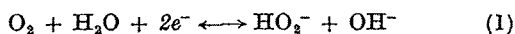


Fig. 1. Potential against the reversible hydrogen electrode (RHE) of smooth platinum electrode as a function of peroxide concentration. Dotted lines intersect at the concentration at which theory indicates change in potential control.

Measurements were carried out in stirred normal potassium hydroxide at 30° C on bright platinum electrodes previously subjected at least three times to a triangular potential sweep between 0.8 and 1.05 V. The effect of increasing peroxide concentration on the open circuit potential at 0.2 atmosphere of oxygen (Fig. 1) was a decrease in the potential of 30 mV for each decade increase in concentration below 10<sup>-4</sup> molar peroxide. Above 10<sup>-4</sup> molar peroxide, the potential shows only a small dependence on peroxide concentration of the order of -3.5 to -8 mV for each tenfold increase in concentration. Increasing oxygen pressure (Fig. 2) from 1 to 100 atmospheres increased the potential 30 mV per decade of oxygen pressure below 10<sup>-4</sup> molar peroxide and approximately 5-10 mV at concentrations of peroxide above 10<sup>-4</sup> molar.

The data for lower concentrations of peroxide obey the Nernst expression for the reversible reaction



as was observed by Berl<sup>1</sup> for carbon, and verifies the presence of the oxygen-peroxide couple on platinum. Geometrical extrapolation of the data and extrapolation of the results by means of the Nernst equation for the oxygen-peroxide couple to molar peroxide and 1 atm. of oxygen gives a standard potential of 0.762 V (against the reversible hydrogen electrode in the same system), compared with the thermodynamic value of 0.752 V. The small difference from the theoretical value can be attributed to depletion of the surface peroxide concentration from the bulk value because of its heterogeneous decomposition. This good agreement between experiment and theory is an additional confirmation of the controlling presence of the oxygen-peroxide couple.

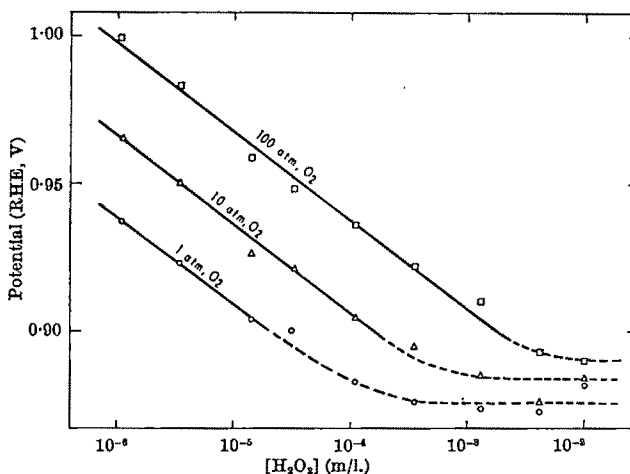
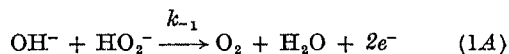
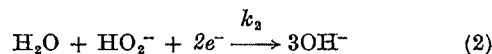


Fig. 2. Potential of smooth platinum as a function of peroxide concentration and oxygen pressure. Solid lines indicate pure Nernst behaviour.

At concentrations of peroxide greater than 10<sup>-4</sup> molar a mixed potential is established which is determined by the catalytic decomposition of peroxide, as has been previously pointed out by Hoare<sup>7</sup>, the Gerischers<sup>4</sup>, Winkelmann<sup>5</sup> and Giner<sup>6</sup>. The appropriate reactions are



and



where  $k_1$  and  $k_2$  are the specific reaction rate constants for reactions 1A and 2. The observed open circuit potential is the potential at which the polarizing currents for the two half-cell reactions are equal. Solution of the kinetic equations corresponding to these reactions, assuming transfer coefficients of 0.5, yields a peroxide-independent potential, reported previously<sup>4-7,10</sup>. Using a value of 0.35 for a transfer coefficient of reaction (2), an increase of 5.0 mV for a tenfold increase in peroxide is obtained, in agreement with Fig. 1. The potential is also a function of the logarithm of the oxygen pressure in agreement with the data of Fig. 2, and a function of the logarithm of the ratio  $k_1/k_2$ . The specific rate constants can vary independently of each other, and depend, of course, on the state of the electrode.

We conclude that the rest potential of a platinum or any so-called inert electrode in an aqueous, oxygenated system can be described quantitatively in terms of both



the oxygen-peroxide couple and the mixed potential system based on reactions 1A and 2.

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### Spectral Correlation of Transients with their Parent Derivatives in Aqueous Solution

THE spectra of many aromatic radicals have been summarized by Land<sup>1</sup> and Habersbergerova *et al.*<sup>2</sup>. The present experiments show a strict correlation between the spectra of some such radicals and their parent aromatic molecules. Cyclohexadienyl radicals derived from benzene and monosubstituted aromatic substances, with side groups directing electrophilic substitution into the *o* and *p* positions of the benzene ring, that is, with a negative  $\sigma$  Hammett function, were investigated by Dorfman *et al.*<sup>3</sup>. The effects of side groups with positive  $\sigma$  Hammett functions have been investigated only in benzoic acid<sup>4,5</sup>, nitrobenzene<sup>6</sup> and nitrosobenzene<sup>7</sup>. These observations have now been extended to the hydroxyl, hydrogen and electron adducts of benzaldehyde, acetophenone, benzonitrile and trimethylphenylammonium (TMPA) salt.

All the reagents used were of 'AnalaR' grade except for the triphenylammonium salt. Redistilled, deaerated water was used throughout the experiments. Pulse radiolysis<sup>8</sup> was used to generate hydroxyl radicals, hydrogen atoms

and hydrated electrons. Hydroxyl radicals were investigated in solutions saturated with nitrous oxide in which hydrated electrons are converted to hydroxyl radicals. Hydrogen atoms were investigated in solutions of pH 2. In the latter case hydroxyl radicals contributed to the transient spectra; the hydroxyl transient spectra were graphically subtracted. Hydrated electrons were investigated in either neutral or alkaline solutions in which hydroxyl radicals were scavenged by formate ions. The radiation doses were measured with a secondary emission chamber using a Fricke dosimeter for calibration. The monochromator was adjusted to give a band width of 10 nm.

Hydroxyl radicals react with aromatic compounds by addition giving hydroxycyclohexadienyl radicals<sup>9</sup>. The measured positions of the absorption maxima of the substituted hydroxycyclohexadienyl radicals are compared in Table 1 with those of the primary bands of the spectra of the corresponding parent molecules. In addition Table 1 gives the bathochromic shifts of these aromatic compounds caused by substitution, relative to the benzene primary band energy (*A*), and those of the substituted hydroxycyclohexadienyl radicals relative to the hydroxycyclohexadienyl radical band energy (*B*). Table 1 includes for comparison the results of other workers. The ratio *B/A* is approximately constant and has a mean value of 0.93.

The spectral characteristics of transients formed on reaction of hydrogen atoms with monosubstituted aromatic substances are very similar to the corresponding hydroxyl adducts and are given in Table 2 in an analogous way to Table 1. The ratio of *B/A* in this case has a mean value of 0.92.

In the spectra of both hydroxyl and hydrogen adducts the constant ratio relationship is less well obeyed by monosubstituted benzene derivatives with charged substituents.

Anions are formed when hydrated electrons react with the compounds listed in Table 3. The transient anions have spectra of a similar pattern to that of the parent molecules, with two well separated maxima but distinctly different from those seen in hydroxyl and hydrogen adducts. The transients from benzonitrile, benzoate and nitrobenzene gave a pH dependent protonation reaction. The anions derived from acetophenone and benzaldehyde

Table 1. HYDROXYL ADDUCTS

Aromatic substance	Primary band		<i>A</i>	Hydroxycyclohexadienyl radical		<i>B</i>	$\frac{B}{A}$	Refs.
	$\lambda$ nm	$\nu$ cm <sup>-1</sup>		$\lambda$ nm	$\nu$ cm <sup>-1</sup>			
Nitrobenzene	268.5	38-800	0.232	410	24-400	0.238	1.02	†
Benzaldehyde	249.5	40-050	0.186	380	26-400	0.175	0.94	*
Acetophenone	245.5	40-750	0.174	370	27-100	0.154	0.88	*
Benzoic acid	230.0	43-500	0.116	345	29-000	0.094	0.81	*
Benzonitrile	224.0	44-600	0.098	340	29-400	0.082	0.84	*
Bromobenzene	270.0	47-500	0.034	325	30-800	0.038	1.12	3
Benzoate ion	224.0	44-600	0.098	328	30-500	0.047	0.48	4
Chlorobenzene	209.5	47-800	0.028	322	31-000	0.031	1.11	3
Phenol	210.5	47-750	0.029	320	31-200	0.025	0.86	3
Toluene	206.5	48-000	0.017	313	31-600	0.0125	0.71	3
Benzene	203.5	49-200	—	313	32-000	—	—	3
TMPA sulphate	203.0	49-300	—	305	32-800	—	—	*

$$A = \frac{\nu_{C_6H_5} - \nu_{C_6H_5X}}{\nu_{C_6H_6}}$$

$$B = \frac{\nu_{C_6H_5OH} - \nu_{C_6H_5OHX}}{\nu_{C_6H_5OH}}$$

\* This work.

† Cercek, B., personal communication.

Table 2. HYDROGEN ADDUCTS

Aromatic substance	Primary band		<i>A</i>	Hydrocyclohexadienyl radical		<i>B</i>	$\frac{B}{A}$	Refs.
	$\lambda$ nm	$\nu$ cm <sup>-1</sup>		$\lambda$ nm	$\nu$ cm <sup>-1</sup>			
Nitrobenzene	265.0	37-800	0.232	410	24-400	0.238	1.02	†
Benzaldehyde	249.5	40-030	0.186	380	26-400	0.175	0.94	*
Acetophenone	245.5	40-750	0.174	370	27-100	0.154	0.89	*
Benzoic acid	230.0	43-500	0.116	345	29-000	0.094	0.82	*
Benzonitrile	224.0	44-600	0.098	340	29-400	0.082	0.89	*
Benzene sulphonamide	217.0	44-600	0.067	328	30-400	0.0590	0.89	*
Bromobenzene	210.0	47-500	0.034	320	31-250	0.0323	0.95	*
Chlorobenzene	209.5	47-800	0.028	318	31-400	0.0265	0.95	*
Toluene	206.5	48-500	0.017	315	31-800	0.0155	0.92	*
Benzene	203.5	49-200	—	310	32-500	—	—	*
TMPA sulphate	203.0	49-300	—	305	32-800	—	—	*

$$A = \frac{\nu_{C_6H_5} - \nu_{C_6H_5X}}{\nu_{C_6H_6}}$$

$$B = \frac{\nu_{C_6H_5} - \nu_{C_6H_5X}}{\nu_{C_6H_5}}$$

\* This work.

† Cercek, B., personal communication.

Table 3. ELECTRON ADDUCTS

	Aromatic substance		<i>A</i>	Radical anion		<i>B</i>	$\frac{B}{A}$	Refs.
	Primary band			Primary band				
	$\lambda$ nm	$\nu$ cm <sup>-1</sup>		$\lambda$ nm	$\nu$ cm <sup>-1</sup>			
Benzoate ion	224.0	44-600	0.098	313	32-000	0.072	0.740	*
Benzonitrile	224.0	44-600	0.098	310	32-300	0.063	0.645	*
Acetophenone	245.5	40-750	0.174	305	32-600	0.055	0.310	*
Benzaldehyde	249.5	40-050	0.186	295	33-900	0.017	0.091	*
Nitrobenzene	268.5	38-800	0.232	285	35-100	0.017	0.091	†
Benzene	203.0	49-200	—	290	34-500	—	—	12

$$A = \frac{\nu C_6H_5 - \nu C_6H_5X}{\nu C_6H_5}$$

$$B = \frac{\nu C_6H_5 - \nu C_6H_5X}{\nu C_6H_5}$$

\* This work.

† Corcek, B., personal communication.

have been defined in isopropanol-water mixtures<sup>9</sup>. The ratio  $B/A$  is not constant in this case but decreases with increasing wavelength of the maximum of the primary band of the parent molecule, as shown in Table 3. All these molecules have substituents with positive  $\sigma$  Hammett functions.

It appears that reactions with hydroxyl (or hydrogen) radicals produce the same structure, namely, a substituted hydroxy (or hydro)-cyclohexadienyl radical from mono-substituted benzenes. Although the hydrogen and hydroxyl radicals have different electrophilic character ( $\rho_H = 0.71$  and  $\rho_{OH} = -0.41$ )<sup>10,11</sup>, the directing forces of as different substituents as  $-NO_2$  and  $-CH_3$  did not result in an obvious change in the transient spectra. The hydrogen and hydroxyl radicals may therefore be non-selective in their point of attack. The incoming hydrogen or hydroxyl radicals are located in the cyclohexadienyl radical outside the plane defined by the conjugated system. It is noteworthy that the influence of the substituents results in the same bathochromic shifts in the aromatic and the cyclohexadienyl systems.

It is expected that the relationships exhibited by the data in Tables 1 and 2 are generally applicable and therefore can predict the positions of the maximal absorptions of as yet unknown monosubstituted cyclohexadienyl hydroxyl and hydrogen adducts in water.

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## BIOLOGY

### Fate of Unejaculated Spermatozoa

THE production of spermatozoa does not cease in sexually inactive males<sup>1-3</sup>, and it becomes necessary to examine the means of disposal of the surplus spermatozoa. The two most likely ways are either that they are voided in the urine or that they are resorbed in the epididymis or vas deferens. Early workers favoured the first of these

mechanisms because they found spermatozoa in the urine of rats, guinea-pigs, dogs, and men<sup>4,5</sup>. In 1931, however, Simeone and Young<sup>1</sup> reported that the vas deferens of the guinea-pig contained large numbers of degenerating spermatozoa and, as only small numbers of sperm were found in the urine, they concluded that resorption was the principal mechanism for disposing of the surplus spermatozoa.

Resorption has since been generally accepted as the principal means by which spermatozoa are removed from the epididymis, although doubts have been raised from time to time because histological investigations have usually failed to show extensive degeneration or phagocytosis of spermatozoa in the epididymis and vas deferens<sup>6-9</sup>. In rams and bulls with fistulated vasa deferentia, a constant flow of sperm through the fistula has been noted<sup>10-12</sup>, and observations on the elimination of radio-opaque material from the epididymis also suggest that the contents of the vas deferens pass into the urethra<sup>13,14</sup>.

The question cannot, however, be settled without quantitative estimates both of the number of spermatozoa produced and of the number resorbed or eliminated in the urine. Bielanski and Wierzbowski<sup>15</sup> reported as many as  $1.4 \times 10^9$  spermatozoa per day in the urine of rams, and this figure was probably of the same order as that of the daily production of spermatozoa by the testes<sup>16</sup>. We have therefore compared the daily sperm production (DSP) of rams, as estimated by an exhaustive ejaculation method<sup>17</sup>, with the output of spermatozoa in the urine by the same rams when sexually inactive.

Four Merino rams 5-7 years old that had been maintained in an animal house on a constant ration for 1 year were used. The rams had been subjected to exhaustive ejaculation tests at intervals of 6-10 weeks for the preceding 2 years. In each test eighteen ejaculates were collected on day 1, eighteen on day 2, and fifteen per day on days 3 and 4. The mean number of spermatozoa ejaculated per day on days 3 and 4 was taken as an estimate of the DSP. The number of spermatozoa per ejaculate drops sharply on days 1 and 2 but is fairly constant on days 3 and 4 (ref. 17). Table 1 gives the estimated DSP, together with the mean number of spermatozoa voided per day in the urine. Variation in the number of spermatozoa voided from day to day is large, and urine was therefore collected for 12-17 days. Collection was not begun for 15 days after the exhaustive ejaculation, as it was found that the output of spermatozoa in the urine was severely depressed for several days after the rams had been ejaculated a number of times. For rams A and B the estimate given in Table 1 is the mean of two ejaculation tests, one before and one after the period of urine collection.

In order to collect urine, a ram was put in a harness so that he could stand but not lie down, and a collecting apparatus<sup>18</sup> made of silicone rubber was strapped under the prepuce. The urine ran into a siliconed bottle containing 300 ml. of a 0.7 per cent solution of saponin containing 0.4 per cent formalin. The saponin prevented the sperm from agglutinating and the formalin inhibited bacterial growth. The bottles were changed daily and the total number of spermatozoa voided daily was estimated using a haemocytometer. Four separate samples were counted for each ram every day.

Table 1. DAILY SPERM PRODUCTION (DSP) ESTIMATED BY EXHAUSTIVE EJACULATION COMPARED WITH THE DAILY OUTPUT OF SPERM IN THE URINE OF THE SAME RAMS WHEN SEXUALLY INACTIVE

Ram	Mean daily output of sperm in the urine (in millions)	DSP estimated by exhaustive ejaculation (in millions)	No. of sperm in urine as percentage of estimated DSP
A	6,440 ± 1,210*	8,870 ± 750*	73
B	8,830 ± 1,650	5,710 ± 250	155
C	4,670 ± 450	7,060†	66
D	4,670 ± 540	6,410†	73

\* Standard error.

† Only two estimates available for these rams.

The results (Table 1) indicate that the mean number of spermatozoa voided daily in the urine of rams is of the same order as the estimated DSP (for all rams the mean daily number of sperm in the urine was 88 per cent of the mean estimated DSP). This further supports the suggestion that, at least in sheep, resorption in the epididymis, vas deferens or ampulla is not very important as a mechanism for disposing of the surplus spermatozoa in sexually inactive males. If this is so, then counting the number of spermatozoa in the urine of unejaculated males may be a simple method of estimating DSP in a variety of animals. Further investigations are in progress on the usefulness and accuracy of the urine method of estimating DSP. The estimates obtained by these investigations are being compared with those obtained by direct cannulation of the efferent tubules of the testis<sup>19</sup> in the same ram. Results are available to date for two rams. The mean number of sperm voided each day in the urine was 88 per cent and 102 per cent, respectively, of the DSP as estimated by testicular cannulation.

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### Narciclasine: an Antimitotic Substance from *Narcissus* Bulbs

A SYSTEMIC research programme for the detection of eventual antigrowth factors in plant extracts revealed a potent antimitotic substance which was isolated from several varieties of *Narcissus* bulbs. The substance was called "narciclasine". Antitumour activity was observed by Fitzgerald *et al.*<sup>1</sup>, who used crude preparations of *Narcissus* bulbs. They found that pure alkaloids from the same source appeared to be inactive.

The substance was detected because of its very strong inhibiting activity on the growth of the radicles of wheat grains; crude extracts corresponding to 5 mg/ml. wet weight of the bulb gave an almost complete inhibition.

This test, which will be described in detail elsewhere<sup>2</sup>, allows good quantitative evaluations, and it was used throughout the procedure for the extraction and purification of the active principle. The bulbs, collected during the winter, were ground and extracted with 95 per cent ethanol. The ethanol was distilled out and the water residue repeatedly extracted with *n*-butanol. The butanol was taken to dryness at low temperature under reduced pressure, the residue extracted with hot acetone and the insoluble part discarded. The residue from acetone evaporation was chromatographed on a cellulose powder column, using acetone + 10 per cent water as eluent, and the active fraction was isolated by the same solvent using preparative circular paper chromatography, and utilizing its yellow fluorescence as a guide. From the paper it was extracted by methanol; its dry residue was dissolved in as little an amount of methylcellosolve as possible, and eight volumes of warm water added. A precipitate formed that was discarded by centrifugation. From the supernatant, kept overnight in the refrigerator, "narciclasine" precipitated as impure crystals. After repeated crystallizations from the same solvent mixture, from ethanol-water, or from glacial acetic acid, white crystals (melting point = 234°-235°), in the form of rosettes of long needles, were obtained. The yield varied from 30 to 100 mg/kg wet weight according to the variety of the bulbs used.

The substance was soluble in methanol, ethanol, *n*-butanol, acetone, ethylene, glycol, methylcellosolve and acetic acid; it was insoluble in water and in apolar solvents. It showed an intense yellow fluorescence in the ultra-violet region, gave a deep violet colour with ferric chloride and a blue colour with Folin Ciocalteu reagent. The reaction with diazotized sulphanilic acid and with diazo blue B was positive. It easily reduced ferricyanide in sodium carbonate solution. The Dragendorff reaction for alkaloids and the ninhydrin reaction were negative. The ultra-violet spectrum showed a sharp peak at 252 mμ with an  $E_{1\text{cm}}^{1\%}$  of 920. The structure of "narciclasine" has been almost fully elucidated<sup>3</sup>. This investigation suggests that it probably occupies a key position in the biogenesis of alkaloids of the Amarillidaceae. It inhibits the growth of the wheat grain radicles at very low dosages, and the inhibition is proportional to the log of concentration (Figs. 1 and 2). When tested on *Escherichia coli* Hpr (from the Microbiological Institute of the Milan University) in nutrient broth, a 50 per cent bacteriostatic inhibition of growth was induced by a concentration of 8 μg/ml. after 24 h.

"Narciclasine" was assayed for its action on cells of sarcoma 180 in ascites form, by intraperitoneal and subcutaneous injection and by oral administration at various dosages. A sharp antimitotic activity was also observed

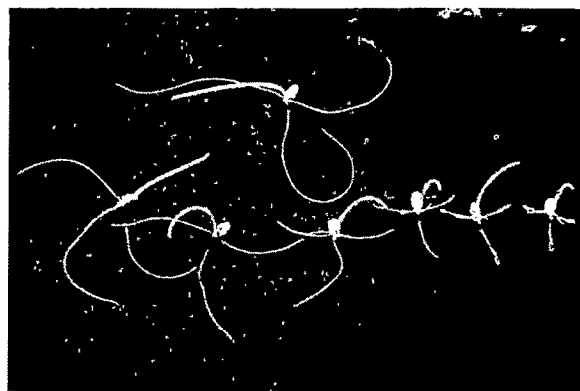


Fig. 1. Wheat grains inhibited by narciclasine at concentrations increasing from 0.05 to 0.5 μg/ml. (lower row) in comparison with a control (upper row).

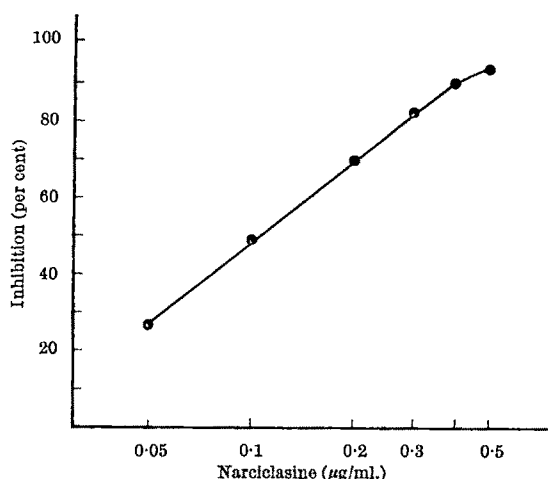


Fig. 2. Linear relationship between the per cent inhibition of growth of the lateral radicles and the log of concentration of narciclasine.

at very low doses and the subcutaneous route appeared the most effective. A colchicine-like effect was present at 0.5 mg/kg. A drastic decrease of the number of mitoses was seen 2 h after injection of 0.9 mg/kg, and after 4 h no more mitotic figures could be detected. As the dosage was increased the mitoses disappeared more rapidly and disrupted cells with globular clumps of chromatin appeared more frequently.

Mitoses reappeared sometimes at 18 h after the injection according to the dosage and the route of administration used. From an analysis of its antimitotic effect, "narciclasine" seems to act essentially as a metaphasic or a preprophasic poison and to have a mitoclastic activity especially at high doses. An  $LD_{50}$  of 5 mg/kg has been determined in mice by subcutaneous injection.

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### Phagocytosis in Mammalian Hair

ON the basis of electron microscope investigations of the human hair follicle Birbeck and Mercer<sup>1</sup> suggested that pigment granules enter the cortical cells by phagocytosis. Swift<sup>2</sup> gave support to this suggestion by illustrating the close association between pigment granules and cortical cell membranes in human hair. During recent investigations on a range of less common animal hairs we have obtained much additional evidence in support of the view that phagocytosis is responsible for the ingestion of pigment granules into cortical cells.

Birbeck and Mercer<sup>1</sup> stated that they had never observed granules in the cuticular cells, although Swift (personal communication) has only rarely seen single granules in the cuticle of human hair. My work on guanaco and vicuña hair (two species of *Llama*), and swamp wallaby hair, has revealed the not infrequent presence of pigment granules within cuticular cells. To my knowledge this has not been previously reported.

Hairs were first reduced with a 0.5 molar solution of thioglycolic acid and then treated with a 0.05 molar solution of silver nitrate in accordance with the method of Dobb *et al.*<sup>3-5</sup>. Treated fibres were embedded in 'Araldite', sectioned on an ultratome, collected from deionized water on to uncoated 'New 200' grids, and examined in an electron microscope.

While single pigment granules have fairly frequently been observed in cuticular cells, the most interesting example is shown in Fig. 1, where a cluster of small gran-



Fig. 1. Thin section of the cuticle of swamp wallaby hair (silver treated,  $\times 24,000$ ).

ules is clearly evident in a cuticular cell of swamp wallaby hair. Guanaco hair also occasionally has clusters of this type, but so far the greatest incidence of these pigment inclusions has been found in the swamp wallaby hair, which also shows excessive pigmentation of the cortical cells, not only in large clusters, but also in strings, which often contain more than twenty granules.

In the cortex of swamp wallaby hair, in particular, there is ample evidence to support the view that a phagocytotic process is used to facilitate the entry of pigment granules into cortical cells, because there are numerous examples of the association between pigment granules and cortical cell membranes; and it is suggested that phagocytosis is also responsible for the inclusion of pigment granules in the cuticular cells.

The fact that pigment granules are rarely observed in cuticular cells of human hair, but are frequently found in the other hairs studied, suggests that differences exist between the various hairs in some of the stages of fibre growth in the region of the papilla. This point will require further investigation.

I thank Dr. J. Sikorski for his advice and encouragement in this work.

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### Time required for Tumour Initiation by *Agrobacterium tumefaciens* on Pinto Bean Leaves

THE mechanism of tumorigenesis by *Agrobacterium tumefaciens*, the organism responsible for crown-gall tumours in plants, may be subject to critical experimental analysis in a host-pathogen complex only when the precise time during which tumour initiation occurs and the time required for the initiation of individual tumours are known. Knowledge of these time requirements and the factors influencing them may also provide insight into the number and kinds of processes involved in crown-gall tumour formation.

The initiation of crown-gall tumours in other host systems is remarkably sensitive to temperature<sup>1,2</sup>. The induction of normal tumours takes place at temperatures as high as 27° C but is completely suppressed at 32° C. By maintaining infected plants at 32° C and then at 25° C

for varying periods of time, Braun and Mandle<sup>3</sup> were able to show that tumour initiation occurred in *Kalanchoe* stems within 10–14 h at the lower temperature, if the plants were subject to the lower temperature at least 24 h after inoculation. The requirement of a 24 h preliminary period was interpreted as a host conditioning phase, as it proceeded in the absence of the bacterium<sup>4</sup>. In a previous paper<sup>5</sup> we have shown that treatments of infected pinto bean leaves at elevated temperatures are most effective in inhibiting tumour initiation if the treatment is begun 9–10 h after infection. Analysis of these results suggested this to be the time at which half the potential number of tumours was successfully initiated.

We have used temperature shifts between 27° and 32° C to determine the period during which tumour initiation takes place on the pinto bean leaf and to estimate the time required for individual tumour initiation.

Procedures for the pinto leaf crown-gall bioassay have been described<sup>6,7</sup>. The highly virulent strain B6 of *A. tumefaciens* was used to initiate the tumours. Eight infected plants (sixteen primary leaves) in an 8 in. pot were used to determine the effect of each temperature variation. The pots which contained the plants were placed in shallow pans of water in a forced air incubator at 32 ± 0.2° C or in an 8 × 10 ft. culture room at 27 ± 1° C immediately after inoculation. All treatments finished 72 h after inoculation and the plants were moved to a greenhouse. The tumours were counted eight days after inoculation. The infected plants were kept in the dark throughout the temperature treatment.

Raising the temperature from 27° to 32° C for 72 h immediately after inoculation (Table 1) allowed only a few per cent of normal tumour initiation to take place. The inhibiting effects of the higher temperature began after 16–18 h of treatment and with increasing time at 32° C a logarithmic decrease was observed in the number of tumours subsequently initiated. The six independent experiments in Table 1 were chosen to show that the amount of inhibition obtained by treatment at 32° C was independent of the number of tumours developing on the leaf, the variation in number of tumours in different experiments being caused by different concentrations of bacteria in the inocula and variations in leaf sensitivity.

In Table 2, three separate experiments are reported in which varying periods at 27° C replaced portions of the

72 h period at 32° C. Lowering the temperature by 5° for 2 h resulted in definite increases in the number of tumours initiated, regardless of the time at which the temperature was lowered. Extending the time at the lower temperature further increased the number of tumours. When the periods at 27° C were started immediately after inoculation, the number of tumours initiated increased logarithmically with time at this temperature. When the low temperature periods were begun 10 or 24 h after inoculation, however, the increase in tumour initiation with time at 27° C seemed to occur at two different rates, as though there were two distinct classes of tumours.

The control values designated as 100 per cent in each experiment in Table 2 represent the maximum number of tumours to be expected if, once the temperature has been lowered (to 27° C) (after 0, 10 or 24 h), it remains at this lower temperature for the rest of the 72 h period. A 10 h treatment at 32° C typically results in an increase in the number of tumours, while a 24 h treatment at this temperature results in about a 20 per cent reduction compared with the number of tumours observed when the entire 72 h period is at 27° C. If the temperature is lowered to 27° C after being kept at 32° C for 10 or 24 h, less time is required at the lower temperature for the same per cent tumours to develop, the longer the delay in subjecting the plants to the lower temperature (Table 3). The time at 27° C necessary to attain 10–100 per cent of maximum tumour initiation is reduced by 3–5 h when a prior treatment at 32° C is given. Thus, some of the events necessary for the initiation of tumours at many potential tumour sites (PTS) must occur at the higher temperature, allowing conversion of the remaining PTS to tumours in less time when the temperature is lowered. These events necessarily precede the process responsible for the lower temperature requirement.

Table 3. TIME AT 27° C REQUIRED TO ATTAIN VARIOUS PERCENTAGES OF MAXIMUM TUMOUR INITIATION, WITH AND WITHOUT PRIOR TREATMENT AT 32° C

Hours at 32° C before start of 27° C periods	Hours at 27° C required for tumour initiation*			
	10	30	50	100
0	7.5	13	16	19
10	4	10	12	14
24	4	9	12	—

\* Percentage of maximum number of tumours (see Table 2).

Two stages in temperature sensitivity were shown previously in this host<sup>5</sup>. The events which lead to the development of temperature sensitivity may be identical to those occurring at 32° C in these experiments, resulting in a shorter time requirement at 27° C to attain resistance to 32° C. Both kinds of experiments, therefore, are consistent in indicating a time requirement at many PTS before a temperature sensitive stage is observed.

From Table 2 it is obvious that tumour initiation by the crown-gall bacterium can occur on pinto bean leaves within 2 h at a conducive temperature. Thus, the minimum time required to convert a normal cell to a tumour cell must be less than 2 h and may be as short as a few min. Because the period during which tumours are initiated extends for at least 19 h following inoculation, preparation for tumour initiation must occur on the part of the bacterium, the host, or both at many PTS. Previous work has indicated that bacteria at many PTS require a period of metabolic activity, and possibly growth and cell division, before they can initiate tumours<sup>8,9</sup>. Braun<sup>2</sup> has shown that tumour, plant and inciting bacterium can grow well at 32° C, and concluded that this temperature inhibits tumour initiation by preventing the tumorigenic agent from reaching the host cell. We have reached similar conclusions in examining the inhibitory effects of short temperature treatments<sup>5</sup>. The bacterium may therefore be responsible for a portion of the lag in tumour initiation at many sites.

The pinto leaf host cells may also have to reach a critical state before tumorigenesis takes place, as has been demonstrated in the case of *Kalanchoe* stem tumours<sup>4</sup>. The generation of wound cambium in *Kalanchoe* proceeds

Table 1. NUMBER OF TUMOURS INITIATED BY *A. tumefaciens* ON PINTO BEAN LEAVES PLACED AT 27° OR 32° C FOR 72 H IMMEDIATELY AFTER INOCULATION

Experiment number	Mean number of tumours per leaf* 27° C	32° C	Per cent of 27° C control
1	9.6	0	<5
2	53.4	1.25	2.3
3	63.3	0.75	1.0
4	89.0	1.1	1.2
5	96.0	2.3	2.4
6	167.0	6.8	4.1

\* Mean of sixteen leaves per sample.

Table 2. NUMBER OF TUMOURS INITIATED ON PINTO BEAN LEAVES WHEN PORTIONS OF A 72 H TREATMENT AT 32° C ARE REPLACED BY PERIODS AT 27° C BEGUN AT 0, 10, OR 24 H AFTER INOCULATION

Hours at 27° C	Mean number of tumours per leaf*		
	0 h	10 h	24 h
0	2.3	1.2	1.1
2	4.4	4.1	3.1
4	4.2	8.6	7.6
6	—	15.0	12.0
8	17.0	18.0	15.0
10	16.0	22.0	33.0
12	28.0	42.0	33.0
		(50 per cent)	(50 per cent)
14	36.0	82.0	—
	(50 per cent)	—	—
18	78.0	—	—
Controls 72 h at 27° C	96.0	65.0	89.0
	(100 per cent)	—	—
10 h at 32° C + 62 h at 27° C	—	85.0	—
	—	(100 per cent)	—
24 h at 32° C + 48 h at 27° C	—	—	71.0†
	—	—	(100 per cent)

\* Mean of sixteen leaves per sample.

† Calculated from a mean per cent inhibition obtained from four comparable experiments.



faster at 32° C than at 25° C (ref. 10), showing that events leading to the cell divisions associated with wound healing may be promoted at 32° C. Because a critical stage in the wound healing process may be required for tumour initiation, these findings suggest that a host cell conditioning requirement may also be involved in determining the time required for pinto leaf tumour initiation.

A further alternative may be envisaged if the tumour inducing agent produced by the bacterium cannot accumulate. Under this restriction both the bacterium and the host cell would have to be in a competent state at the time of tumour initiation. Treatments inhibiting either might then determine whether tumour initiation occurs at individual sites, as well as the precise time of occurrence. The available evidence does not allow a decision as to the relative contribution of these alternatives to the 19 h requirement for the conversion of all PTS.

The time at 27° C during which pinto leaf tumours become able to withstand a temperature of 32° C is similar to the low temperature period necessary for tumour formation in stem systems<sup>3</sup>. The pinto leaf system, however, does not require the 24 h preliminary treatment which was shown to be a host conditioning phase<sup>4</sup> and the parameter measured is the number of tumours rather than the tumour size. Since the size of stem tumours seems to be directly related to the number of bacteria introduced into the wound<sup>11</sup>, as are the number of tumours per leaf in the pinto leaf assay, the close agreement between the low temperature time requirement in these two systems may be of significance.

The above results show that at 27° C the process of initiation of crown-gall tumours can proceed through the 32° C sensitive stage on the primary pinto bean leaf in a minimum time of under 2 h. Because all of the PTS are not able to withstand a temperature of 32° C until 19 h after inoculation, other events must take place at many sites before tumour initiation and these events determine the time at which the initiation process starts at these sites.

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### Effects of a Soil Humic Compound on Root Initiation

HUMIC compounds are acidic dark coloured and predominantly aromatic substances that occur in soil organic matter in concentrations which range from close to zero to near 100 per cent. Because of their exchange capacity and ability to complex metal ions and hydrous oxides, these compounds affect the availability of nutrients to plant roots and biological systems, and also play an important part in the genesis of soils. Little is known about the more direct effects of humic compounds, such as those effects caused by uptake by plants and influence on plant metabolism. According to Russian workers<sup>1</sup> small concentrations of humic compounds, that is, up to 60 p.p.m.,

enhance root development and growth of a number of plants. Khristeva<sup>1</sup> believes that these compounds enter the plant during the early stages of growth and are supplementary sources of the polyphenols that serve as respiratory catalysts.

Heavy summer rainfalls may bring concentrations of humic compounds of the order of several thousand p.p.m. into the soil solution. The object of this investigation was to determine the possible influence of these higher concentrations on the initiation of roots.

The humic compound used originated from a podzol Bh horizon. Methods of extraction, purification and drying, as well as a number of physical and chemical characteristics of this organic matter, have been described previously<sup>2</sup>. The extracted and purified organic matter was soluble in both alkali and acid, so that, according to the definition accepted in soil science, it was fulvic acid.

Root initiation was measured as follows<sup>3</sup>: cotyledons were removed from 11-day-old bean seedlings (*Phaseolus vulgaris* L., C.V. 'Contender') which were grown on vermiculite; roots and etiolated stem tissues were rejected by trimming 2 in. below the cotyledonary node. The resulting leafy stem cuttings were placed in individual vials, each containing 0.25 ml. of 1/40 molar sodium bicarbonate solution either with or without fulvic acid. The cuttings completely absorbed the solutions within 3 to 3.5 h and were then trimmed through the cotyledonary node to leave 2-in. hypocotyl segments which were planted vertically (node-end exposed), 1.875 in. deep, in pots of 'Perlite' previously drenched with nutrient solution. Potted segments were overwrapped with polyethylene and held at 78° F to develop roots. The segments were removed from the 'Perlite' after six days and roots and initials counted under a magnifier-illuminator. Root counts reported here are averages of twenty-two segments.

Root formation was stimulated by fulvic acid concentrations greater than 500 p.p.m. (Fig. 1), reached a maximum at 3,000 p.p.m. and then remained more or less constant up to 6,000 p.p.m., which was the highest concentration tested. The pH of the 1/40 molar sodium bicarbonate solution was 8.4; pH values of bicarbonate solutions containing 1,500, 3,000 and 6,000 p.p.m. of fulvic acid were 7.1, 6.6 and 5.8, respectively. Treatments with pure 1/40 molar sodium bicarbonate solution (pH 8.4) produced the same root counts as treatments with distilled water (pH 5.5).

The presence of substantial numbers of COOH, phenolic and alcoholic hydroxyl groups in this fulvic acid<sup>4</sup> suggested that these groups might, at least in part, be responsible for the root initiation properties of the fulvic acid. Thus, each of the principal functional groups was blocked selectively by methods described elsewhere<sup>5</sup>. These preparations were dissolved in 1/40 molar sodium bicarbonate solution and administered at concentrations of 1,500 p.p.m. Blocking

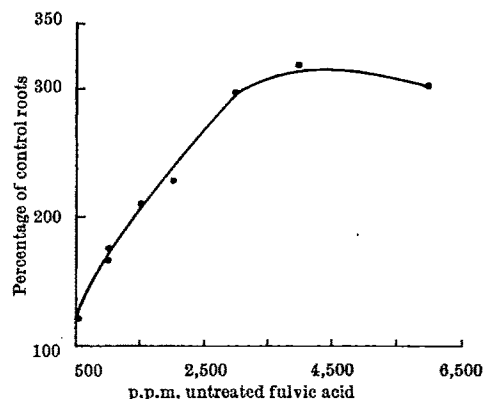


Fig. 1. Effect of fulvic acid concentration on root initiation in beans. Average number of roots and initials on control segments:  $8.1 \pm 0.5$  (= 100 per cent). Controls received 1/40 molar sodium bicarbonate solution but no fulvic acid.

of practically all hydroxyl groups by acetylation reduced root initiation considerably, but blocking of COOH groups by esterification had no effect. Simultaneous blocking of both COOH and hydroxyl groups reduced root initiation to values of control samples which received 1/40 molar sodium bicarbonate solution only. This suggested that both types of functional groups in the fulvic acid were involved concurrently in reactions which resulted in increased root initiation.

A possible explanation for the experimental results lies in the known ability of the fulvic acid to form stable water-soluble complexes with di- and tri-valent metal ions<sup>4</sup>. Thus it is possible that the fulvic acid may aid in the movement of metal ions which can only be transported with difficulty within the plant. Iron is known to be essential to cell division and to the growth of roots<sup>5</sup>, but its mobility within the plant system appears to be severely restricted. In our tests the addition of 8 per cent ferric iron to 1,500 p.p.m. of fulvic acid resulted in a significant increase in root initiation over the application of 1,500 p.p.m. of fulvic acid alone.

These results suggest that humic compounds have significant effects on the rooting of plants in natural soils.

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### Significance of Selenocystathionine in an Australian Selenium-accumulating Plant, *Neptunia amplexicaulis*

*Neptunia amplexicaulis* (Domin.) is a selenium-accumulating herbaceous legume endemic to Central Queensland. This species can accumulate selenium to more than 4,000 p.p.m. on a dry matter basis and is the main cause of clinical selenosis in livestock in some regions<sup>1</sup>. In previous work it was shown that <sup>75</sup>Se-selenite is metabolized by this species predominantly into ethanol-soluble compounds, with negligible labelling of the protein fraction<sup>2</sup>. This distribution contrasted strongly with the predominant conversion of <sup>75</sup>Se-selenite to seleno-amino-acids in peptide linkage in the proteins of several common pasture and cereal species<sup>2</sup>. The identity of the seleno-compounds present in *N. amplexicaulis* is therefore of considerable interest.

Three seedlings (with an average selenium content of 123 µg/seed) were grown for 6 weeks, to the stage of four compound leaves, in nutrient solution cultures which contained no added selenium<sup>3</sup>. The root systems were coiled in a small beaker and immersed in 4 ml. water containing 0.4 mc. sodium <sup>75</sup>Se-selenite (equivalent to 8 µg selenium). This solution was absorbed by the plants in 6 h and was followed by 0.001 molar calcium chloride solution over 24 h and finally nutrient solution<sup>3</sup> one-tenth of the strength over 64 h. The plants were continuously illuminated in a controlled environment cabinet over the period of absorption and metabolism of the selenium-75. They were then gathered, dissected into roots, stems and leaves, and frozen. Each tissue sample was ground under liquid nitrogen to a fine powder and extracted by stirring for 15 min with 50 ml. 80 per cent aqueous ethanol at room temperature. The extract was filtered, concentrated by rotary film evaporation at 35°

and 2 ml. was transferred to a small centrifuge tube. The pigment was removed from the leaf and stem samples with negligible loss of radioactivity by gentle extraction with 2 ml. ether. The aqueous solution was concentrated under a stream of nitrogen to a volume of 0.25 ml. and subjected to high voltage electrophoresis as a 30 cm band on acid-washed 'Whatman 3 MM' paper ('Miles Hivolt' apparatus, 6.0 kV, 300 m.amp. for 10 min in a pyridine/acetic acid buffer<sup>4</sup>, pH 5.3). Scanning revealed a small radioactive peak caused by the selenite ion and a larger peak (90–95 per cent of the total radioactivity in the extract) in the neutral amino-acid region, which was eluted with water. The solution was concentrated by freeze-drying, subjected to high voltage electrophoresis (6.0 kV, 300 m.amp. for 45 min in acetic/formic acid buffer, pH 2.0) and the dried electrophoretogram was autoradiographed overnight. The patterns of radioactivity were the same in root, stem and leaf tissues, with predominant labelling in two bands which were eluted. Each band (designated 1 and 2) was further purified by paper chromatography in *n*-butanol-acetic acid-water<sup>5</sup> and examined further.

The first band, which contained one-third of the radioactivity, was ninhydrin-positive and migrated identically with authentic selenocystathionine when the following separation procedures were used: paper chromatography in the solvent systems<sup>6</sup> *n*-butanol-ethanol-water, *n*-butanol-acetic acid-water and *n*-butanol-pyridine-water; high voltage electrophoresis at pH 9.2 (borate buffer<sup>4</sup>, 35 min, 'Whatman No. 1') and pH 2.0 (see above); and ion exchange chromatography at pH 4.7 on 'Whatman DE-20' paper<sup>5</sup>. Exact correspondence of ninhydrin-positive spots with autoradiograph images with or without added authentic selenocystathionine was observed in superposition tests. No evidence was obtained for the presence of compounds other than selenocystathionine in the first band.

Hydrogenolysis with Raney nickel<sup>6</sup>, followed by paper chromatography of the products in *n*-butanol-acetic acid-water, yielded only two ninhydrin-positive spots of comparable intensity, identified as alanine and  $\alpha$ -amino-butyric acid. Authentic selenocystathionine behaves similarly<sup>7,8</sup>.

Two-thirds of the radioactivity was contained in the second band, which was ninhydrin-positive and behaved as a neutral amino-acid on electrophoresis at pH 5.3 and 6.1. The unknown compound migrated faster than selenocystathionine on electrophoresis at pH 9.2, but slower at pH 2 and was clearly separate. Djenkolic acid and lanthionine both behaved similarly to the unknown compound on electrophoresis; the unknown compound, however, migrated between djenkolic acid and lanthionine on chromatography for 4 days in *n*-butanol-acetic acid-water<sup>4</sup>. On hydrogenolysis, the unknown compound yielded predominantly alanine; djenkolic acid and lanthionine yield only alanine. Related sulphur compounds have also been reported from seed of the legumes, *Albizia julibrissin*<sup>9</sup>, *Dichrostachys glomerata* and *Neptunia oleacea*<sup>10</sup>, which are all members of the Mimosaceae. Because analogous selenium compounds were not available for comparison the identity of this material remains in doubt.

The free amino-acids present in seeds of *N. amplexicaulis*, collected from seleniferous areas in Queensland, were also examined. It was shown by the above methods that selenocystathionine was a prominent amino-acid; additional colour tests with iodine-azide and nitroprusside reagents<sup>4</sup> were also consistent with this identification. The unidentified material of the second band was also present; both compounds are being isolated in milligram amounts for further characterization.

Selenocystathionine has previously been isolated from *Astragalus pectinatus*<sup>11</sup> (Papilionaceae) and *Lecythis ollaria*<sup>8</sup> (Myrtales) and identified in *Stanleya pinnata*<sup>12</sup> (Cruciferae). It is of interest that Se-methylselenocysteine is the principal seleno-amino-acid in several accumulator species (seven *Astragalus* spp.<sup>7,13</sup>, *Oenopsis condensata*<sup>7</sup>

(Compositae), *Stanleya pinnata*<sup>7</sup>). This compound could not, however, be detected in *N. amplexicaulis*.

The important characteristic which emerges from the distribution of selenium in *N. amplexicaulis* is its presence predominantly as two soluble non-protein amino-acids. Irrespective of whether <sup>76</sup>Se-selenite<sup>2</sup> or <sup>76</sup>Se-selenate was administered, 95 per cent of the <sup>76</sup>Se was soluble from the shoots and 75 per cent from the roots. The residual material was resistant to attack by proteolytic enzymes and after further tests was concluded to be elemental selenium<sup>2</sup>. The presence of selenium predominantly in the non-protein amino-acid fraction appears to be a characteristic of the accumulator species so far investigated. In non-accumulator plants, incorporation of selenium into protein amino-acids is the dominant feature<sup>2</sup>.

Accumulator species, therefore, may have evolved mechanisms for exclusion of selenomethionine and selenocysteine from proteins, which could be a factor in the tolerance of these species to high levels of selenium. No free or protein-bound selenocysteine or selenomethionine could be detected in *N. amplexicaulis*<sup>2</sup>. Exclusion of selenomethionine and selenocysteine from proteins might occur after their synthesis, for example, by specificity of amino-acid activating enzymes, or intracellular localization discrete from sites of protein synthesis. Alternatively these seleno-amino-acids may not be synthesized; in *N. amplexicaulis* the accumulation of selenocystathionine suggests an enzymatic discrimination against selenium analogues in the interconversion of cysteine and methionine. This hypothesis is supported by investigations of the selenium compounds present in the genus *Astragalus*. Whereas Se-methylselenomethionine is the principal soluble seleno-compound synthesized by four non-accumulator species, Se-methylselenocysteine is the soluble form synthesized by seven accumulator species<sup>14</sup>. Both selenomethionine and Se-methylselenomethionine were identified in pasture species<sup>2</sup>.

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### Light Requirements of Buried Seeds

It is well known that the upper layers of many types of soil contain large populations of buried, dormant seeds estimated to amount to many thousands per acre in some instances<sup>1</sup>. Many of these buried seeds are viable and germinate readily when the soil is cultivated or disturbed in other ways. There is good evidence that such seeds can remain dormant in the soil for long periods; indeed, evidence has recently been presented that seeds which have lain in the soil for as much as 1,700 years are still viable<sup>2</sup>.

The cause of this failure of buried seeds to germinate is not understood, although it has been suggested that high concentrations of carbon dioxide in the soil, lack of light or need for mechanical abrasion may be involved. It appears to be commonly held that "carbon dioxide narcosis", which was shown by Kidd<sup>3</sup> to occur in seeds of *Brassica alba*, is the factor most likely to control the dormancy of other species, but there is little direct evidence to support this view. On the other hand, the role of light in the germination of many species of seed is well known. Our present experiments suggest that a requirement for light may be the most important factor regulating the dormancy of many buried seeds.

The experiments were carried out at Aberystwyth on agricultural soil which had been continuously under pasture for at least 6 years, but which had been arable land in the past. After removing the top 2 cm of soil, samples were taken at various depths down to 10 cm. These operations were carried out in complete darkness. The soil was divided into 3 kg samples and sieved (3 mm mesh) under weak green light. Each sample was then divided into two equal portions. Each of these was then spread out in a thin layer in trays which were then maintained in a moist condition, either in light or in complete darkness in an unheated greenhouse. The subsequent emergence of seedlings was recorded at regular intervals.

Tables 1 and 2 summarize the results of such experiments over a period of 12 months. The emergence figures are the means of six replicates with the emergence in the dark expressed as a percentage of the total emergence in light. The most commonly emerging dicotyledonous species are shown in Table 2. We did not attempt to identify grass species.

Table 1 shows that there was a mean emergence in the dark of 4.4 per cent for grasses and 7.8 per cent for dicotyledonous species, giving a total emergence in the dark of only 6.1 per cent of the total emergence. The seeds which germinated in the dark may have been relatively fresh seeds which had been buried for only a short time, or old seeds which had been stimulated to germinate by the mechanical abrasion involved in sampling and sieving. The species shown in Table 2 as germinating in the dark trays were always represented in the light trays in much higher numbers.

From the results it would appear that a light requirement for germination is the chief factor controlling the

Table 1. MEAN NUMBER OF SEEDLINGS EMERGING AFTER 28 DAYS

Date	Condition	Grasses	Dicotyledonous species
November 1964	Light	14.0	7.0
	Dark	0.5 (3.6)*	0.5 (7.1)
August 1965	Light	19.0	7.0
	Dark	0.6 (3.2)	0.5 (7.1)
October 1965	Light	9.2	11.0
	Dark	0.6 (6.5)	1.0 (9.1)

All figures are means of six replicates.

\* Figures in parentheses express germination in the dark as a percentage of the total germination.

Table 2. SPECIES LIST FOR LIGHT AND DARK TREATED TRAYS

Light	Dark
1. <i>Hypochaeris radicata</i>	
2. <i>Leontodon autumnalis</i>	
3. <i>Rumex crispus</i>	
4. <i>Senecio jacobaea</i>	
5. <i>Senecio vulgaris</i>	
6. <i>Sonchus asper</i>	
7. <i>Tripleurospermum maritima</i>	
8. <i>Papaver dubium</i>	
9. <i>Plantago lanceolata</i>	
10. <i>Plantago media</i>	
11. <i>Polygonum persicaria</i>	<i>Polygonum persicaria</i>
12. <i>Polygonum aviculare</i>	
13. <i>Sinapis arvensis</i>	<i>Sinapis arvensis</i>
14. <i>Spergula arvensis</i>	<i>Spergula arvensis</i>
15. <i>Stellaria media</i>	
16. <i>Trifolium repens</i>	
17. <i>Aphanes arvensis</i>	
18. <i>Atriplex hastata</i>	
19. <i>Cerastium vulgatum</i>	
20. <i>Chenopodium rubrum</i>	
21. <i>Chrysanthemum segetum</i>	
22. <i>Myosotis arvensis</i>	
23. <i>Veronica persica</i>	

dormancy of buried seeds. This conclusion was fully confirmed by the results of the following field experiment.

Holes approximately 70 cm square were dug in a randomized experimental plot in the same field from which the samples in the earlier experiment had been taken. These holes were dug to depths of 5 cm, 15 cm and 30 cm in complete darkness except for weak green artificial illumination. They were covered either by sheets of glass or by sheets of asbestos, so that the surface of the soil was exposed to light or maintained in darkness, respectively. The number of seedlings which emerged was recorded, using a 0.25 square metre quadrat, placed centrally in the hole at 10, 21 and 35 days, again using only weak green illumination for the dark series. The average results of four replicates are shown in Fig. 1. These figures show the complete dependence of the germination of buried seeds on light, and also indicate a close relationship between the number of seedlings and depth.

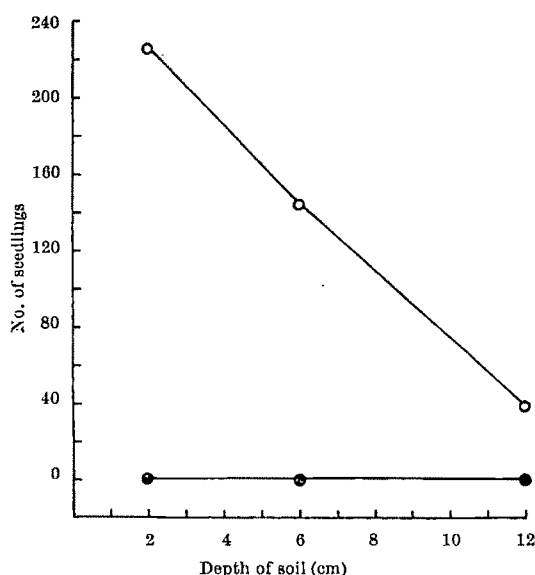


Fig. 1. Number of seedlings per 0.25 square metre after 35 days. O, Light; ●, dark.

When the asbestos covers were removed from the holes after 35 days and replaced by sheets of glass, there was a rapid emergence of seedlings, but the emergence attained only about 50 per cent of the final value for continuously exposed plots.

Tests carried out on freshly harvested seeds of a range of species, to determine their light requirements, have shown that of the species listed in Table 2, numbers 1 to 7 inclusive are promoted by light, 8 to 16 are indifferent to light and 17 to 23 have not yet been tested.

The possibility that light is the principal factor controlling the germination of buried seeds after cultivation or other disturbances of the soil has probably been rejected because the seeds of many of the species involved have not been shown to require light when the fresh seed has been tested. The observation that some species of seed only required light when taken from soil samples suggests either that the burial of the seeds has in some way modified their reaction to light, or that in a given species there may always be a low percentage of seeds, usually so small as to be insignificant in germination tests, which require light, and that it is these seeds which remain dormant when buried. Borthwick *et al.*<sup>4</sup> have been able to induce light sensitivity in previously insensitive varieties of lettuce by heat treatment: and our own work has shown that by prolonged cold treatment it is possible to induce light sensitivity in seeds of locally collected *Stellaria*

*media*—the freshly harvested and after-ripened seeds of which appear to be indifferent to light.

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## PHYSIOLOGY

### Enhanced Muscle Regeneration and Increased Strength in Myopathic Animals treated with Ethylenedinitramine

DIFFERENTIATION of skeletal muscle is activated as a function of replication<sup>1</sup>. This predicts that enhancement of growth in a myogenic system will increase the yield in new muscle. Excessive proliferation, however, can cancel muscle differentiation<sup>2</sup>. In employing the theory it is therefore necessary to stipulate that increased growth shall not reach deleterious levels (although just what these levels are cannot yet be specified). The properties of ethylenedinitramine (EDNA)<sup>3,4</sup> seemed, intuitively, to satisfy this condition. EDNA enhanced the regeneration of muscle under several specific conditions.

EDNA was converted to its ammonium salt and dissolved in physiological saline. Young, adult Swiss-Webster mice (for description see ref. 5) which had been wounded in their tibialis anterior muscles 45 h earlier received 0.1 mg of EDNA by intraperitoneal injection. Wounds were fixed 2 days later (96 h after wounding) and were examined histologically. Those from treated animals showed more regeneration than controls by a factor of almost four (Table 1). The effects of EDNA were much less pronounced when the compound was applied before or 24 h after wounding (Table 1). EDNA did not stimulate grossly premature unfolding of the signs of differentiation of new muscle. This was judged from the results of the following experiment: three mice with 45 h old wounds were given EDNA as before; the tissues, together with those of three untreated controls, were fixed at 84 h; that is, about 10 h before the advent of myotubes. (In regenerating mouse muscle, myotubes first appear in small numbers at 94.5 h after wounding. Within 1 h the density of myotubes increases almost tenfold and tends to hold steady until early in the sixth day when there is again another increase in number. These are unpublished observations of Pietsch and McCollister.) Serial examination of each section revealed not a single myotube among the controls or the EDNA treated specimens.

In another series the tibialis anteriors of male C57BL/6J mice with the same birthdate were punctured aseptically. Animals were divided into two equal groups. At 67 h those of one group received 0.1 mg of EDNA intraperitoneally. The others were untreated. All animals were given the equivalent of 10  $\mu$ c. of L-lysine labelled with carbon-14 (uniformly labelled with 200 mc./mmole) intraperitoneally

Table 1. EFFECTS OF EDNA ON REGENERATION IN MUSCLE AFTER SURGICAL INJURY

Experiment	Cases	Muscle fibres (regeneration/injured)	Index
Untreated	5	0.442 $\pm$ 0.076 S.D.	100.00
EDNA, 24 h before wounding	4	0.685 $\pm$ 0.077 S.D.	154.97
EDNA, 24 h after wounding	3	0.682 $\pm$ 0.045 S.D.	149.77
EDNA, 45 h after wounding	5	1.759 $\pm$ 0.220 S.D.	397.96

The above specimens were fixed and processed 96 h after wounding for reasons given in refs. 6-8. The rationale in the timing of experiments may be found in ref. 1. Three sections, about 10 per cent of the wound area, were randomly selected for counting in each case. S.D., Standard deviation.

Table 2. INFLUENCE OF EDNA ON SYNTHESIS OF ACTOMYOSIN IN REGENERATING MOUSE MUSCLE

	Specific activity*		Means
	Aliquot 1	Aliquot 2	
Controls			5,090
1	5,487	5,318	
2	4,660	4,762	
3	5,164	5,160	
EDNA-treated			7,997
1	6,325	6,206	
2	8,564	8,765	
3	8,818	9,009	

\* (D.p.m.  $^{14}\text{C}/\text{O.D.}_{275}$  actomyosin  $\times \text{O.D.}_{275}$  sarcoplasmic proteins) minus specific activity of actomyosin from uninjured tibialis anterioris.

117 h ( $\pm 3$  min) after wounding. Animals were killed 161 h after wounding, and their tibialis anterior muscles were dissected for the extraction of actomyosin. Synthesis of this protein complex was assessed by liquid scintillation counting. Specific activities, recorded in Table 2, were of the order of about 30 per cent to 80 per cent greater in EDNA treated animals than among controls.

The aforementioned evidence encouraged experiments with genetically dystrophic mice (*Re 129/dydy*). Muscle regeneration is spontaneous in these animals. Male *dydy* mice born within 3 days of each other were divided into two groups of three animals. One group was maintained on ground 'Purina' chow and the other on the food taken from the same bag and blended with 0.2 per cent (w/w) EDNA. The tibialis anterior muscles of these animals were prepared for histological examination 13 days later. There were appreciably greater numbers of myotubes in the muscles of the three EDNA treated animals than in their controls. To specify this in quantitative terms, eight sections (approximately 10 per cent of the muscle) were randomly selected from the tibialis anterior of a control and an experimental animal that happened to have been litter mates. The number of myotubes estimated for each unit area showed that the factor of increase in regeneration was approximately two (Table 3). During normal regeneration myotube nuclei increase progressively<sup>7,8</sup>. The numbers of nuclei in each myotube reflect maturation and therefore serve as an index of the quality of regenerative activity. Tubes with smaller numbers signal the advent of new foci of regeneration. Those with greater numbers indicate more advanced stages of differentiation. In control dystrophic specimens the number of nuclei in a myotube tended to cluster between four and ten, and in no control case were there more than twenty nuclei in a tube. In the EDNA treated cases the number of nuclei in each myotube varied considerably; there were many instances in which the number exceeded

Table 3. INFLUENCE OF EDNA ON REGENERATION IN GENETICALLY DYSTROPHIC MOUSE SKELETAL MUSCLE

Section	'Purina' chow (new fibres/mm <sup>2</sup> )	Section	'Purina' chow + EDNA (0.2 g/kg) (new fibres/mm <sup>2</sup> )	F
1	5.00	1	12.50	
2	5.17	2	15.18	
3	5.75	3	13.68	
4	11.32	4	15.18	
5	6.29	5	16.49	
6	5.77	6	16.96	
7	5.62	7	13.20	
8	7.89	8	11.38	
Means	6.60 $\pm$ 1.97 S.D.		14.32 $\pm$ 1.83 S.D.	57.749*

The bellies of left tibialis anterioris were trimmed into rectangular blocks and were sectioned tangential to the anterior surface. The data in the table were obtained for randomly selected sections of one control and one experimental case. These sections were examined with a dissecting microscope to ensure uniform density and while in place they were sized with a stage micrometer. The number of sections in which counts were made constituted approximately 10 per cent of the total. Tissues were alike with respect to density per unit area, size of non-regenerative muscle fibres and extent of degeneration.

\* Normal distribution of F (99 per cent) for 7 d.f. = 7.00.

Table 4. INFLUENCE OF EDNA ON REGENERATIVE ACTIVITY AS INDICATED BY THE NUMBERS OF NUCLEI IN A MYOTUBE

	Nuclei in myotubes (grouped)					
	2-3	4-5	6-10	11-15	16-20	21-37
Control	2	45	45	7	1	0
EDNA-treated	13	14	44	16	8	6

Using the specimens described in the legend to Table 2, ten sections were randomly selected; nuclei were counted in each myotube of each section. Eighty-two myotubes were found in the control sections and 149 in the experimentals. Values were equated to a hundred myotubes for simplicity.

twenty (see Table 4 for more rigorous examination of this point).

Dystrophic mice maintained on control or experimental regimens over a period of several months showed no differences in survival rates or body weight. Muscular dystrophy was in no sense "cured". After about 8 consecutive weeks, however, EDNA treated animals had gained feeble, although measurable use of their hind limbs (Table 5). Slow motion films of animals taken before treatment and after several months of feeding on EDNA revealed a pumping motion in the hind limbs that was not observable at the outset of the investigation nor in control animals.

Table 5. INFLUENCE OF EDNA ON CLIMBING ABILITY OF DYSTROPHIC MICE

Animal	Diet	No. of attempts	No. of climbs	Percentage climbs
Normal*	'Purina'	10	10	100
Normal*	'Purina' plus EDNA	10	10	100
Dystrophic†	'Purina'	8	2	25
Dystrophic†	'Purina' plus EDNA	10	9	90

There were four animals in the dystrophic, untreated group and five in each of the others. Each animal was tested twice. Learning became a variable in further testing. Treated animals continued to climb successfully. The untreated animals quickly learned, however, that falling bore no untoward consequences and eventually they made no attempts to sustain themselves. These factors contributed spuriously to the weight of the argument. The results recorded above represent the first two attempts made by the animals to accomplish the climbing task. The two successful climbs among untreated animals were achieved without the use of hind limbs. The treated animals were able to use their hind limbs and did so in all attempts.

\* *Re 129/Dydy*.

† *Re 129/dydy*.

Effects of EDNA also were evaluated in pure bred beagle dogs. The dogs suffered a low-grade myopathy that had been induced by prolonged vitamin E deficiency and copper and selenium deprivation<sup>9-11</sup>. There were eight dogs, four from each of two litters born 4 days apart. Animals were brought into the laboratory as weanling pups. They were immediately started on the vitamin E deficient regimen with deionized water for drinking. A salt mixture free of copper and selenium was added to their diet along with vitamins other than E (refs. 9-11). One dog from each litter was kept on a normal kennel ration throughout the study. The deficient regimen was assayed with weanling rabbits (because of high sensitivity to vitamin E deficiency). Rabbits succumbed in 29 weeks. Estimating from the data of others<sup>12</sup>, our methods were roughly 40 per cent efficient in producing muscle lesions. Dogs were trained for 9 months. Each animal was taught to pull a sledge counterpoised with his body weight. The test course was 48 m, linear, smooth-surfaced, air conditioned and in a pressure-regulated room immediately adjacent to the kennel. Each animal ably and eagerly negotiated the 48 m, reproducing his performances within a fraction of a second. Weights could be varied  $\pm 10$  per cent without altering performance. Preliminary tests were conducted until there was no question that deficiently fed dogs went through the course more slowly than their normally fed siblings. In the test itself, each dog was clocked through the course at roughly the same time of day, twice weekly for 42 consecutive weeks. The test interval was subdivided into four experimental periods (I-IV). Each dog's performance was analysed individually on the basis of differences between periods. Comparisons between dogs were made on the basis of differential rather than absolute values. No EDNA was administered to any dog during period I. During period II three deficient dogs were maintained on a daily dose of 10 mg/kg of EDNA fed by capsule; the other three deficient animals were untreated. During period III EDNA was withheld. In period IV two deficient but previously untreated dogs were given the compound. Dogs, of course, were appropriately isolated at all times.

Data compiled in Table 6 and analysed in Table 7 leave little doubt that EDNA acted in some manner to enhance pulling capacity. About 4 consecutive weeks of treatment with the compound were required before beneficial effects were manifested. About a week after withdrawal of EDNA the previously treated dogs showed



Table 6. INFLUENCE OF EDNA ON PULLING CAPACITY OF DOGS

Dog	6-13 Weeks (I)	16-29 Weeks (II)	30-34 Weeks (III)	38-42 Weeks (IV)
Duke	19.7 ± 0.54	19.5 ± 0.52	20.0 ± 0.17	19.3 ± 0.85
Pete	18.7 ± 0.34	18.9 ± 0.46	19.1 ± 0.46	18.4 ± 0.44
Joe	24.6 ± 0.75	23.8 ± 3.13	24.8 ± 0.80	20.8 ± 0.49*
Spike	23.1 ± 0.71	22.6 ± 1.00	24.0 ± 0.48	23.4 ± 0.25
Pat	26.4 ± 1.37	20.5 ± 0.54*	24.0 ± 0.98	25.7 ± 1.40
Mike	22.6 ± 1.30	20.8 ± 0.81*	24.8 ± 1.39	24.3 ± 0.63
Dan	28.1 ± 1.26	23.9 ± 1.51*	30.3 ± 2.04	23.2 ± 0.02*
Andy	28.1 ± 1.41	27.5 ± 3.24	28.3 ± 0.51	20.8 ± 0.42*

Duke and Pete were on a normal diet throughout the investigation; all others were on the vitamin E deficient ration with deionized drinking water. Dogs were isolated during their entire time in the laboratory.

Roman numerals correspond approximately to the periods in Table 5. Time in sec; course, 48 m; load, body weight.

\* Dogs given EDNA.

Table 7. INFLUENCE OF EDNA ON STRENGTH OF MYOPATHIC DOGS AS A FUNCTION OF PERCENTAGE OF CHANGE IN PULLING TIME FOR DIFFERENT PERIODS IN THE INVESTIGATION

	$\Delta$ I-II (6-15 weeks versus 16-24 weeks) $t(P, 0.01)$		$\Delta$ II-III (16-29 weeks versus 30-34 weeks) $t(P, 0.01)$		$\Delta$ III-IV (30-34 weeks versus 38-42 weeks) $t(P, 0.01)$	
	$\Delta$ per cent = 3.06		$\Delta$ per cent = 5.84		$\Delta$ per cent = 5.84	
Duke	-1	1.39	+1	4.00	-3	1.85
Pete	+1	1.64	+2	1.95	-1	1.53
Joe	+3	0.96	+4	2.80	-16†	18.53*
Spike	-2	1.87	+6†	6.54*	-2	5.41
Pat	-22†	40.97*	+17†	8.01*	+7	2.72
Mike	-7†	7.00*	+19†	6.45*	-2	1.73
Dan	-14†	10.42*	+26†	7.03*	-23†	15.60*
Andy	-2	0.69	+2	3.52	-26†	40.11*
Dogs on	Pat, Mike, Dan		None		Joe, Dan, Andy	

Duke and Pete had normal diet throughout.

\*  $P < 0.01$ .

† Significant enhancement in strength or decrease in pulling time.

‡ Significant decline in strength or increase in pulling time.

marked increases in time through the course; that is, they had become measurably weaker after the treatment was discontinued. Reintroduction of EDNA led to significant increases in speed, again after about 4 weeks.

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## Action Potential Phenomena in Experimental Bimolecular Lipid Membranes

A MOLECULAR mechanism which develops multivibrator kinetics such as those shown in Figs. 1 and 2 has been constructed in experimental bimolecular lipid membranes by the methods given here. These electrokinetic phenomena are indistinguishable from cellular action potentials and include thresholds, refractoriness, delayed rectification, bistable flip-flop kinetics, anodal break excitation, self-regenerative changes of membrane potential accompanied by decreases in conductance, and spontaneous rhythmic firing, the frequency of which can be precisely controlled by varying the temperature and the applied electrical polarization. They are also reversibly blocked by local anaesthetics such as cocaine at physiological concentrations (2 per cent) and are modified in various ways by acridine and phenothiazine derivatives including chlorpromazine at concentrations of about 1  $\gamma$ /c.c.

The membranes, separating two aqueous phases, are made by methods presented elsewhere<sup>1,2</sup>. The membrane solution is composed of 2.5 per cent commercial purified sphingomyelin in  $\alpha$ -tocopherol:chloroform:methanol,

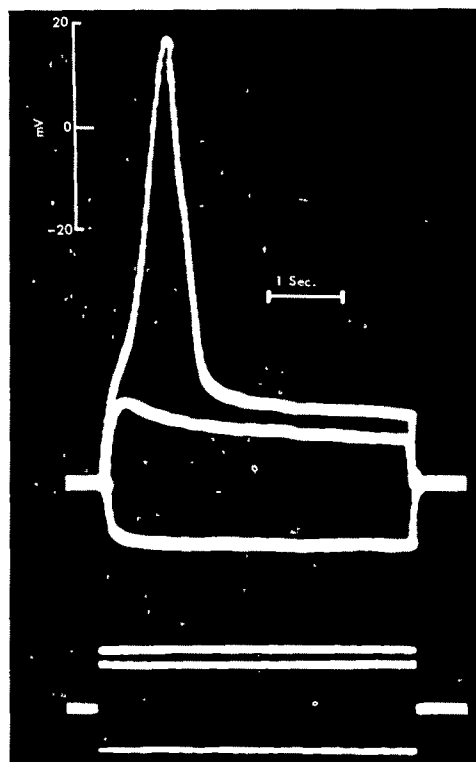


Fig. 1. Action potential and subthreshold response in an experimental bimolecular lipid membrane (upper) in response to applied rectangular constant currents (lower). Experimental conditions as in Fig. 3 except that potassium sulphate was used inside and 2 mg phosphatidyl serine were added to 1 c.c. of the membrane solution of the following composition: 0.06 g crude winter steer heart lipids, 0.05 g  $\alpha$ -tocopherol, 0.04 g tetradecane in 1 c.c. 3:2 chloroform:methyl alcohol. After the resting potential and delayed rectification were fully developed as in Fig. 3, protamine sulphate was added to give  $10^{-4}$  g/c.c. in the inside compartment. A few seconds of depolarization to +10 mV was required to initiate the protamine effect. After this action potentials appeared on stimulation as shown. Their threshold, amplitude and duration were constant provided the interval between stimuli exceeded the relative refractory period of about 10 sec. The resting membrane resistance was about  $10^8 \Omega \text{ cm}^2$ . Large decreases (10-30 times) and sometimes small increases in resistance accompany the action potentials, depending on the lipids. The geometry of the experimental system precludes the recording of propagated action potentials because the potential is macroscopically uniform over the entire membrane.

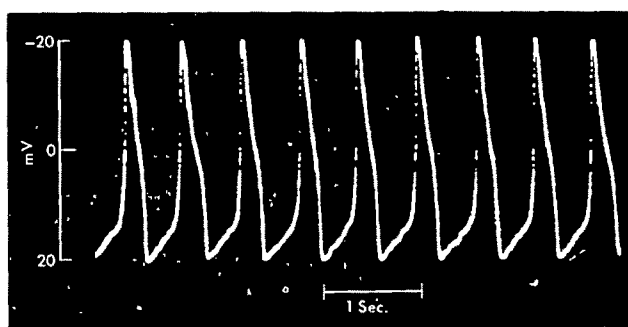


Fig. 2. Rhythmic action potentials. Experimental conditions as in Figs. 1 and 3 except that membrane lipid was purified sphingomyelin and potassium monohydrogen orthophosphate was used as electrolyte. Temperature  $40^\circ \text{C}$ . In sphingomyelin membranes spontaneous rhythmic firing in the absence of external polarization often occurs. It requires careful titration of EIM and protamine but is not yet under complete experimental control. With small depolarizing currents, however, rhythmic activity can always be induced and maintained for hours. Membrane lifetimes of 30 min-3 h are the limiting factor in this state. The rhythmic action potentials often show an initial spike and plateau like those found in heart muscle. The firing frequency increases with temperature. In this preparation the quasi-stable resting level was positive on the inside and the action potentials go towards the usual cationic resting potential level, that is, are inverted because of a slight excess of protamine. A small steady outward current maintains the rhythmic state. The resting membrane resistance was about  $5 \times 10^8 \Omega \text{ cm}^2$ . Except for the EIM these action potentials have also been made entirely from synthetic components (stearyl sphingomyelin and poly-arginine;  $\alpha$ -tocopherol). Synthetic aldosterone, 10 mg/c.c., generally strengthens the membranes.

5:3:2. The commercial product is usually repurified by silica gel *G* column chromatography as follows (Trams, E., personal communication). One gram of sphingomyelin is heated to boiling point in 20 ml. petroleum ether. Methanol is added until the solution is clear. This is then mixed with 10 g of 200 mesh silica gel and eluted from a column at 2 c.c./min with 60 c.c. each of petroleum ether; petroleum ether:chloroform, 1:1; chloroform:methyl alcohol, 1:1, 1:2, 1:3, 1:4 and 0:1. The last three fractions are collected under nitrogen, pumped dry and stored under nitrogen in glass ampoules below 0° C. Cadmium precipitates of crude acetone or methanol extracts of winter beef heart followed by removal of the cadmium<sup>3</sup> also provide useful membrane lipids.

After the formation of the membrane in 5 millimolar histidine chloride, pH 6.8, at 25° C, one compartment (denoted as the inside) is brought to a concentration of 10–50 mmoles/l. monohydrogen potassium orthophosphate or potassium sulphate and to 10<sup>-4</sup> g/c.c. of a crude proteinaceous excitability inducing material (EIM) obtained from *Aerobacter cloacae* A.T.C.C. 961 grown overnight in egg white media pH 6.8 at 37° C and stabilized by pan drying at 50° C or by lyophilization. As shown in Fig. 3, the membrane resistance then falls from 10<sup>8</sup> Ω cm<sup>2</sup> to 2 × 10<sup>6</sup> Ω cm<sup>2</sup> and a negative resting potential of about 50 mV develops on the inside in a few minutes. Under these conditions the system produces a strongly developed voltage restoring effect with applied depolarizing currents called delayed rectification. Protamine sulphate is next titrated into the inside compartment to an approximate concentration of 10<sup>-4</sup> g/c.c. After carefully adjusting the ratio of protamine to EIM, fully developed action potentials appear which include the single response to an applied pulse in Fig. 1 and the rhythmic firing shown in Fig. 2, as well as the other characteristics already mentioned.

The four components of the fully developed system are chemically specific. Histones, cholinesterase and other basic proteins cannot substitute for protamine. The protamine cannot act in the absence of EIM, which implies that it couples either directly or indirectly by way of the lipids with EIM in the membrane. This coupling requires sphingomyelin and is depressed by increasing the ratio of lecithin to sphingomyelin. EIM is itself sensitive to endopeptidase but not to exopeptidase (Trams, E., and Bukovsky, J., personal communication). It has a molecular weight of less than 10<sup>6</sup> and is dialysable through 'Schleicher and Schuell B20' membranes. It has also been found in acetone powders of human red cell ghosts and brewers yeast as well as in *Aerobacter* and *Serratia*. It has been purified two-thousand-fold after growing the bacteria in synthetic media and is then active at 10<sup>-8</sup>–10<sup>-9</sup> g/c.c. (Bukovsky, J., and Kushnir, L., personal communication). The final component, the salt gradient, also controls the nature of the electrokinetics. Monovalent anions tend to generate bistable flip-flop kinetics while phosphate or sulphate ions tend to generate monostable or unstable multivibrator kinetics, that is, single or rhythmic action potentials. Calcium (II) ions added in millimolar concentrations block rhythmic firing and change the thresholds. Except for secondary differences, EIM and protamine will function when placed together on either side of the membrane or in opposite compartments. Thus the direction of the ion gradient provides the only sense of polarity across the membrane.

It is thought that EIM develops channels which selectively conduct cations, develop potentials according to

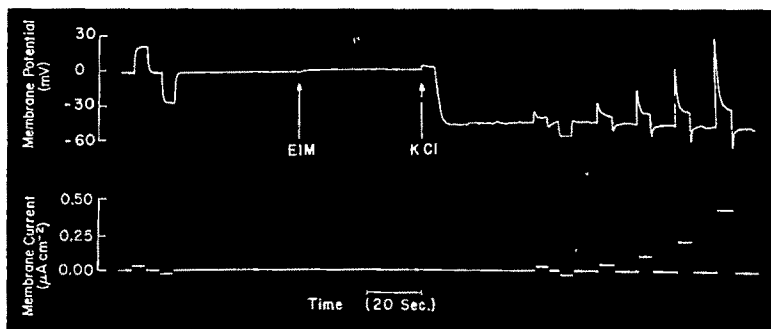


Fig. 3. The development of conductance, resting potential and delayed rectification in a bimolecular membrane. The membrane, 1.0 mm<sup>2</sup> in area, was formed at 25° C, separating two aqueous compartments containing 5 mmolar histidine chloride buffer at pH 6.8. Test pulses were applied through series resistors which were usually kept higher than the membrane resistance so that the membrane current was constant during the pulse. The membrane potential was recorded with a differential cathode follower<sup>16</sup>. The first test of the membrane resistance was made by applying ±80 mV through a series resistance of 2 × 10<sup>8</sup> Ω which gave the two voltage pulses on the left. At the arrows, 0.1 c.c. of an 0.05 g/c.c. solution of crude EIM and 0.1 c.c. of a 2 moles/l. potassium chloride solution were added to one compartment of 4 c.c. and stirred. Shortly thereafter the membrane resistance began to fall and a resting potential appeared which was 50 mV negative on the side of the potassium chloride, defined as the inside. Within 2 min a strong delayed rectification appeared, that is, the membrane resistance and potential decrease during the applied constant outward currents. Under these conditions, the steady state current voltage, *I*(*V*), curve shows that the ratio of the membrane resistance (*R* = *V*/*I*) at the resting potential to that at zero potential can be as large as 1,000:1. After addition of EIM the conductance continues to rise linearly for periods from 30 min to several hours depending on lipid type and can reach 10<sup>-1</sup> mhos cm<sup>-2</sup>.

the Nernst equation and contain a dual gating mechanism corresponding to the two negative resistance region existing in the *I*(*V*) curve published elsewhere<sup>4</sup>. Protamine then apparently converts the selective conductance of a fraction of these channels to anions while leaving the gating mechanism unaltered. Thus two channel populations arise and the interplay between the voltage coupled permeability changes and the opposing electromotive forces of the two channel types generate the observed electrokinetic phenomena. This interpretation is consistent with the general requirements of the Hodgkin-Huxley theory for action potentials in squid nerve<sup>8</sup>, although in this case anion channels substitute for sodium channels, and a universal gating mechanism generates the permeability functions. Action potentials, produced by alternating cation-anion permeability, are found in algae, *Chara*<sup>9</sup> and *Nitella*<sup>7</sup>, and voltage controlled chloride permeability also contributes to action potentials in electroplax<sup>8</sup>. Although EIM does not show any appreciable selective conductance among the monovalent cations, this feature can be introduced into experimental bilayers by certain macrocyclic antibiotics such as valinomycin and nonactin, which develop as much as 160 mV between 0.10 molar solutions of sodium and potassium chloride<sup>9</sup>.

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### Coupling a Sensory Artefact to the Brain

THE construction of artificial analogues of sensory receptors has recently become practicable. There are a number of electronic and electro-mechanical techniques for converting external stimuli into discharge trains similar to those initiated by receptors in the afferent peripheral nerves. It is more difficult, however, to devise satisfactory ways of coupling this type of mechanism to the brain so that the sensory influx provides information resulting in the evolution of a realistic motor programme. Although progress has been made in unravelling the "sensory coding" along some receptor channels<sup>1,2</sup>, much remains to be learned about the nature of sensory signals, their spatio-temporal organization and their interaction patterns. This communication briefly describes an attempt to couple a sensory artefact to brain, which uses a biologically destructive stimulus as an input source and which feeds a neural centre, the output of which has been empirically determined to be appropriate to such an input.

The radiation detector used in these experiments consisted of a small mercury two transistor power supply driven by batteries, which fed a Raytheon scintillation tube counter the output of which drove a Schmidt trigger feeding a small amplifier. Each radiation count resulted in the production of a square wave of 0.5 msec duration, adjustable in amplitude from 0.5 to 2 volts; this served as stimulus. The entire system was built up of miniaturized elements and mounted in an impact resistant plastic case slightly smaller than a cigarette packet which was fitted to a leather neck and chest harness worn without apparent discomfort by the cat. Stimuli were fed over a short length of insulated wire to a Winchester miniature octagonal plug mating with a female socket previously implanted in the skull. The implanted electrodes consisted of two stainless steel enamelled 30 gauge wires with tips sharpened to about 50 $\mu$  and separated from each other by 3 mm. A slightly coarser central shaft maintained the intertip distance and gave a firm purchase to the electrode holder of the stereotaxic instrument during implantation. Once the electrodes were in place and the dental cement had set, the central shaft was cut off close to the skull while the two leads were soldered into appropriate terminals on the under surface of the indwelling plug.

Four stimulation sites were used: ventro-postero-lateral (VB) and dorso-medial (DM) nuclei of thalamus, mesencephalic tegmentum (Teg), and sensori-motor cortex (Cx). Stimulation of the last site was achieved using a pair of stainless steel screws the blunted tips of which penetrated the dura. Parameters for position of the depth stimulation sites were selected through the use of the stereotaxic charts of Jasper and Ajmone-Marsan<sup>3</sup>. The position of the electrode tips was later checked histologically.

Of the five animals on which this report is based, each was provided with at least two sites for stimulation. Three cats presented dorso-medial and tegmental stimulation sites, while two carried ventro-postero-lateral and sensori-motor cortex stimulating electrodes. Four other animals were eliminated because of unsatisfactory histological control data and one because of apparent difficulties with the electrode insulation.

We used a radiation source of 1 mr. and a Heathkit Geiger-Müller radiation counter. The source was contained in a small cylindrical metal case about 2 cm in diameter and 1 cm in height and was already hidden when the animal was brought into the room. Different sites were chosen for each run to prevent the animal from associating a particular area with unpleasant sensations. The animal was allowed freedom to wander in a room (approximately 10 ft. by 14 ft. in size) which contained a number of familiar objects (feeding pan, box, crumpled paper) in or behind one of which the radioactive source was hidden. Animals were tested on alternate days, and every other run with the radiation sensor in place was a

"cold" run to prevent association of the pack with stimulation. Finally, each animal carried the pack on a total of only six occasions, three of them being tests with the source. The total stimulation period varied from 1 to 3 min, so that the chances of significant changes in the junction between tissue and electrodes were small. The histological controls were examined for evidence of damage at the electrode tips and changes were invariably minimal. Each run was watched by three observers, at least one of whom was ignorant of the site at which the radioactive source was hidden, and even with the character of the trial. Results were noted by each observer, then pooled and compared after all runs were finished and the animal had been killed.

Table 1 summarizes the electrode placements and the results attained in this investigation. In each of three dorso-medial implanted animals, entry into the radiation field resulted in immediate increase in motor activity, with crouching, tail lashing, and rapid head movement, accompanied by some variation of propulsive or retro-pulsive activity serving to move the animal out of the field. In every case except one, this field escape activity which removed the animal from the effective radius of the source (2-5 ft.) was completed in 3 to 10 sec. As soon as this was achieved, there was noticeable diminution of rapid motor activity, and a more leisurely sniff and search routine was reinstituted. On one occasion, cat R 5 froze at the border of the effective field (about 5 ft. from source) and remained in crouching stance, pilo-erected and tail lashing for at least 30 sec before slinking away.

Results of stimulation in the three other sites were variable, and in no case produced locomotor activity serving to remove the animal from the radiation field.

The introduction of sensory data from an artificial receptor directly into the central nervous system raises the problem of presenting such data in a meaningful manner. Because present knowledge of coding mechanisms is still inadequate to the task, other stratagems are necessary. The introduction of artificially induced signal trains into neural centres which produce empirically determinable outputs is one possibility, and has been of use in the self stimulation studies reported by Olds<sup>4,5</sup> and others.

In the present situation, an empirically demonstrated effect of dorso-medial stimulation is the output to which a stimulus from an appropriate input is matched. The studies of Roberts<sup>6</sup> among others have emphasized the effects of dorso-medial nucleus stimulation in cats, which include fear-like crouching and skulking with dilated pupils and—as stimulation voltages are raised—locomotor escape activity executed in a low slinking posture. In man, lesions performed stereotaxically in the dorso-medial nucleus produce apparent freedom from the suffering component in intractable pain<sup>7-9</sup> as well as much of the anguish in certain psychotic syndromes<sup>10</sup>. Anatomical analysis of this area suggests a very complex presynaptic

Table 1

Animal	Dorso-medial	Experimental results		Sensori-motor cortex
		Tegmental	Ventrobasal	
R2	Increased motor activity, tail lashing, backing out of field	Heightened sniff and search routines, looking around		
R3		No obvious effect		Raising right hind limb, licking foot
R5	Increased motor activity, pupillary dilatation, crouching, then forward slinking motion out of field			Turning head to right, preening right side
R7	Ears back, staring over one shoulder or other, slinking forward or sideways out of field		Scratching flank	
R10		Constant head turning, easily startled	Preening fur over right side	

field which combines elements of the ascending brain stem and thalamic reticular formations, the spinothalamic bundle, intrathalamic relays, tecto-tegmental systems, massive corticofugal bundles from prefrontal cortex and finer ascending components from hypothalamus<sup>11-15</sup>. Its position would appear to be critical for the integration of a number of specific, non-specific and autonomic components associated with nociceptive stimuli and vigilance. The output of the field appears associated in man and animals with very disagreeable effects often associated with motor patterns programmed to remove the organism from the stimulus if possible. In these experiments, all three of the dorso-medial implanted animals promptly instituted motor activity of one sort or another tending to remove them from an area apparently associated with discomfort or suffering. Because the source of these stimuli was a radiation detector and the intensity of intracerebral stimulation was proportional to the strength of the radiation field, it follows that the degree of motor escape activity instituted by our cats was some function of the intensity of the radiation field.

The possibility of directly mating artefacts to brain clearly remains limited until more is learned about the coding systems and about the techniques for artificially innervating neural fields. For most applications, it seems sufficient to convert stimuli for which no biological receptors exist into forms which can be interpreted. There may, however, be advantages in the introduction of certain physicochemical stimuli into the brains of animals unable to use artificial devices for converting such stimuli into usable data.

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### Impulses from Single Nerve Fibres recorded in Man using Microelectrodes

Our knowledge of impulse patterns in the different types of afferent nerve fibres is based chiefly on animal experiments. It is true that Hensel and Boman<sup>1</sup> recorded action potentials from single cutaneous nerve fibres in man using the microdissection method, but this method cannot be used in routine clinical examinations. Moreover, it only allowed investigation of the cutaneous nerves and not of other types of nerve fibres. We therefore considered it of interest to develop a method for recording from single

nerve fibres in man using microelectrodes. For this purpose glass-coated platinum-iridium wire electrodes were used, made according to the method described by Wolbarsht and Wagner<sup>2</sup>. The wire had a diameter of 400 $\mu$ . It was electrolytically polished to a tip of 0.5–1 $\mu$ . As shown by Wolbarsht and Wagner, it is possible to free the metal tip from the glass insulation over a surface of optional size and thereby obtain electrodes of different impedance. In our electrodes the impedance ranged between 40 and 100 k $\Omega$  at 1,000 c/s. The electrodes were sterilized by immersion in boiling water for 20 min and then connected to a preamplifier with a cathode follower, the action potentials being displayed on an oscilloscope. A loud-speaker and a tape recorder with a frequency response of 400–19,000 c/s were also used.

The experiments were carried out on six healthy subjects between the ages of 20 and 30 years. All recordings were made from the ulnar nerve. After immobilizing the arm by embedding half the upper arm, the forearm and part of the hand in sandbags, the ulnar nerve just above the wrist was localized by determining the point on the skin at which maximal nerve action potentials were obtained when the little finger was stimulated electrically. The skin was cleaned both with 0.5 per cent bensenon spirit and with a 2 per cent chloramine solution and the micro-electrode was then inserted perpendicular to the skin surface into the nerve with the aid of a micromanipulator. Insertion of the microelectrode sometimes caused slight pain, but when it was held still with the tip in the nerve no pain was felt. A few of the subjects had mild paraesthesia in the hypothenar and/or little finger a few hours after the experiment, but none of them found this disagreeable, nor has any untoward reaction been encountered during the 6 months following the experiments.

Impulse activity was obtained from several hundred units and it was possible to record activity for several minutes—sometimes up to 20 min—from about seventy of these units in response to repeated skin stimulation or passive movements of the fingers.

Electrodes with high impedance were comparatively selective and recorded action potentials of 100–250  $\mu$ V amplitude which appeared to be unitary (Fig. 1A). They had an initial positive phase of very short duration, 0.2–0.5 msec, followed by a low negative phase of longer duration, which for the most part was due to the lower limiting frequency characteristics of the tape recorder. Conversely, electrodes with lower impedance generally picked up activity from more than one unit (B) and sometimes recorded composite action potentials varying in shape and amplitude from one discharge to another and

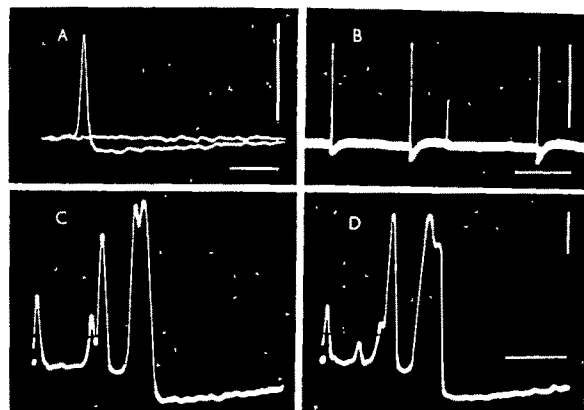


Fig. 1. Unit activity during tactile stimuli to the hypothenar (A) and fourth finger (C, D) and during passive abduction of the thumb (B). In every case the stimulus preceded the beginning of the sweep and outlasted the sweep. Records were obtained by playing back the tape recorder. In C and D the sweep was triggered by the first action potential, the foot of which is therefore not fully visible. Calibrations: in A, C and D, 200  $\mu$ V and 0.5 msec; in B, 200  $\mu$ V and 20 msec.

evidently originating from several units. Such action potentials are shown in *C* and *D*. They were evoked by gently stroking the dorsal surface of the first phalanx of the fourth finger. The shape of the discharges in *C* and *D* indicates that, on the whole, the same elements were activated in both instances.

The skin stimuli consisted of gentle stroking with the finger or a soft paint brush, or light pressure with the finger or a small blunt metal rod. Skin stimuli generally evoked brief discharges from several units but sometimes only a single discharge from one unit. It was possible to map out the receptor areas of the cutaneous fibres with a fair amount of exactitude. Movement of an interphalangeal joint elicited impulse activity which often continued during the greater part of the movement, but different units were active in different phases of the movement. A few units were activated by passive abduction of the thumb, which stretches the thumb adductor muscles. They responded with one or a few discharges during the movement. It is obviously not possible to state that the activity obtained emanated from muscle receptors as other deep and superficial receptors may also have been activated by the stimulus. It is, however, noteworthy that units activated by this as well as by other stimuli were all rapidly adapting. So far no units with tonic activity have been encountered.

The present investigation has established the possibility of recording impulses from single nerve fibres in man using microelectrodes without causing any appreciable discomfort or demonstrable injury. This technique presents new possibilities of studying the activity in different types of receptors in normal and pathological conditions.

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## Intracellular Responses from the Grasshopper Eye

THE compound eye of the grasshopper (*Schistocerca gregaria*) consists of several thousand ommatidia which are arranged in an orderly manner along the radii of the eye. Each ommatidium contains from five to seven cells arranged so that their inner edges meet and form the rhabdome. A capillary microelectrode was introduced into a single ommatidium following a procedure described by Fuortes<sup>1</sup>. All experiments were performed on preparations mounted in a perfused oxygenated chamber. Light from an 8 W incandescent filament was attenuated as desired by neutral filters. At zero attenuation, the stimulus intensity was approximately  $3 \times 10^{15}$  photons (absorbable between 4 and  $6 \times 10^3 \text{ Å}$ )  $\text{cm}^{-2} \text{ sec}^{-1}$ . Penetration of ommatidial cells is presumably reflected by a negative potential of 40–70 mV which, in darkness, may remain constant for periods up to 2 h in exceptional cells. This potential decreases during illumination.

The response recorded intracellularly to a brief rectangular step of light consists of two readily identifiable parts—a transient and a late component (Fig. 1*A*). The transient component has been observed for 5.4 log units of stimulus attenuation. The transient increases in amplitude from a barely observable response and undergoes several modifications of configuration until, at the highest intensities of the stimulus, it reaches saturation very close to the resting membrane level. A plot of amplitude against the logarithm of the attenuation is sigmoid in

shape. The late component follows the transient and can persist, often at a rather constant amplitude, for several hundreds of milliseconds. The relation between the amplitude of the late component and the logarithm of the stimulus intensity is approximately linear.

The two components are increased after a long step of light (Fig. 1, right column). The responses in this sequence are quite similar to those observed in the dragon fly<sup>1</sup> except that the potential oscillation following the transient is more damped in *Schistocerca* and usually appears only at intermediate intensities of stimulation. In contrast with the response in *Limulus*<sup>2</sup> and the drone<sup>4</sup>, nerve impulses were not observed in this preparation.

A third component of the response to light which is not easily observable is a spike on the ascending limb of the transient. This is occasionally seen as a discrete entity but more often as a notch on the rising transient. It is barely noticeable on slow sweeps (Fig. 1*A*) but can be seen more clearly on faster sweeps (Fig. 1*B*). The spike becomes quite evident, in some cells, during hyperpolarization (Fig. 1*C*). In those cells which respond with an early spike, depolarizing current in darkness evokes this spike (Fig. 1*D*) but no other components of the response to light.

The passage of current through the microelectrode changes the potential across the cell membrane. Steady hyperpolarizing currents increase the change of potential evoked by light stimulation (Fig. 1*C*) but depolarizing currents decrease it. Fig. 2 is a plot of the transient

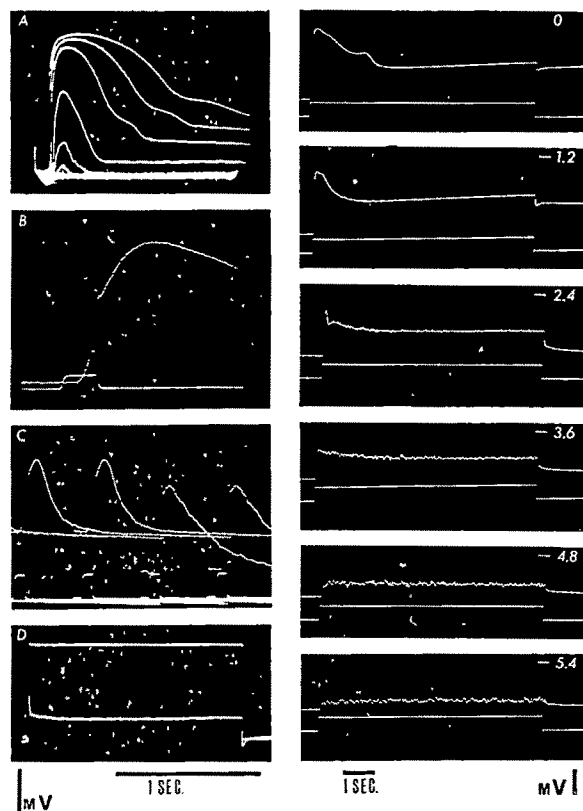


Fig. 1. Left column: *A*, superimposed responses to 25 msec steps of light of log attenuation 0, 0.6, 1.2, 2.1, 3.0, 3.9 and 4.8; impedance pulse is to far left. Interval between grid lines is 100 msec; *B*, response to zero attenuation stimulus of duration (lower trace) 22 msec for a different cell; *C*, response to zero attenuation stimulus at 1/sec before (left) and during (right) steady hyperpolarization of 2.5 nanoamperes for a different cell; stimulus duration, lower trace, is 22 msec; *D*, response to 1.5 sec depolarizing pulse of 3.5 nanoamperes in darkness; impedance pulse, on far left, precedes current pulse. Right column: responses to illumination with steps of light 7 sec long. The numbers in the top right-hand corner are the logarithm of relative light intensity. Discrete miniature potentials<sup>2</sup> are seen in the records at the greatest attenuations of stimulus which sum to give a "noisy" appearance to the response. Lower beam indicates duration of stimulus. For the entire sequence the time line is 1 sec and the calibration is 20 mV.



amplitudes evoked by three intensities of light during the passage of constant current.

Fig. 3 shows the relationship of the current and voltage of the cell of Fig. 1D. The relationship between current and voltage is linear for a short range before a hyperpolarizing response and rectification appear. Plots of the relationship of current and voltage from cells exposed to constant background light have different slopes from cells in darkness (Fig. 4). Membrane resistance is decreased in illuminated cells.

These observations have much in common with those made in *Limulus*, the dragon fly, the drone and the

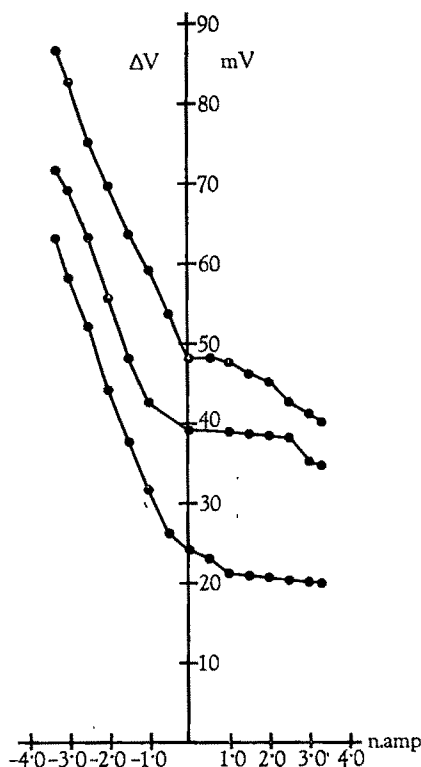


Fig. 2. Relation between the amplitude of a light evoked transient and the intensity of a steady current through the cell membrane. The top, middle and lowest plots are the responses to stimuli of log attenuation 0, 1.2 and 2.1 respectively. Duration of the stimulus was 22 msec.

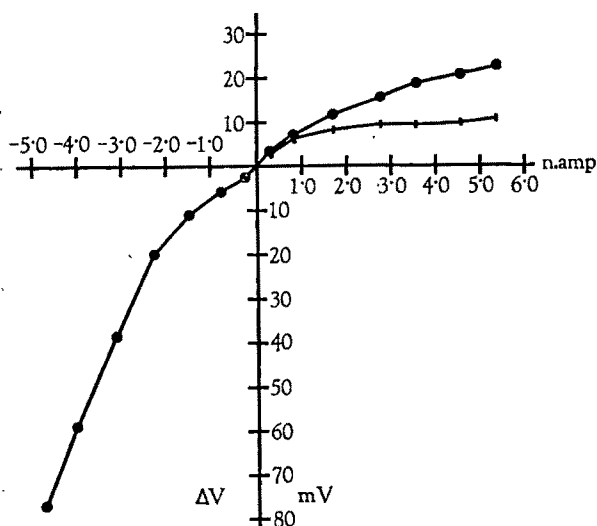


Fig. 3. Relation between the amplitude of the voltage change and the intensity of the current pulse through the cell membrane for the same cell as Fig. 1D. The response to depolarizing pulses (right) consists of a spike (closed circles) and a steady state component (bars). Slope of line through the linear portion of the curve, on either side of zero, is 7.7 Mohm.

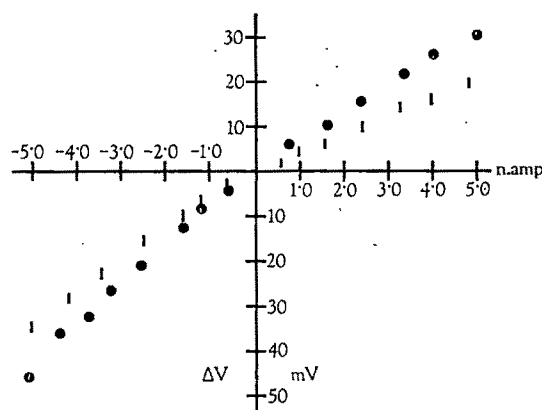


Fig. 4. Current-voltage relation in a cell which did not exhibit an early spike. Circles and bars represent responses obtained in darkness and with zero attenuation background light respectively.

worker honeybee<sup>4</sup>. It seems reasonable to assume that after a light stimulus there is an increase of membrane conductance in the visual receptors which generates potentials with a transient and late component. In *Limulus*, the multiple spikes which are observed on this potential are probably generated at a distance from the recording site and represent axonal discharges<sup>3</sup>. In *Schistocerca*, however, light or currents produce only a single spike which increases in amplitude when stimulation is increased. I therefore suggest that this spike is a local response generated by the receptor and not a reflected response from the cell axon.

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## MICROBIOLOGY

### Possible Extracellular and Intracellular Bactericidal Actions of Mouse Complement

THE pathogenicity of Gram-negative bacteria for mice has been related to their resistance to human or guinea-pig complement *in vitro* by Maaløe<sup>1</sup> and by Rowley<sup>2</sup>, while Jenkin<sup>3</sup> has suggested that serum factors are also involved in killing by macrophages. The use of human and guinea-pig complement in these experiments emphasizes the fact that no one has yet demonstrated bactericidal activity by mouse complement *in vitro*. Haemolytic activity, however, can be measured if special precautions are taken<sup>4</sup>.

Spiegelberg *et al.*<sup>5</sup> found that the clearance of dead *Escherichia coli* given intravenously to Swiss mice was reduced if complement had been removed by a previous injection of aggregated gamma globulin. By contrast, however, Stiffel *et al.*<sup>6</sup> failed to reduce the phagocytosis of *Salmonella typhimurium* when they de complemented Swiss mice by injection of egg albumin with anti-egg albumin. Moreover, clearance was the same in B10.D2 mice described by Rosenberg and Tachibana<sup>4</sup> as genetically lacking in complement.

This communication reports the results of some preliminary experiments using strains of *E. coli* sensitive to

complement (*WF* 96) and resistant to complement (*WF* 82) in *B10.D2* "old line" and *B10.D2* "new line" mice. These strains of mice are genetically identical except that the "old line" is genetically deficient in a component of complement probably corresponding to human  $C_6$  (refs. 7-9).

Bacteria were grown into the logarithmic phase in a minimal glucose medium<sup>10</sup> with added  $^{14}\text{C}$ -glucose. Under these conditions it is thought<sup>11</sup> that labelling is predominantly in the bacterial cell wall. Each strain of mouse was injected intravenously with each strain of *E. coli* in a dose of  $10^8$  living bacteria/100 g mouse. Blood clearance was followed by taking 0.05 ml. samples at intervals and measuring both the content of carbon-14 and the viable count.

At the end of 30 min the mice were bled and killed, and measurements of the carbon-14 content and viable counts were made on homogenates of liver, spleen and lungs. A calibration curve was made relating carbon-14 to viable counts in dilutions of the original inocula in which the viable count is known to equal the total count as measured by a Coulter counter. Total bacterial counts were then calculated for all samples of blood and organs on the assumption that there had been no loss of label and no bacterial multiplication. Control experiments using labelled *coli* in inactivated serum showed no loss of carbon-14 over a half-hour period but the viable count increased by about a quarter. If there is similar multiplication *in vivo* our calculated total counts are too low by about one-quarter because division reduces the activity of carbon-14 per bacterium.

Figs. 1 and 2 show the clearance curves for total and viable *E. coli* *WF* 96 (*C'* sensitive) and *WF* 82 (*C'* resistant) in *B10.D2* "old" and *B10.D2* "new" lines. Table 1 gives the corresponding phagocytic indices calculated from the early part of the clearance slopes as<sup>12</sup>

$$K = \frac{\log C_1 - \log C_2}{t_2 - t_1}$$

where  $C_1$  and  $C_2$  are the numbers of bacteria/ml. of blood at times  $t_1$  and  $t_2$  respectively. We have used the terms  $K_t$  and  $K_v$  according to whether  $K$  has been calculated from the total or viable counts.

For the *WF* 96 strains, which are sensitive to complement,  $K_t$  is the same in both strains of mice.  $K_v$  is

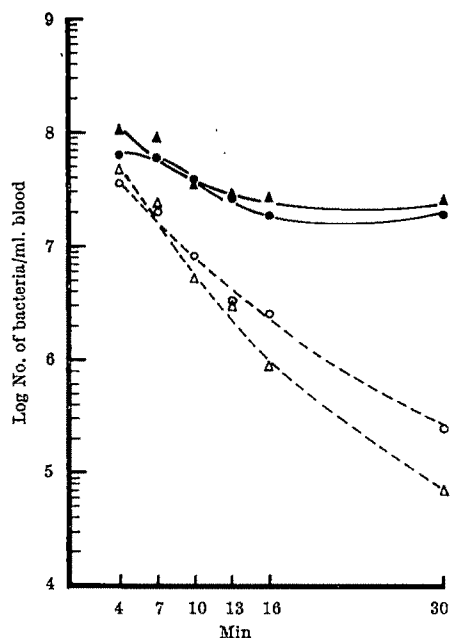


Fig. 1. Change of total and viable counts with time of *E. coli* *WF* 96 (*C'* sensitive) in the blood of *B10.D2* "old line" and "new line" mice. —●—, Total count "old line"; —▲—, total count "new line"; - - ○ - -, viable count "old line"; - - Δ - -, viable count "new line".

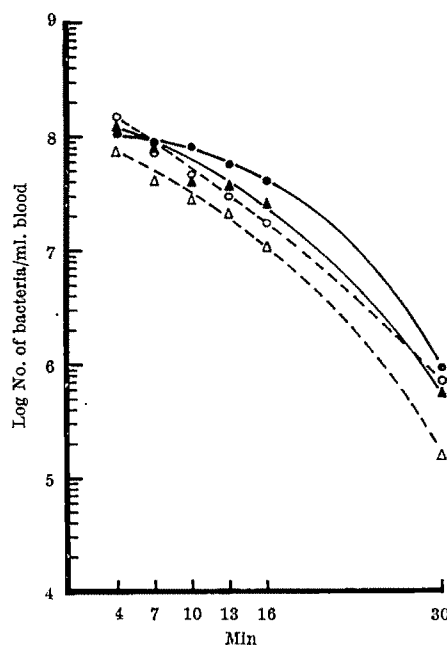


Fig. 2. Change of total and viable counts with time of *E. coli* *WF* 82 (*C'* resistant) in the blood of *B10.D2* "old line" and "new line" mice. —●—, Total count "old line"; —▲—, total count "new line"; - - ○ - -, viable count "old line"; - - Δ - -, viable count "new line".

roughly three times greater in mice with normal complement but only twice as great in the mice deficient in complement. In contrast, for the strains of *WF* 82, which are resistant to complement,  $K_t$  and  $K_v$  do not differ significantly and are the same in both strains of mice.

The finding that complement has little effect on  $K_t$  agrees with the results of Stiffel *et al.*<sup>6</sup>. The high  $K_v$  relative to  $K_t$  for *WF* 96 suggests killing of this organism in the circulation. The fact that in *B10.D2* "old line" mice this effect is less marked with *WF* 96 and absent with *WF* 82 suggests that it is due to complement.

The difference between the total and the viable counts of *WF* 96 in the blood increases markedly with time particularly in "new line" mice (Fig. 1). A much smaller difference develops with *WF* 82 (Fig. 2). Benacerraf, Sebestyen and Schlossman<sup>13</sup> found no difference in the clearance of live or dead *coli*, but they were measuring bacteria labelled with phosphorus-32 and not viable counts. If many bacteria are being killed in the circulation the question arises as to whether this is humoral or due to circulating phagocytes. Examination of stained blood smears showed 3,500-5,500 white cells/mm<sup>3</sup> with few bacteria in either cells or serum. Further investigation and differential counting are clearly required.

Table 2 gives the number of viable bacteria as a percentage of the total count in various organs. Underestimates of total count as already described explain why there are occasionally more viable than "total" bacteria.

The proportion of *WF* 96 surviving is greater in "old line" than in "new line" mice, by a factor of ten in the liver and of up to fifty in the lungs. The results are similar in the spleen except for an unexpectedly high proportion of survivors in one "new line" spleen. In contrast *WF* 82 shows much smaller differences between the two types of mouse and indeed in the liver survives rather less well in "old line" mice.

Table 1

Strain of mouse	<i>C'</i>	<i>WF</i> 96 ( <i>C'</i> sens.)		<i>WF</i> 82 ( <i>C'</i> resist.)	
		$K_t$	$K_v$	$K_t$	$K_v$
<i>B10.D2</i> "old line"	$C'_6$ defective	0.043	0.088	0.046	0.050
<i>B10.D2</i> "new line"	Normal	0.052	0.136	0.063	0.055

Phagocytic indices for *C'* sensitive *WF* 96 and *C'* resistant *WF* 82 strains of *Escherichia coli* in different strains of mice.  $K_t$  is calculated from total and  $K_v$  from viable bacterial counts.

Table 2. PER CENT OF BACTERIA FOUND VIABLE IN MOUSE ORGANS

Organ	Bacteria	Strain of mouse	
		B10.D2 "old line" (C <sub>3</sub> defective)	B10.D2 "new line" (normal C <sub>3</sub> )
Liver	WF 96	23	2
	(C <sub>3</sub> sens.)	23	2
		210	18
	WF 82	29	81
	(C <sub>3</sub> resist.)	45	100
Spleen	WF 96	74	169
		24	1
		35	13
	WF 82	38	111
		35	60
Lungs		81	64
		116	69
	WF 96	10	0.6
		25	0.8
	WF 82	51	0.9
		8	4
		15	7
		15	13

Thus within the organs the results again suggest that complement is important in killing bacteria, perhaps intracellularly. Unfortunately, because of the unexpected finding that considerable killing occurs in the circulation the results within the organs could also be explained on the basis that bacteria sensitive to complement are killed first and phagocytosed afterwards. Clearance experiments in whole animals are incapable of distinguishing between these two possibilities.

When evaluating the general significance of the results the possibility of species differences must be borne in mind. Roantree and Rantz<sup>14</sup> found that strains of *E. coli* isolated from patients with bacteraemia were likely to be resistant to complement. In rabbits, Roantree and Pappas<sup>15</sup> using a viable count method found that resistant strains of *coli* disappeared more slowly than sensitive strains at first and were more likely to reappear later. Rother and Rother<sup>16</sup> found that labelled killed *Salmonella typhi* were cleared from the blood at the same rate in normal rabbits and in a strain deficient in C<sub>3</sub>.

We agree with Rother *et al.*<sup>16</sup> and Stiffel *et al.*<sup>8</sup> that absence of a C<sub>3</sub> component has no effect on phagocytosis by the reticuloendothelial system. The absence of such a component, however, impairs the killing of an organism sensitive to complement.

Mouse complement may be inactive *in vitro*, but it is clearly bactericidal *in vivo*, probably extracellularly but perhaps intracellularly as well.

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## Wasting Syndrome in Thymectomized Immature Baboons (*Papio Species*) after Infection with Adenovirus Type 12

INDUCTION of neoplasms in newborn hamsters by the injection of adenovirus type 12 has been well established<sup>1-4</sup>. This virus has been found primarily in "normal" humans and then only infrequently<sup>5</sup>. The data presented here on the inoculation of immature baboons with adenovirus type 12 were obtained from a study designed to ascertain whether or not this virus would produce tumours. In order to enhance the oncogenic potential of the virus, thymectomy and splenectomy, irradiation and an immunosuppressive drug ('Imuran': 6-(1-methyl-4-nitro-5-imidazolyl) thiopurine) were used either singly or in combination.

The baboons (*Papio* sp.) were immature animals of ages varying from 3 months to approximately 3 yr with the majority under 1 yr of age. Experimental animals were maintained in strict isolation and were fed the standard ration used at this institution<sup>6,7</sup>.

The Hui strain of adenovirus type 12 originally obtained from Dr. J. J. Trentin (Baylor University School of Medicine) and adapted to baboon kidney cells (BKC), was used in this study. Repeated serum neutralization (SN) tests were performed (antiserum supplied by Dr. J. G. Winn, Communicable Disease Center, Atlanta, Georgia) to ensure specificity. Newborn hamsters were inoculated for oncogenicity. Passage of the virus back through HeLa or KB cells was done in order to maintain the titre. The titre remained virtually unchanged throughout the course of the experiment, that is, 10<sup>-3.5</sup>–10<sup>-4</sup> TCD<sub>50</sub>/0.1 ml. The stock virus remained free of pleuropneumonia-like organisms.

Each baboon received 50 ml. of undiluted virus suspension divided into intraperitoneal, subcutaneous, intrapulmonary and intravenous doses, with the exception of two animals (A-352 and A-373) which received 2.0 ml. by the same routes. Thymectomy and splenectomy were performed using standard surgical techniques<sup>8</sup>. Total body irradiation was from a cobalt-60 source which, when monitored, averaged 350–450 r. for each animal. 'Imuran' was given intraperitoneally in dosages analogous to those employed in human studies, that is, 3–4 mg/kg body weight daily.

Baboons were divided into groups receiving adenovirus type 12 either alone or in combination with: thymectomy and splenectomy; thymectomy, splenectomy and irradiation; 'Imuran'; thymectomy, splenectomy and 'Imuran'. In addition as controls two animals received only thymectomy and splenectomy; one animal thymectomy, splenectomy and irradiation; and two animals (D-101 and D-103) were maintained as uninoculated controls (Table 1).

Serological and virus isolation procedures employed in this study are considered standard for this laboratory and published elsewhere<sup>9</sup>.

Of thirteen immature baboons inoculated with adenovirus type 12 and which had either thymectomy, splenectomy, 'Imuran', irradiation or combinations of these procedures, six (46.2 per cent) have died at varying periods of time, 1–2 yr after inoculation (an additional animal (A.D-284) died by accidentally hanging itself in its cage). One thymectomy-splenectomy control and the only control animal with thymectomy and splenectomy plus irradiation died approximately 2 months and 1.5 yr following surgery and irradiation respectively. Although these two animals did not receive virus, they were kept in the same room with the infected animals and as a result were possibly exposed to infection. If all the thymectomized animals are considered, the mortality rate is 8 of 16 (50.0 per cent). Of the ten baboons considered as controls, five animals received only adenovirus type 12; three were thymectomized and splenectomized (one of these was also irradiated); and two animals were simply

kept in the same room with all these other animals. There has been one death (A-534) in the non-thymectomized-splenectomized group. These data, along with the experimental procedure used on each animal, are given in Table 1.

The mortality in the experimental series of animals is considerably in excess of the usual death rate in the Foundation's baboon colony, which has been 6-10 per cent over the past 3 yr.

During the course of the experiment a syndrome developed in this series of animals which resembled the "wasting" or "runt disease" reported in rodents<sup>10-13</sup>. X-ray evidence showed that seven of the animals had periosteal elevations particularly in the long bones (some more pronounced than others). These animals had a hunched posture with abnormal gait and general signs of debilitation. In addition to the above bone changes, nine of the animals evidenced weight loss. Two of the animals which received only virus showed marginal loss of weight and questionable bone changes on X-ray (Table 1). One of the three control animals died approximately 1-1.5 yr after inoculation. Alopecia occurred only in those animals which experienced weight loss and/or bone changes.

The animals which exhibited the most marked symptoms were A-594, A-539 and the two 'Imuran'-adenovirus type 12 animals, AD-283 and AD-289. Although the latter two did not develop bone changes, their weight and hair loss were extensive. The animal that killed itself

(AD-284) also exhibited early indications of this syndrome before it died.

Haematological studies performed regularly on each animal revealed only the development of transient leucopenia, eosinophilia and mild anaemia following irradiation<sup>14</sup>. Histological examinations have also failed to indicate conclusively or to suggest a cause of death. Bone lesions showed marked calcium depletion but no inflammatory or tumour cells. The compact bone layer was extremely thin as shown by fractures which occurred in two animals (A-539 and A-594). Histological examination of the parathyroids did not reveal anything.

All attempts to isolate an aetiological agent from any of the affected or dead animals have been unsuccessful. Bacteriological and mycological cultures have failed to reveal a pathogen. Attempts to recover adenovirus type 12 by passage of tissues in BKC, HeLa and WI-38 cells were negative. Four viral agents have been isolated from stool specimens. Neutralization tests indicated that these agents were not adenovirus type 12.

Development of antibodies as measured by complement-fixation (CF) and neutralization (SN) tests was extremely sporadic (Table 2). Animals which received a small virus inoculum (2.0 ml.) developed antibodies as expected, but erratic results were obtained in animals inoculated with 50 ml. of undiluted adenovirus type 12. Because of these results the animals were challenged with inactivated polio virus vaccine (Salk) obtained commercially. Again, the results were inconsistent and of little value for elucidat-

Table 1. EXPERIMENTAL PROCEDURE AND DISPOSITION OF IMMATURE BABOONS USED IN ADENOVIRUS TYPE 12, THYMECTOMY, SPLENECTOMY, 'IMURAN', AND RADIATION STUDY

Animal No.	Thymectomy-splenectomy	'Imuran'	400 r.	A <sub>12</sub>	Deaths	Periosteal changes	Weight loss	Hair loss
A-532				7-31-64		?	+	-
A-533				7-31-64		?	+	-
A-534				7-31-64	1-12-66	?	-	-
A-352				6-26-63†		-	-	-
A-373				8-6-63†		-	-	-
A-592	6-17-64			7-31-64	9-21-64	-	-	-
A-593	6-3-64			7-31-64		-	-	-
A-594	6-10-64			7-31-64	7-8-65	+	+	+
A-537	4-29-64		8-17-64	8-24-64		-	-	-
A-453	2-5-64		8-17-64	8-24-64		-	+	+
A-531	5-6-64		8-17-64	8-24-64		-	-	-
A-540	4-22-64		8-17-64	8-24-64	9-8-64	-	-	-
AD-283		4-29-64		7-31-64	8-9-65	-	+	+
AD-284		4-29-64		7-31-64	6-2-65*	-	+	+
AD-289		4-29-64		7-31-64		-	+	+
A-538	3-25-64	4-6-64		7-31-64	8-23-64	-	-	-
A-539	4-1-64	4-6-64		7-31-64		+	+	+
A-591	5-20-64	6-4-64		7-31-64		+	-	+
Surgical controls								
D-86	1-22-64					-	-	-
A-536	1-8-64				6-2-64	-	-	-
A-450	2-12-64		8-17-64		10-29-65	?	+	+
D-101	Uninoculated and untreated					-	-	-
D-103						-	-	-

\*Accidental death.

† Received 2.0 ml. undiluted virus; all other animals received 50.0 ml. virus.

Table 2. CF AND SN ANTIBODY RESPONSE OF IMMATURE BABOONS TO ADENOVIRUS 12 INOCULATIONS AFTER THYMECTOMY, SPLENECTOMY, 'IMURAN' AND RADIATION

Animal No.	Treatment	Preinoculation		1 month		2 months		Serum collected after:		9 months		12 months	
		CF	SN	CF	SN	CF	SN	6 months		CF	SN	CF	SN
A-532	A <sub>12</sub>	-	-	-	+	-	-	-	-	-	-	-	-
A-533	A <sub>12</sub>	-	-	-	-	-	-	-	-	-	-	-	-
A-534	A <sub>12</sub>	-	-	AC	-	AC	-	-	-	-	-	-	-
A-592	TSIA <sub>12</sub>	-	-	-	+	-	Died	-	-	-	-	-	-
A-593	TSIA <sub>12</sub>	-	-	+	+	+	+	-	+	-	+	+	+
A-594	TSIA <sub>12</sub>	-	-	+	+	+	+	-	+	-	+	Died	+
A-537	TSRA <sub>12</sub>	-	-	-	-	-	-	-	-	-	-	+	-
A-453	TSRA <sub>12</sub>	-	-	-	-	-	-	-	-	-	-	+	-
A-531	TSRA <sub>12</sub>	-	-	-	+	-	+	AC	+	-	+	AC	+
A-540	TSRA <sub>12</sub>	-	-	-	-	-	-	-	-	-	-	-	-
AD-283	IA <sub>12</sub>	-	-	-	-	-	-	-	-	-	-	-	-
AD-284	IA <sub>12</sub>	-	-	-	+	-	+	-	+	-	+	-	-
AD-289	IA <sub>12</sub>	-	-	-	-	-	-	-	-	-	-	-	-
A-538	TSIA <sub>12</sub>	-	-	-	-	-	Died	-	-	-	-	-	-
A-539	TSIA <sub>12</sub>	-	-	-	+	-	+	AC	+	AC	+	AC	-
A-591	TSIA <sub>12</sub>	-	-	-	-	+	-	-	-	-	-	-	-
D-86	TS	-	-	-	-	-	-	-	-	-	-	-	-
A-450	TSR	-	-	-	-	-	-	-	-	-	-	-	-
A-352*	A <sub>12</sub>	ND	ND	+	+	-	+	-	+	-	+	-	+
A-373*	A <sub>12</sub>	ND	ND	-	+	-	+	-	+	-	+	-	+

Except for control animals A-352 and A-373 virus inoculums were 50 ml. undiluted virus. Control animals received 2.0 ml. undiluted virus. T, Thymectomy; S, splenectomy; R, 400 r.; I, 'Imuran'; A<sub>12</sub>, adenovirus type 12.

\* Animals inoculated at birth. Mothers were free of adenovirus type 12 antibody.

+ Antibodies present.

ing the immunological capability of these animals. A larger number of animals are evidently required in order to draw any definite conclusions.

Although "runt" or "wasting" disease has been reported in rodents following neonatal thymectomy and inoculation with various viral agents, this observation has been confined to newborn animals<sup>10-12</sup>, and has not been reported in a sub-human primate. A "runt" or "wasting" type syndrome was produced in immature (3 months to 3 yr) baboons which had been thymectomized-splenectomized and inoculated with adenovirus type 12 as well as in animals receiving the immunosuppressive drug 'Imuran'. Antibody production in these animals to both adenovirus type 12 and killed (Salk) polio vaccine was extremely erratic. Whether this reflects immunological incompetence associated with a reduction in the antibody production mechanism as described by Miller and others<sup>13,15-20</sup> or an overwhelming of the capability to produce antibodies by the large virus inoculum (50 ml.) used requires further investigation.

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### Adaptation and Growth Characteristics of Influenza Virus at 25° C

THERE have been many attempts to adapt influenza virus to growth at lower temperatures. For many viruses such as polio<sup>1-3</sup>, Japanese encephalitis<sup>4</sup> and measles<sup>5</sup>, a relationship has been observed between virulence and the ability to grow at lower temperatures, and this has been used in the selection of attenuated variants.

This study is an attempt to select a cold variant of influenza virus and to obtain practical data regarding multiplication of the virus in animal, tissue culture and embryonate eggs. A comparison of the ability of this

cold variant and the original strain to grow in different hosts and at different temperatures will be reported. The virus was propagated in primary chick kidney tissue cultures (CKTC) the management of which has been described elsewhere<sup>6</sup>, and in white Leghorn embryonate eggs between 10 and 11 days old.

Schematic presentation of the passage history of A<sub>2</sub>/AA/6/60 influenza virus  
Virus isolated in chick kidney tissue culture (CKTC) at 36° C

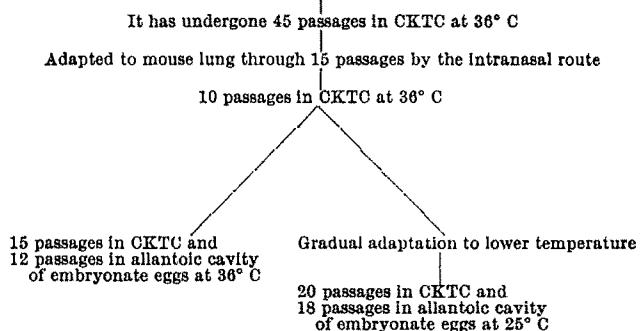


Fig. 1.

Fig. 1 is a schematic presentation of the passage history of the Asian strain (A<sub>2</sub>/AA/6/60) of influenza virus, which had been isolated from throat washings directly in chick kidney tissue culture at 36° C. After passage in tissue culture, the strain was adapted to mouse lung and then back again to tissue culture at 36° C. The process of growing the virus by gradually lowering the temperature of incubation was then undertaken.

Primary CKTC was infected by washing the monolayers free from serum and then allowing the virus inoculum to adsorb for 1 h at room temperature. Excess virus was removed and fresh maintenance media composed of double strength Eagle's basal medium<sup>7</sup> containing no serum was added. The incubator was 'BOD' equipped with a control system and a fan to ensure a uniformly low temperature with variation of  $\pm 0.1^\circ\text{C}$ . No selection or purification of the initial virus preparation was carried out before adaptation to growth at lower temperatures.

A sudden sharp decrease in the temperature of incubation was not successful. A gradual lowering of temperature was found essential for optimal growth (Table 1). The method followed was to grow the virus at an arbitrary temperature for 10 passages before lowering the temperature by 3° C. The data show that the infectious yield of virus from the initial passage at the particular temperature used was always about three logs lower than the corresponding tenth passage, where the infectious yield is maximal. No change in titre of the virus was noticed on further passages. Any further decrease in temperature of incubation did not seem to have additional advantages. This strain of virus was adapted to growth at 25° C,  $\pm 0.1^\circ\text{C}$ , initially in chick kidney tissue culture. It was then passed into embryonate eggs at 25° C so that no further adaptation was required. The pattern of growth of the virus in both host systems was approximately the same. Here the original strain refers to the virus exclusively passed at 36° C, while the cold variant refers to the virus passed at 25° C.

Table 1. INFECTIOUS TITRE OF INFLUENZA VIRUS (A<sub>2</sub>/AA/6/60) DURING GRADUAL ADAPTATION TO LOWER TEMPERATURES IN CHICK KIDNEY TISSUE CULTURE (CKTC)

Temperature used in °C	No. of passages at each temperature	Infectious titre (EID <sub>50</sub> /ml.) First passage	Tenth passage
36	60	7.5	7.7
33	10	5.7	7.5
30	10	5.0	7.3
27	10	4.3	7.3
25	10	4.0	7.0



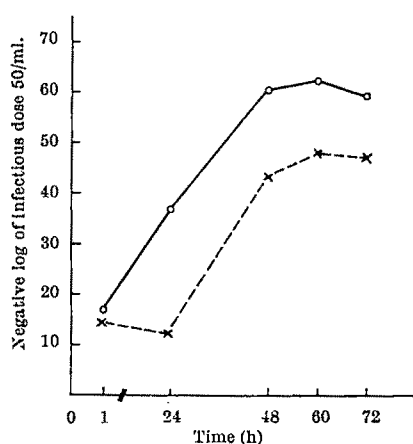


Fig. 2. Growth curves of *A<sub>2</sub>/AA/6/60* strain of influenza virus and its cold variant in chick kidney cells. ○—○, Cold variant (25° C); × — × — ×, original strain (36° C).

Haemagglutination titre of the above growth curves

h after infection	Cold variant	Original strain
1	<2	<2
24	<2	<2
48	8	<2
60	32	2
72	128	16

A comparison of the growth of the original strain of the virus and its cold variant at 25° C is shown in Fig. 2. Primary chick kidney cultures were infected with high multiplicity of the virus at 25° C. Samples were taken at designated intervals and titrated at 36° C for infectious yield. It is apparent that the virus titre of the cold variant starts rising early and achieves a higher value than the titre of the original strain of influenza virus. The same pattern holds true when haemagglutination titre is determined, as shown in the legend to Fig. 2. The virus was found to propagate quite readily in both tissue culture and embryonate eggs at 25° C and the maximal value was reached between the second and the third day. Virus collection was always carried out at 72 h after infection. At 25° C the metabolic rate of tissue culture cells and growth of embryo is decreased. Moreover, cytopathic change, although evident, developed more slowly.

When growth patterns of the two strains were compared at 36° C the shape of the two curves was almost identical, but the maximal yield of the cold variant was lower at 36° C as compared with the original strain. There was no difference, however, in the appearance of cytopathic effects or in the extent of involvement of the cultures on infection with the two strains at 36° C.

The next approach was to compare the growth of both lines of virus at different temperatures. A pool of the two strains was made and titrated in embryonate eggs simultaneously at the three different temperatures, 25°, 36° and 40° C. Table 2 shows that the cold variant exhibits a markedly decreased ability to grow at 40° C—the titre is  $10^{-3.0}$  *EID<sub>50</sub>* as compared with the titre  $10^{-8.7}$  *EID<sub>50</sub>* of the original strain. The infectious titre of the cold variant is lower at 36° C, but higher at 25° C. The results also suggest that the original strain grows less efficiently at 25° C. The data presented permit the assumption that the main cause of differing values of the infectious titre at different incubation temperatures is the varying degree of thermoinactivation which exists and that 40° C can be used as a marker for characterization and differentiation of the two strains<sup>8,9</sup>. The relative stability of

Table 2. EFFECT OF DIFFERENT TEMPERATURES ON THE INFECTIOUS TITRE OF THE ORIGINAL STRAIN (*A<sub>2</sub>/AA/6/60*) AND ITS COLD VARIANT

Virus	Infectious titre ( <i>EID<sub>50</sub>/ml.</i> )		
	25° C	36° C	40° C
Original strain	4.5	9.0	8.7
Cold variant	6.3	7.5	3.0

this marker in the cold strain of the virus is at present under investigation.

Because we are also interested in determining if any degree of attenuation for mice is achieved by this process, as reported for other viruses, the original strain had been adapted to grow in mouse lung. Mice were then infected, using comparable egg infectious doses, by the intranasal route, with the original strain and its cold variant, and at selected intervals four mice in each group were killed and their lungs ground and titrated.

Fig. 3 shows the growth characteristics of the original strain and its cold variant in mouse lung. It is apparent that the cold variant shows moderate and delayed growth in the lung, the pattern resembling that of an unadapted virus; no lung lesions were evident in 72 h. The original strain, however, shows vigorous growth in the lung with no apparent delay in rise in the infectious titre, and maximum yield is reached 24 h after infection. Lung lesions were evident from the forty-eighth hour.

Table 3. RESPONSE OF MICE TO INFECTION WITH THE TWO STRAINS OF INFLUENZA VIRUS (*A<sub>2</sub>/AA/6/60*)

Dilution used to infect mice	Cold variant (25° C)		Original strain (36° C)	
	Mortality ratio	HI titre*	Mortality ratio	HI titre*
$10^{-1}$	4/10	1,024	10/10	—
$10^{-2}$	0/10	1,024	10/10	—
$10^{-3}$	0/10	512	9/10	1,024
$10^{-4}$	0/10	128	3/10	1,024
$10^{-5}$	0/10	<16	0/10	32

\* Haemagglutination-inhibition antibody at 21 days after infection.

Table 3 shows the comparative antibody response as well as the mortality ratio with different dilutions of the two lines. It is evident that there is a definite attenuation with a marked drop in the *LD<sub>50</sub>* mouse titre of the cold variant, regardless of whether the strain was passed in embryonate eggs or tissue culture. The virus seems to lose most of its ability to kill mice, but does infect, as evidenced by the good antibody response obtained. Infection with  $10^{-1}$  and  $10^{-2}$  dilutions of the original strain showed 100 per cent mortality, while infection with the  $10^{-3}$  and  $10^{-4}$  dilutions shows 90 per cent and 30 per cent deaths respectively. There was a good antibody response with the high dilutions used. This corresponds to infection of mice with the cold variant strain at  $10^{-2}$  and  $10^{-3}$  dilutions with no mortality and comparable antibody response. Even with infection at  $10^{-4}$  of the cold variant which is only 100 infectious doses, a good antibody titre was evident.

This line of research was considered encouraging in relation to qualities essential for an effective live virus vaccine<sup>10</sup>. Manipulations to attempt further characterization as well as adaptation of other recent strains of *A<sub>2</sub>* and type B influenza viruses are being carried out.

We have successfully adapted influenza virus to growth at 25° C in both tissue culture and embryonate eggs.

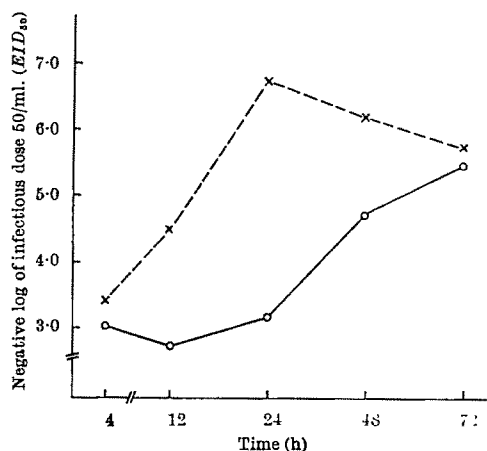


Fig. 3. Growth curves of *A<sub>2</sub>/AA/6/60* and its cold variant in mouse lung. ○—○, Cold variant (25° C); × — × — ×, original strain (36° C).

The cold variant appears to be heat labile and its growth at 40° C can be used as a marker of differentiation. A marked reduction in mortality of infected mice was observed. The cold variant appears to infect mice without overt pathological response, while stimulating good antibody responses. Thus a significant degree of attenuation has been achieved.

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### Do Certain Colicines and Phages share Common Receptors?

THE receptors of bacterial cells for colicines have not so far been studied in great detail, but there is some evidence<sup>1,2</sup> for the assumption that the role of these receptors is to fix colicines specifically on to the bacterial surface and to enable them to have inhibitive effects like phage receptors<sup>3</sup>. Although it is generally accepted, this hypothesis has never been proved<sup>4</sup>. The main support for it came from Fredericq's later experiments, which showed a cross-resistance between certain colicines and phages. Consequently they are both supposed to be adsorbed on to common receptors. Thus colicine *K* should share a common receptor with the phage *T6* (ref. 5), colicine *M* with the phages *T1* and *T5* (ref. 6), etc.

All the receptors of *T*-phages are localized in the bacterial cell wall. Attempts have been made to define them in terms of the biochemical composition of the *Escherichia coli* *B* cell wall: the *T6* receptor is situated in the protein part of its lipoprotein layer<sup>7</sup>, while the *T5* receptor seems to be a lipopolysaccharide-lipoprotein complex<sup>8</sup>. The *T1* receptor has not yet been defined chemically.

From experiments with stable L-forms of *Proteus mirabilis* it is known that these cells which are devoid of their cell walls have lost their phage receptors and therefore are absolutely resistant to the action of phages<sup>9</sup>.

Recently a stable L-form of *E. coli* *B* has been isolated<sup>10</sup>. Preliminary experiments showed that this stable L-form, too, is resistant to the phages of the *T*-series. Thus it became possible to compare the action of both colicine *K* and phage *T6* on normal rods and on the cells of the L-form, that is, on cells with and without cell walls.

Plates of 1.5 per cent meat-peptone agar containing 15 per cent horse blood serum, 1.5 per cent or 3 per cent sucrose and 0.1 per cent magnesium sulphate (MgSO<sub>4</sub>·7H<sub>2</sub>O) were used in our tests. On to these, 6–8 days old broth culture of the stable L-form of *E. coli* *B* was densely seeded for every experiment and (a) broth suspension of the phage *T6* (10<sup>6</sup>, 10<sup>8</sup>, 10<sup>10</sup> particles/ml.), (b) broth solution of the colicine *K* (10<sup>10</sup>, 10<sup>11</sup> lethal units/ml.), and (c) broth (control) were dropped on to the dry surface. (For colicine *K* we used a filtrate of a broth culture of the strain *E. coli* *K* 235; the participation of the other colicine produced by this strain<sup>11</sup> was excluded by cultivating

the producer in meat-peptone broth under heavy aeration.) The experiment was repeated with various modifications.

In all cases the results were the same: the stable L-form of *E. coli* *B* is completely resistant towards the phage *T6* as well as towards all other phages of the *T*-series; however, at the same time, it remains fully sensitive towards colicine *K*. Together with its cell wall the L-form has evidently lost its receptors for *T*-phages, but not its colicine *K* sensibility, which means that the action of the colicine does not depend on previous adsorption on to a specific receptor in the cell wall.

Unfortunately, it is hardly possible to perform a similar experiment with the colicine *M* and phages *T1* and *T5*; it would be necessary to have a stable L-form of *E. coli* *B* resistant to colicine *V*, from which the colicine *M* cannot be separated. But the colicine *M* does not attack normal cells of *E. coli* *B*, which are specifically resistant to colicine *V*, in spite of the fact that the *B* strain is the classical indicator for *T*-phages. In this case, therefore, it is not possible to suppose a common receptor.

We conclude that the analogous sensibility of some strains towards certain colicines and phages cannot be simply explained by the assumption of common receptors. Reeves<sup>12</sup> has also stated that this assumption is open to serious criticism. The cross-resistance has never been found in 100 per cent of the strains covered.

The observation that specific cell wall receptors are not needed for colicine action at all—at least with certain colicines—will be thoroughly discussed elsewhere.

These experiments were performed at the Institute of Microbiology and Experimental Therapy, Jena.

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### Bacteriocinogeny in Strains of *Providencia* and *Proteus morganii*

BACTERIOCINS<sup>1</sup> are a distinctive class of antibiotics produced by bacteria. Bacteriocins are proteinaceous and their action is limited to strains of the same or closely related species as the producer organisms.

Strains of a number of different families produce bacteriocins (bacteriocinogenic), but until the recent discovery of bacteriocin production in strains of *Proteus hauseri*<sup>2</sup> the *Proteus-Providencia* group were regarded as unique among the Enterobacteriaceae in being non-bacteriocinogenic<sup>1</sup>. This communication describes the presence of these antibiotics in strains of *Providencia* and *P. morganii*. The organisms used comprised the seventeen *P. morganii*, twenty-two *P. rettgeri*, twenty-four *Providencia*, twenty-eight *P. hauseri* and other strains of Enterobacteriaceae previously used<sup>3,4</sup>. A further seventy-



seven strains of *P. morganii* (labelled NM) and thirteen National Collection of Type Culture strains of *Providencia* were also investigated. The broth, solid media and phage techniques were those previously used<sup>5,6</sup>. The method for detection of bacteriocins was that of Craddock-Watson<sup>2</sup> with the use of 'Difco MacConkey' agar. Strains of *P. rettgeri*, *P. morganii* and *Providencia* were tested intraspecifically both for bacteriocinogeny and as bacteriocin indicators. Subsequently, all strains of the other species mentioned were tested for susceptibility to the bacteriocins. Many of these organisms are lysogenic<sup>3,4,7</sup> and phage activity was distinguished as follows: clear areas of inhibition on 'MacConkey' agar were cut out and suspended in 1 ml. broth containing a few drops of chloroform to sterilize the suspension. After the chloroform had been bubbled off, dilutions of the suspensions were spotted on a newly poured layer of the indicator organism. Lack of serial transmissibility of the inhibitory effect was taken to favour bacteriocin-like activity.

None of the *P. rettgeri* but streaks of five *Providencia* and twelve *P. morganii* strains produced areas of inhibition on indicator organisms (Fig. 1). No phage activity was demonstrated in the inhibition zones and the producer organisms were non-lysogenic by methods previously used<sup>3,4,7</sup>. The zones were confined in width to the original vertical streaks of producer organisms and there was little or no diffusion of the inhibitory agent even with prolonged periods of incubation before application of the indicator cross-streaks. Often the inhibition was confined to two narrow lines with normal growth of the indicator in between resembling the "D zones" encountered by Abbott and Shannon<sup>8</sup> (Fig. 2). Small doses of ultra-violet light applied to a 4 h growth of the vertical streak of the producer always changed the "D zone" of a system to a clear area of inhibition. Producer strains were active on a number of strains of the same species and the agent produced by *P. morganii* strain NCTC 2818 inhibited forty-three of the *P. morganii* strains. All seventeen agents could be distinguished by their spectrum of activity. None of the producer strains are inhibited by their own agents although some are sensitive to heterologous agents. The only extraspecies activity demonstrated was that of the inhibitory agent produced by *Providencia* strain NCTC 9190 on the *P. rettgeri* strain

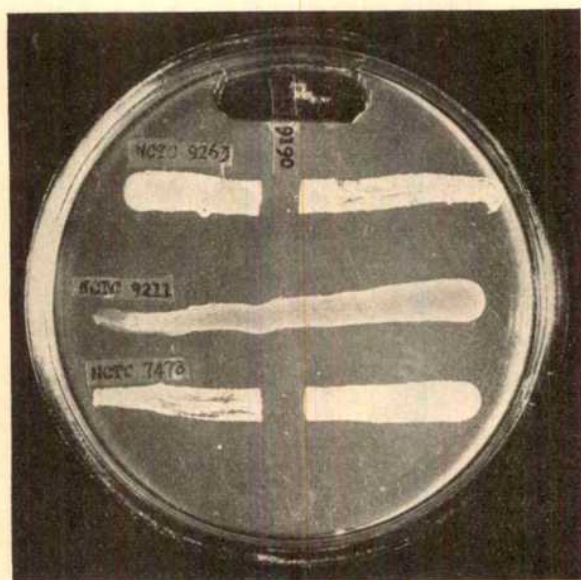


Fig. 1. *Providencia* strain NCTC No. 9190 was streaked across 'MacConkey' agar and incubated at 37° C for 24 h. The growth was killed with chloroform vapour and scraped off with a sterile glass slide. Cross-streaks of *Providencia* strain NCTC 9263, *Proteus rettgeri* strain NCTC 7478 and *Providencia* strain NCTC 9211 were made and the plate incubated for 18 h. Strain NCTC 9211 is resistant to the bacteriocin produced by NCTC 9190. The other strains are sensitive.

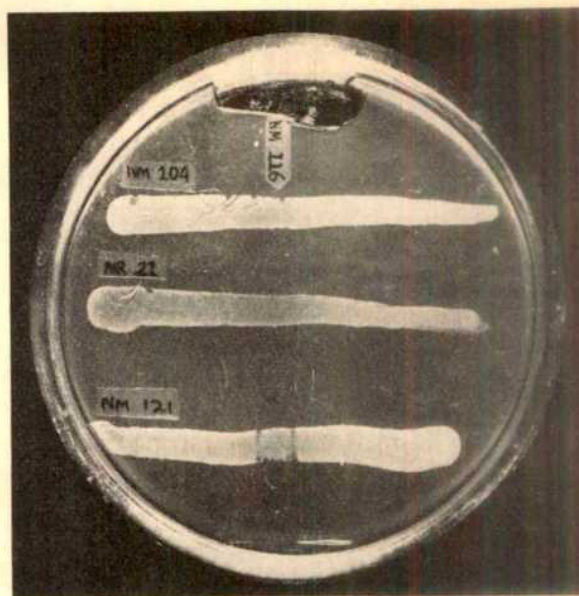


Fig. 2. *Proteus morganii* strain NM 116 was streaked across 'MacConkey' agar and incubated at 37° C for 24 h. The growth was killed with chloroform vapour and scraped off with a sterile glass slide. Cross-streaks of *P. morganii* strains MR 21 and NM 104 and 121 were made and the plate incubated for 18 h. Strain MR 21 is resistant to the bacteriocin liberated by NM 116. The other strains show a "D zone" reaction of sensitivity described in the text.

NCTC 7478. Indeed, the latter strain is now used as indicator for this agent instead of the original *Providencia* indicator strain NCTC 9263. Table 1 lists the producer strains and one each of their indicators. No stably resistant mutants of an indicator strain have yet been isolated and in this respect the systems resemble *Pseudomonas aeruginosa*-pyocine interactions<sup>9</sup>.

Table 1. INCIDENCE OF BACTERIOCINOGENY IN *Proteus morganii* AND *Providencia* STRAINS

No. investigated	Bacteriocinogenic	Incidence (per cent)	Indicator*
37 <i>Providencia</i>	NCTC 9190	13.5	NCTC 7478
	" 9207		" 9283
	" 9246		" 9235
	" 9248		" 9264
	" 9298		" 9213
94 <i>P. morganii</i>	NCTC 2818	12.7	MR 21
	MR 336		47
	NM 29		NM 112
	" 30		" 105
	" 32		" 125
	" 38		" 130
	" 39		" 112
	" 107		" 129
	" 116		" 121
	" 120		NCTC 2818
	" 126		NM 104
	" 127		NCTC 7381
	"		"
	"		"

\* Only one indicator strain per bacteriocin is listed.

Overnight broth cultures of inhibitory strains sterilized with chloroform and centrifuged to clarity had inhibitory effects on indicator organisms. Titrations were done by spotting dilutions on 'MacConkey' agar. When the spots had dried the plates were covered for 5 min with filter paper disks which had been soaked in a suspension of the indicator strain. Results were read after about 16 h at 37° C. Inhibitory titres up to 1/20 were obtained. Titres ten-fold higher could be obtained by applying small doses of ultra-violet light to logarithmically growing broth cultures in shallow layers, and then incubating in the dark for 6 h before addition of chloroform. All the agents are bactericidal as determined by subculturing from clear areas of inhibition to fresh agar. Solutions of the bactericidal agents diffuse 1 cm or more overnight from a central sealed hole in 'MacConkey' agar as determined by application of the indicator filter disk the next morning. The reason why little or no diffusion is detected by the

cross-streak method may be because the agents adsorb to producer organisms. The agents are retained by dialysis tubing and this method was used to obtain a fifty-fold increase in titre. Inhibitory titres could not be increased by centrifugation at 24,000g for 2 h, and differential centrifugation procedures used to concentrate *Proteus* phages for electron microscopy resulted in total loss of activity. The agent liberated by *P. morganii* strain NCTC 2818 is inactivated at 70° C for 30 min but not at 65° C. The other agents are inactivated at 65° C. The agents are susceptible to the proteolytic enzymes of *P. vulgaris* strain OX19 as determined by a modification of the method of Fredericq<sup>10</sup>. The agents of *P. morganii* strains NCTC 2818 and NM 38 are least susceptible, while those produced by *Providencia* strains are totally inactivated. The detection of the agents is dependent on the medium used. In this respect they resemble the variability of colicine production in different fluid media<sup>11</sup>. No activity could be demonstrated on nutrient agar or in 'Difco' nutrient broth and this may explain previous failures to demonstrate this type of activity in the species concerned.

The bactericidal agents described conform to the definition of bacteriocins and the only member of the *Proteus* group which has not yet proved bacteriocinogenic is thus *P. rettgeri*.

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### Effect of Calcium Ions and Pentachlorophenol on the Respiration of *Micrococcus lysodeikticus*

THERE has been much work on the effect of divalent cations and of uncouplers of oxidative phosphorylation on mitochondrial respiration, but little is known about the effect of these substances on bacterial respiration at whole-cell level.

We have examined the effect of calcium ions on the endogenous respiration of *Micrococcus lysodeikticus*, and the effect of pentachlorophenol, an effective uncoupler of the oxidative phosphorylation of membrane fragments of *M. lysodeikticus*<sup>1</sup>, has also been examined.

The conditions of culture of *M. lysodeikticus* will be described elsewhere (unpublished results of Fujita and Ishikawa). The cells were washed well with 5 mmolar *tris* hydrochloric acid, pH 7.4, containing 1 mmolar EDTA before use. A Clark type oxygen electrode was used; it was equipped with a plastic cell with an internal volume of about 3 ml. The experiments were carried out at 22° C–23° C.

In Fig. 1 the effect of added calcium ions on the cellular respiration in *tris* buffer is shown. The stimulation of the respiration by calcium ions occurs at a rate much slower than in a similar experiment with mitochondria<sup>2</sup>. Thereafter the rate of respiration progressively decreased until it became less than the initial rate. This decreased respiration after stimulation, on addition of calcium ions, was temporarily designated as "calcium ion-inhibited respiration". The addition of 0.1 mmolar pentachlorophenol affected the calcium ion-inhibited respiration only slightly if at all, but a later addition of 1.4 mmolar inorganic phosphate reactivated the calcium ion-inhibited

respiration. If pentachlorophenol was added to the cells suspended in *tris* hydrochloric acid buffer containing no calcium ions, the oxygen consumption (calcium ion-free respiration) was inhibited immediately and the inhibition was removed by addition of phosphate (Fig. 2).

When the reaction proceeded in phosphate buffer the addition of calcium ions induced a progressive decrease in the rate of respiration as in *tris* buffer and the addition of pentachlorophenol released the oxygen consumption (calcium ion-inhibited respiration) instantaneously (Fig. 3).

We therefore recognized that pentachlorophenol plus inorganic phosphate released the calcium ion-inhibited respiration, whereas pentachlorophenol alone inhibited the calcium ion-free respiration. The latter inhibition was relieved by a subsequent addition of phosphate.

If it is possible to assume that the calcium ion-inhibition of respiration mentioned here reflects the accumulation of inactive intermediate as a result of coupling between electron transfer and cation incorporation<sup>3</sup>, it can be concluded that pentachlorophenol plus inorganic phosphate dissociate the non-phosphorylated intermediate which is involved in the incorporation of calcium ions. Fragments of the sonicated protoplasmic membrane of *M. lysodeikticus* have been observed to undergo a progressive inhibition of the oxidation of NADH in the presence of calcium ions (unpublished results of Fujita and Ishikawa). There is, however, no satisfactory interpretation of the inhibition of respiration by pentachlorophenol in the absence of inorganic phosphate.

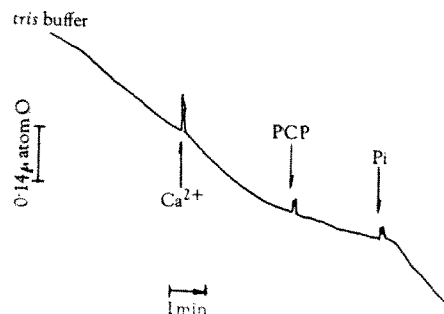


Fig. 1. Effect of calcium ions and pentachlorophenol on the endogenous respiration of *M. lysodeikticus*. *Tris* hydrochloric acid, pH 7.4 (15 μmoles) and 0.1 ml. of cell suspension were in a total volume of 3.5 ml. Further additions were made as follows: 6.6 μmoles of calcium chloride; 0.39 μmoles of pentachlorophenol, and 5.0 μmoles of potassium phosphate, pH 7.4. Oxygen consumption was measured with a Clark type oxygen electrode. PCP, pentachlorophenol; Pi, inorganic phosphate.

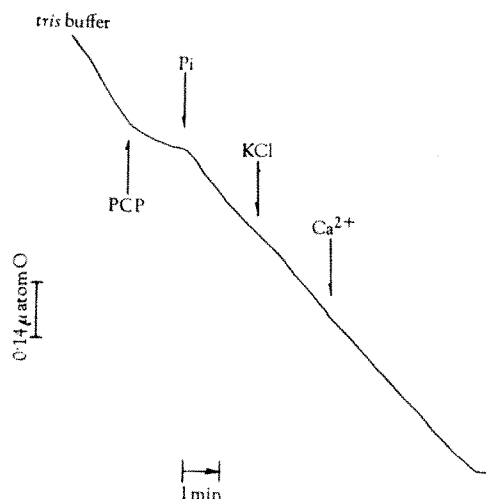


Fig. 2. Effect of pentachlorophenol on calcium ion-free respiration. *Tris* hydrochloric acid, pH 7.4 (15 μmoles) and 0.15 ml. of cell suspension were in a total volume of 3.5 ml. Further additions were 0.39 μmoles of pentachlorophenol; 3.5 μmoles sodium phosphate, pH 7.5; 20 μmoles of potassium chloride, and 1.5 μmoles of calcium chloride.



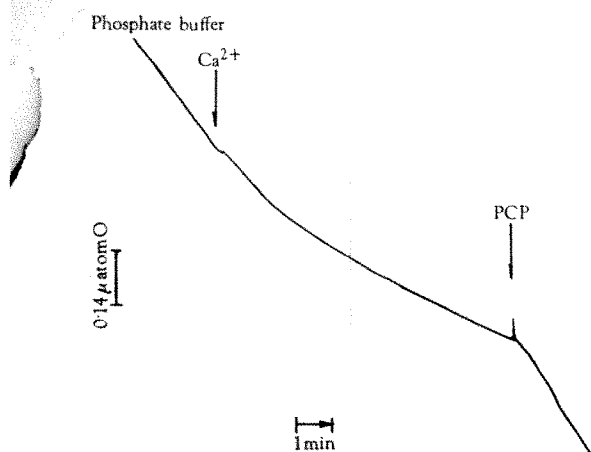


Fig. 3. Effect of pentachlorophenol on respiration inhibited by calcium ions in phosphate buffer. Potassium phosphate, pH 7.4 (5.0  $\mu$ moles) and 0.15 ml. of cell suspension were in 3.5 ml. Additions were 3.3  $\mu$ moles of calcium chloride and 0.39  $\mu$ moles of pentachlorophenol.

It seems that pentachlorophenol is not a bacterial equivalent of dinitrophenol or an oligomycin-type inhibitor in the mitochondrial system.

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## PATHOLOGY

### Increase of Lysosomal Enzymes in Skin Cancers

THE function of the lysosome in the pathogenesis of cancer is unknown. In the normal cell these cytoplasmic structures bound to the membrane are thought to be constituents of an intracellular digestive system. Exogenous material that will eventually be digested is engulfed by a cell membrane<sup>1</sup>. These newly formed lysosomes are thought to be long lived structures involved in numerous digestive events.

Lysosomes are also important after normal cells are injured or die. Hydrolytic enzymes are released and distributed throughout the cell. Lysosomal enzyme activities greatly increase. After cellular death the pH of the cell is probably lowered to quantities where lysosomal enzymes have their maximal activity. Rapid cell autolysis and tissue breakdown ensue. Lysosomal enzymes are markedly increased concomitant to uterine involution, resorption of tadpole tails during metamorphosis, and in rheumatoid synovial tissue<sup>2-4</sup>. The cancer process is a steady invasion and erosion of normal tissue, and so an increase of lysosomal enzyme activity can be anticipated.

In this investigation 90 ICR Swiss female mice, 50-55 days old, were divided into three groups: the shaved skin of the back was exposed once to 0.125 mg of 7,12-dimethylbenzanthracene (DMBA) in acetone; after three weeks 0.25 ml. of 0.033 per cent of the tumour promoter, croton oil in acetone, was applied daily for 19 weeks; a second group of thirty mice received only DMBA; the third group received daily 0.25 ml. of 0.01 per cent of the complete carcinogen, 3-methylcholanthrene in acetone, for 22 weeks.

The number and distribution of tumours were noted weekly. After 22 weeks, all tumours were graded according to size and location. Application of carcinogens was halted. In mice given the DMBA and croton oil the progress of regression of the papillomas and the number of cancers in the mice treated with 3-methylcholanthrene were observed for an additional 6 weeks. Differences between complete carcinogenesis and tumour promotion are summarized in Table 1.

Table 1. DIFFERENCES BETWEEN TUMOUR PROMOTION AND COMPLETE CARCINOGENESIS

Group	Number of papillomas			No. of cancers 28 weeks
	19 weeks	22	28	
DMBA and croton oil	132	127	54	9
3-Methylcholanthrene	34	95	18	71
DMBA only	0	0	0	0

After 28 weeks, several of the skin cancers, untreated skins (DMBA only) and untreated mouse livers were ground with 10 parts by volume of 0.09 per cent sodium chloride to 1 part of tissue by weight with a hand homogenizer. Skin is difficult to homogenize and the lysosomal particles would be damaged, and so no attempt was made to differentiate between bound and free enzyme activities. Four frozen rat livers from 60-day-old rats and an adult pheasant liver were similarly homogenized. Acid phosphatase,  $\beta$ -glucuronidase and cathepsin activities were measured using standard enzyme assays<sup>5-7</sup>. Proteins were determined by the method of Lowry<sup>8</sup>.

Table 2. LYSOSOMAL ENZYME ACTIVITIES IN SKIN CANCERS AND NORMAL TISSUES

Tissue	Cathepsin ( $\mu$ moles of tyrosine/mg of protein/h)	$\beta$ -Glucuronidase $\mu$ moles of phenolphthalein/ mg of protein/h $\times 10^{-3}$	Acid phosphatase nmoles of nitrophenol/mg of protein/h
Mouse skin (DMBA)	(10) 0.0	(10) 0.0	(7) 15.8 $\pm$ 2.6
Mouse skin cancer	(10) 0.41 $\pm$ 0.04	(9) 1.5 $\pm$ 0.25	(9) 26.0 $\pm$ 4.8
Mouse liver	(10) 0.41 $\pm$ 0.08	(10) 0.81 $\pm$ 0.2	(10) 42.0 $\pm$ 8.0
Rat liver	(4) 0.05 $\pm$ 0.004	(4) 3.2 $\pm$ 1.7	(4) 27.1 $\pm$ 3.3
Pheasant liver	(1) 0.85	(1) 0.3	(1) 46.6

Lysosomal enzyme activity greatly increased in skin cancers (Table 2). Mouse skin cancer cathepsin,  $\beta$ -glucuronidase, and acid phosphatase activity were enhanced to a degree comparable with those found in liver which is an organ noted for its great metabolic activity. No cathepsin or  $\beta$ -glucuronidase activity was detected in skin initiated only with DMBA. Nearly 74 per cent of the papillomas induced by 3-methylcholanthrene became cancers (Table 1). Fifty-nine per cent of the papillomas induced by croton oil regressed. No cancers were observed. Forty-nine of the papillomas induced by croton oil remained the same size. Only five of the 132 croton oil papillomas increased in size.

The skin chemical carcinogenic process may be divided into three distinct stages: the original insult to the cell resulting in papillomas, regression of the papillomas or conversion of the papillomas to cancers, and finally growth and maintenance of the cancers.

The original insult to the cell by the carcinogen can result from a disturbance of the digestive function of the lysosome by the carcinogen. In addition, initial damage could be attributed to peroxidation of the lysosomal lipid membrane prematurely releasing hydrolytic enzymes. These two possibilities are not necessarily mutually exclusive. Several carcinogens have been added to HeLa cells<sup>9</sup>, and all those tested appeared first in the lysosomes. Many undigestible particles may accumulate in the lysosome, and so the function of digestion in the lysosome could be impaired. Chemically induced carcinogenesis is a cumulative process which apparently reaches a threshold before tumours form. The first tumours do not appear until carcinogens are applied daily for 5-10 weeks. Progressive accumulation of undigestible carcinogen residues may overwhelm the lysosome, making it unable properly to eliminate its wastes.



Antioxidants protect lipid membranes from peroxidation<sup>10</sup>. Premature damage of the lipid membrane by peroxidation may release hydrolytic enzymes which damage other cellular functions. Several peroxy compounds are carcinogenic<sup>11</sup>. A hydroperoxide of cholesterol is carcinogenic in certain conditions<sup>12</sup>. Peroxidation may change the geometrical isomerism of the unsaturated fatty acids present in the lipid membrane<sup>13</sup>. Permeability of the lysosomal membrane to various biological fluids may be altered. Changes of the hormonal control of the lysosomal membrane may result. If antioxidants are applied simultaneously with croton oil, the incidence of skin papillomas is greatly reduced<sup>14</sup>. Hydrocortisone, a protector of lipid lysosomal membranes, regresses skin tumours<sup>15</sup>. The early reactions of carcinogenesis may be different from events which determine the fate of the early papilloma. Lysosomes may be involved in other stages of carcinogenesis.

Lysosomes are probably important in the fate of the papilloma. Differences between tumour promotion and complete carcinogenesis could be attributed to changes in the hormonal control of the lysosomal membrane (Table 1). In regressing tissues lysosomal enzyme activity is enhanced. Lysosomal enzyme activity is also increased in regressing Flexnor-Jobling tumours<sup>16</sup>. Hormonal control of the lysosomal membrane apparently remains effective in most tumour tissues induced by croton oil. In pre-cancer papillomas, however, hormonal control of the lysosomal membrane can be lost. These papillomas do not regress, but are transformed into cancers.

The early metabolic changes of carcinogenesis and critical reactions important in the fate of the early papilloma could be different from the cellular changes necessary for growth and maintenance of skin cancers. Lysosomal enzyme activities are greatly elevated in the growing cancer (Table 2). The intense enzyme activity at this stage of carcinogenesis could be important in the invasion and destruction of normal tissue or this great enzyme activity may indicate rapidly metabolizing tissue.

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### Phagocytic Activity after Thymectomy

In recent years it was shown that thymectomy soon after birth causes impairment of the immune response of the mature animal. A depression of antibody production was reported in mice<sup>1</sup>, rats<sup>2</sup>, rabbits<sup>3</sup> and guinea-pigs<sup>4</sup>, and a depression of the homograft reaction was observed after thymectomy<sup>5</sup>.

This lack of immunological response has usually been related to the depletion of lymphoid tissues associated

with early thymectomy<sup>5</sup>, although the role of lymphocytes in immunological responses seems to be poorly understood.

Comparatively little attention has been paid to the changes induced by thymectomy in the macrophage system, which is known to be involved in immunological reactions<sup>6-8</sup>.

It was shown by Miller and Moreman<sup>9</sup>, Osoba and Miller<sup>10</sup> and Miller and Howard<sup>11</sup> that mice thymectomized at birth exhibit moderate to marked reticuloendothelial cell hyperplasia and increased phagocytic activity (as measured by the clearance from the blood of colloidal carbon). Experiments by Morrow and Di Luzio<sup>12</sup>, however, did not show any apparent abnormality in the granulopoietic activity of the thymectomized rats: colloidal carbon removal from the blood after a single intravenous injection of 8 mg/100 g body weight occurred at about the same speed both in thymectomized and in sham-operated animals.

Two or three day old Wistar albino rats were thymectomized according to the method of Miller<sup>13</sup>. At 5 weeks of age, the phagocytic activity in the thymectomized animals was evaluated by measuring the rate of removal from the blood of colloidal carbon (*C* 11/1431 *a*). Sixteen milligrams of carbon per 100 g body weight were injected intravenously and blood samples of 0.01 ml. were obtained from the tail veins and haemolysed in 0.1 per cent sodium carbonate. The carbon concentration was estimated spectrophotometrically at 650 m $\mu$  and the granulopoietic index  $K$  ( $\log C_0 - \log C_{30}/30$  min) and the half-time ( $t/2$ ) were calculated. The absence of thymic residues was verified in all cases. For comparison the rate of removal of carbon was estimated in normal animals of the same age and body weight: previous experiments demonstrated that controls behave like unoperated animals.

After a single intravenous injection of carbon no difference could be detected in thymectomized and normal rats. If another dose of 16 mg was injected intravenously after 1 h, the velocity of carbon removal was reduced in normal animals and was very appreciably increased in the thymectomized ones (Table 1, Fig. 1).

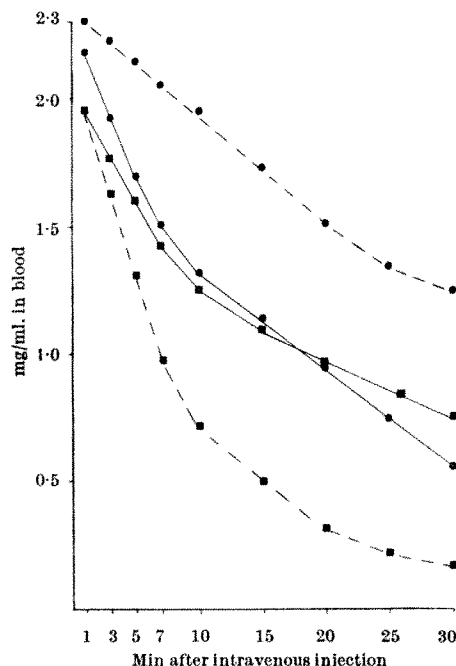


Fig. 1. Colloidal carbon removal from blood after an intravenous injection of 16 mg/100 g body weight (each curve represents the average results of six experiments). ●—● Normal rats; only one dose was given. ○—○ Normal rats; the experiment was carried out 1 h after the injection of an equal dose by intravenous route. ■—■ Thymectomized rats; one dose. □—□ Thymectomized rats; the experiment was carried out 1 h after an equal dose by intravenous route.

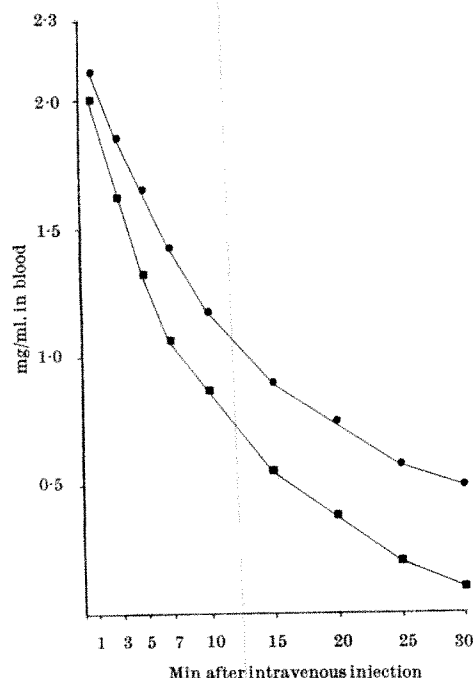


Fig. 2. Colloidal carbon removal from blood after an intravenous injection of 16 mg/100 g body weight. The experiment was carried out 24 h after the injection of an equal dose by intraperitoneal route (each curve represents the average results of six experiments). ●—●, Normal rats. ■—■, Thymectomized rats.

Further experiments were carried out with an intraperitoneal injection of 16 mg of carbon followed by an intravenous injection of the same dose 24 h later. The velocity of carbon removal was higher in the thymectomized animals (Table 1, Fig. 2).

Table 1. COLLOIDAL CARBON REMOVAL FROM BLOOD OF THYMECTOMIZED AND NORMAL RATS AFTER ONE INTRAVENOUS INJECTION OF 16 mg

	Without a previous dose	1 h after dose (intravenous)	24 h after dose (intraperitoneal)
	K	K	K
Thymectomized	0.014 19 ± 0.003 ± 5.56	0.038 7 ± 0.013 ± 2.44	0.043 9 ± 0.010 ± 2.37
Normal	0.019 17 ± 0.002 ± 5.19	0.009 32 ± 0.002 ± 6.85	0.022 12 ± 0.005 ± 2.23

Values are expressed as means ± standard errors. There were six animals in each group.

Differences between thymectomized and normal animals that were not given a previous dose are not significant. Differences are significant ( $P < 1$  per cent or even  $< 1$  per mil.) in the other cases, when a previous dose was given (by intravenous or intraperitoneal route).

Histological investigations showed appreciable hyperplasia of reticuloendothelial cells in the spleen and in the lymph nodes.

It appears that the phagocytic response to stimulation by colloidal suspensions is much higher in thymectomized rats because a larger number of macrophages is involved and possibly because a larger amount of particles is ingested by single macrophages. The velocity of carbon removal from the blood may not be appreciably affected after a single injection, but it is very different, in comparison with normal animals, after repeated injections. The features of the reticuloendothelial system (RES) seem to be very similar in rats and mice after thymectomy; the difference may be only quantitative.

It seems difficult to correlate the reticuloendothelial hyperplasia and the lymphatic depletion with the decreased immune response after thymectomy; in fact the close relationship between the RES, the lymphocytes and the formation of antibodies is still obscure even in normal animals.

Miller and Howard<sup>11</sup> suggest that early thymectomy first induces lymphatic depletion and immunological incompetence; reticuloendothelial cell hyperplasia could

be the result of infection with some virus to which immunologically defective animals may be more susceptible. They pointed out that in spite of the similarity between the graft versus host disease and the neonatal thymectomy syndrome, there is no unequivocal evidence for the operation of autoimmune processes, while experiments with thymectomized germfree mice<sup>14</sup> strongly suggest that cell proliferation (along with destructive lesions) is triggered by some environmental influence.

The possibility that the reticuloendothelial cell hyperplasia is the counterpart of lymphoid aplasia and that both events are contemporarily induced by thymectomy, however, is not excluded.

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## Autoradiography of Rat Lung before and after Birth

THE details of the development of the mammalian lung from late foetal life to maturity are still little known. For several decades many investigators have thought that proliferation in the mammalian alveolar epithelium stops in late foetal life; and that the epithelium then undergoes degeneration and desquamation before birth<sup>1-4</sup>. Bertalanffy and Leblond, using a colchicine technique and classical histological methods, showed in 1953 that the alveolar lining cells in adult rat lung are continuously renewed, just as the surface cells of the intestine and skin. Since then, high resolution autoradiography with tritiated thymidine has been applied to the study of cellular proliferation in adult mice and rats. The rates of cellular proliferation of lung tissue have not been studied in the late foetal or neonatal period either with autoradiographic or colchicine techniques. In order to obtain more information on the proliferative properties of lung in the late foetal and early neonatal periods we undertook an *in vitro* autoradiographic study of the rat lung.

The lungs of four rat foetuses and those of three newborn rats were used. The foetuses were removed from the uterine horn under ether anaesthesia on the seventeenth (two foetuses) and on the nineteenth (two foetuses) days of gestation. The newborn rats were killed 6 h after birth. All animals were killed by decapitation.

Immediately after removal from the thorax, the lungs were cut into small pieces, each approximately 0.1–0.2 cm thick and 0.3 cm in diameter. The slices of lung were incubated in 100 ml. of Ringer's solution containing 100  $\mu$ c. of tritiated thymidine (specific activity 3.0 c./mmole). A mixture of gas composed of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled through the incubation medium for 2.5 h at a rate of flow sufficient to

keep the tissue slices in constant motion. After incubation, the tissues were washed for 2 min each in three 100 c.c. portions of Ringer's solution and fixed for 24 h in neutral 10 per cent formaldehyde solution. After embedding in paraffin, sections were cut from each side of the block at  $5\mu$  to a depth of  $150\mu$ . The slides were deparaffinized, rehydrated, and dipped in NTB<sub>2</sub> 'Kodak' emulsion. The dipped slides were exposed for 4 weeks in a Conrad-Joffe fluid emulsion autoradiography system in a carbon dioxide atmosphere and at a relative humidity below 15 per cent. They were then developed, using 'Kodak 19' developer (3 min), 'Kodak SB5a' stop bath (15 sec), and 'Kodak' acid fixer (twice clearing time). All solutions were maintained at 18° C throughout development. The developed slides were washed for 12–18 h, stained with haematoxylin and eosin, dehydrated and maintained in balsam. The percentage of labelled cells was determined by counting 4,000 cells randomly scattered in areas of lung in each age group. In addition, the number of labelled alveolar lining cells was counted in 1,000 randomly selected alveolar spaces in the foetal and neonatal lungs. This latter procedure was necessary because of the well known flattening of alveolar cells after some hours of respiration. The background count was low and random. The results are shown in Table 1. The percentage of labelled cells was the same in the 17 and 19 day old foetal lungs, and the results were therefore tabulated together. In all the rats studied there was marked uptake of tritiated thymidine in the alveolar epithelium before (6.7 per cent) and after birth (estimated 4.02 per cent). There was a marked reduction in labelling of stromal cells (from 8.9 per cent to 4.0 per cent) after birth. The muscular and fibrous elements of the intrapulmonary branches of the pulmonary artery showed the same labelling in the foetal and early neonatal period. It is interesting to note that the columnar bronchiolar epithelium had an increased number of labelled cells after birth (from 4.8 per cent to 5.4 per cent).

Table 1. LABELLING OF CELLS IN RAT LUNG IN THE LATE FOETAL AND EARLY NEONATAL PERIOD

	Alveolar epithelium (per cent)	No. of labelled alveolar cells/1,000 alveolar spaces	Stroma (per cent)	Respiratory epithelium (per cent)	Pulmonary artery (intrapulmonary branches) (per cent)
Foetal lung (17–19 days of gestation)	6.7	578	8.9	4.8	7.1
Lung (6 h after birth) calculated	4.02	335	4.0	5.4	7.2

The embryological development of mammalian lung has been extensively investigated with classical histological methods. In the past few decades a number of investigators have expressed the view that the mammalian alveolar lining cells do not proliferate in late foetal life, that they degenerate and desquamate up until the time of birth. Short<sup>1</sup> believed that alveolar epithelium was not present in man and guinea-pig after birth. Three years later, Low demonstrated by electron microscopy the presence of continuous alveolar epithelium in the adult mammalian lung<sup>2</sup>. In the same year, Bertalanffy and Leblond demonstrated the continuous renewal of alveolar cells under physiological conditions<sup>3</sup>. They estimated that the percentage of alveolar tissue undergoing mitosis at any single time was between 0.63 and 1.23 per cent in the adult rat and suggested that the cell surplus is excreted with the tracheal secretions. The labelling index of alveolar lining cells and of bronchial epithelium in adult rats and mice was first determined with autoradiography by Schultze in 1960 (ref. 7) and was 1.8 per cent and 1.0 per cent, respectively. Shorter and Titus found a labelling index of 0.25 to 1.0 per cent for alveolar epithelium in the adult mice with autoradiography<sup>8</sup>. The labelling index of bronchial epithelium similarly determined in adult mice was found to be

0.53–1.72 per cent by Meyer zum Gottesberge and Koburg<sup>9</sup>. The kinetics of cell proliferation were studied autoradiographically in 20 day old rat fetuses by Wegener and associates<sup>10</sup>, who demonstrated a placental barrier to tritiated thymidine. The grain count in each nucleus was significantly lower in the foetal than in maternal tissues, including the foetal and maternal sides of the placenta. They found no diurnal variation in the rate of proliferation of different foetal tissues. The lack of variation in the mitotic index of foetal tissues over a period of 24 h is in agreement with the observations of Aschoff<sup>11</sup>, who found no diurnal rhythm in mammalian fetuses and newborn infants. Because of the placental barrier for tritiated thymidine, we feel that *in vitro* labelling is the best method to study cellular proliferation in foetal tissues.

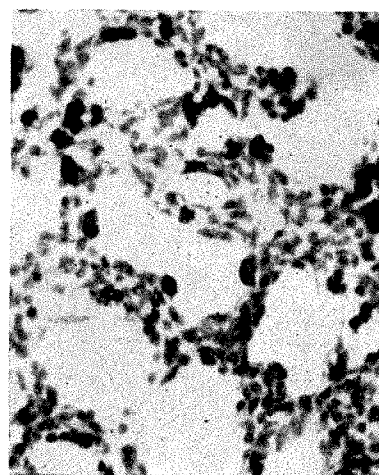


Fig. 1. Lung of newborn rat. Alveolar epithelial cells labelled with tritiated thymidine. ( $\times 804$ .)

Our investigation showed a marked decrease in the proliferative rate of alveolar epithelium (about 40 per cent) and of stromal cells (45 per cent) after birth. If our results are compared with those of other investigators, the proliferative rate of alveolar lining cells is about twice the rate of that of adult animals in the neonatal, and about three times the rate of adults in the late foetal, periods. The increase of proliferation of respiratory epithelium (from 4.8 to 5.4 per cent) after birth is in agreement with the observations of Broman<sup>12</sup> in the cow and man, and of Willson<sup>14</sup> in the mouse, who demonstrated that the number of bronchial lining cell divisions between the trachea and alveolar ducts increased between birth and maturity. The appearance of surfactant has been found to take place about the same time as the beginning of the desquamation of cells in the foetal lung. A surfactant material may therefore be derived from the desquamated cells and the continued loss of cells throughout life may provide a constant source of this material<sup>13</sup>.

The development of rat lung from birth to maturity is at present being studied by the foregoing method. These anatomical data are necessary for the better understanding of various physiological and pathological processes during development.

Future investigations should also be directed toward problems of lung pathology. High resolution autoradiography with tritiated thymidine, by an *in vitro* technique, should be used to study degenerative, neoplastic and inflammatory lung diseases of man. Fresh human surgical material is readily available, the technique is simple, and the equipment necessary is not expensive. Such investigations would provide important new data for the understanding of diseases of the lung.

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## IMMUNOLOGY

### Immunological Reactivity of Lymphocytes in Multiparous Females after Strain Specific Matings

It is now established that multiple interstrain matings may change the immunological reactivity of the female towards the foreign isoantigens of the mating male. This altered reactivity of post-partum females has been investigated in transplantation as well as serological experiments. The results of the two modes of investigation are, however, contradictory. The transplantation experiments have, with one exception<sup>1</sup>, shown a decreased<sup>2-6</sup> or unaltered<sup>7,8</sup> reactivity of the post-partum females against the transplantation antigens of the mating male. Serological investigations, on the other hand, have indicated sensitization of the females after interstrain matings. Haemagglutinins directed towards the antigens of the mating male have been demonstrated in the sera of outcrossed, multiparous females<sup>1,9,10</sup>. These conflicting results could be explained by the assumption that immunological enhancement<sup>11</sup> is responsible for the prolonged survival of tissues in the transplantation experiments. Kaliss and Dagg<sup>1</sup> investigated this hypothesis. Some of their results supported the enhancement hypothesis, but no enhancing effect of serum from multiparous females could be demonstrated after transfer to virgin mice of the maternal strain.

If enhancement were responsible for the decreased resistance of multiparous females to homografts from the mating male, it would then be impossible to detect cell-bound immunity by experiments in which homografts from the mating male are transplanted to these multiparous females. In the spleen assay technique of Simonsen<sup>12</sup> the degree of cell-bound immunity can be measured in a test system where the effect of cell-bound immunity is separated from the effect of humoral antibodies. Simonsen's spleen assay technique has therefore been used in the present work to determine whether cell-bound immunity, directed against the antigens of the mating male, does exist in multiparous females.

Newborn hybrid mice, established by crossing the strains of the female and the mating male, were injected with lymphoid cells from the outcrossed females and the ability of those cells to induce a graft versus host reaction was studied. The working hypothesis was that if the maternal lymphoid cells were sensitized against the transplantation antigens of the mating male, they should

induce a more severe graft versus host reaction, and thus cause a higher spleen index, than that induced by lymphoid cells from virgin females.

Female mice of the inbred strain *CBA* were divided into four groups and females of the strain *C57BR/cd* into three groups. *A* (experimental group): *CBA* or *C57BR/cd* females were mated to males of the inbred strain *A/Sn*; *B* (control group): *CBA* females were mated to *CBA* males; *C* (control group): *CBA* or *C57BR/cd* females were retained as virgins; and *D* (control group): *CBA* or *C57BR/cd* females were immunized against *A/Sn* tissue by inoculation at 2-week intervals (two to four times) with 0.5 ml. of a crude saline suspension of spleen, liver and lymph node cells from two *A/Sn* female mice.

Two weeks to two months after they had delivered the last of their litters, the females were killed; their spleens and lymph nodes were processed through a 60 mesh steel screen and suspended in physiological saline. Fifteen million cells in 0.2 ml. were injected intraperitoneally into (*CBA* × *A/Sn*)*F*<sub>1</sub> and (*C57BR/cd* × *A/Sn*)*F*<sub>1</sub> mice respectively, which were less than 6 days old. In each litter, consisting of at least six animals, one or more animals were injected with 15 million lymphoid cells from either: females mated to *A/Sn* males; females mated intra-strain; virgin females, or females actively immunized against *A/Sn* transplantation antigens. One or more animals in each litter were not injected and served as controls for the calculation of the spleen index.

Eight days after injection the animals were weighed and killed; the spleens were removed and also weighed. The spleen index for each animal injected with lymphoid cells from females of the experimental and control groups was calculated according to Simonsen<sup>12</sup>, relating the spleen and body weights of the experimental animals to the spleen and body weights of the control animals.

The results are shown in Tables 1 and 2. Table 1 shows that the mean spleen index obtained after injecting (*CBA* × *A/Sn*)*F*<sub>1</sub> hybrids with lymphoid cells from *CBA* females, mated intra-strain (group *B*), is the same as that obtained by injection of lymphoid cells from virgin females (group *C*). These spleen indices differ significantly from those caused by injection of lymphoid cells from females actively immunized against the transplantation antigens of the *A/Sn* strain (group *D*).

In the case of *CBA* females mated one to four times with *A/Sn* males, the immunological reactivity of the lymphoid cells of the females against the antigens of the mating male is increased with an increased number of pregnancies; the means of the spleen indices increase from 1.45 to 1.91 after one to four pregnancies. The mean of the spleen index for group *A4* differs from those of groups *B* and *C* (*P* < 0.05). When *C57BR/cd* females are mated to *A/Sn* males the immunological reactivity becomes significantly increased after one pregnancy (Table 2). Group *C* differs

Table 1. MEAN SPLEEN INDICES OF (*CBA* × *A*)*F*<sub>1</sub> ANIMALS INJECTED WITH 15 MILLION LYMPHOID CELLS FROM OUTCROSSED *CBA* FEMALE MICE OR *CBA* FEMALES OF THE CONTROL GROUPS

Source of lymphoid cells	No. of hybrid animals injected	Mean spleen index	Standard error
<i>CBA</i> females mated to <i>A/Sn</i>			
Four litters (group <i>A4</i> )	10	1.91	± 0.113
Three litters (group <i>A3</i> )	13	1.57	± 0.137
Two litters (group <i>A2</i> )	12	1.45	± 0.137
One litter (group <i>A1</i> )	10	1.45	± 0.119
<i>CBA</i> females mated to <i>CBA</i> (group <i>B</i> )	36	1.55	± 0.065
<i>CBA</i> virgins (group <i>C</i> )	38	1.56	± 0.055
<i>CBA</i> females immunized against <i>A/Sn</i> (group <i>D</i> )	33	2.04	± 0.098

Table 2. MEAN SPLEEN INDICES OF (*C57BR* × *A*)*F*<sub>1</sub> ANIMALS INJECTED WITH 15 MILLION LYMPHOID CELLS FROM OUTCROSSED *C57BR* FEMALE MICE OR *C57BR* FEMALES OF THE CONTROL GROUPS

Source of lymphoid cells	No. of hybrid animals injected	Mean spleen index	Standard error
<i>C57BR</i> females mated to <i>A/Sn</i>			
Four litters (group <i>A4</i> )	9	2.10	± 0.174
Three litters (group <i>A3</i> )	14	1.90	± 0.102
Two litters (group <i>A2</i> )	12	1.95	± 0.077
One litter (group <i>A1</i> )	19	1.87	± 0.116
<i>C57BR</i> virgins (group <i>C</i> )	25	1.57	± 0.069
<i>C57BR</i> females immunized against <i>A/Sn</i> (group <i>D</i> )	14	2.62	± 0.161

from groups A4 and A2 ( $P < 0.01$ ) and from groups A3 and A1 ( $P < 0.05$ ). These results (Tables 1 and 2) indicate that, after interstrain matings, the maternal lymphoid cells become sensitized against the isoantigens of the mating male.

The standard errors of the mean spleen indices are rather high (Tables 1 and 2). It is of interest to establish if this is due to technical errors or if there is a true variation in the occurrence and degree of immunization after multiple pregnancies. Such a variation might be expected if the immunization was a result of a chance phenomenon, as for example occasional pathological leakage of the placenta. This question was investigated by comparing the variances of the experimental and control groups. The standard deviation of the spleen indices from animals injected with lymphoid cells from virgin females was considered to represent the methodological error. The variance of this group was compared with the variances of each of the experimental groups by using the  $F$  test. In only one case, when CBA females were mated interstrain three times, was there a larger variance ( $P < 0.05$ ) in the experimental than in the control group. Evidently, the immunization was not subject to any individual variation greater than the methodological error and therefore the immunization seems to be a regularly occurring phenomenon in interstrain pregnancies.

The results of the present investigation indicate that cell-bound immunity, directed against the isoantigens of the mating male can be induced in CBA and C57BR/cd female mice by mating these females to males of the strain A/Sn. This finding supports the results of earlier serological investigations<sup>1,9,10</sup> in showing that outcrossed females become sensitized against isoantigens of the mating male. Whether this means that enhancement is responsible for the prolonged survival of homografts from the mating male in outcrossed multiparous females cannot be concluded from this work. There must, however, be some mechanism which makes the cell-bound immunity, demonstrable with the technique used in this work, ineffective in the rejection of transplants from the mating male, and one plausible explanation is that humoral antibodies may interfere with the homograft reaction.

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### Inhibition of the Mitogenic Factor in Phytohaemagglutinin by an Antiserum

EXTRACTS of some varieties of the bean *Phaseolus vulgaris* can agglutinate erythrocytes<sup>1</sup>, precipitate human cell homogenates and serum proteins<sup>2</sup>, stimulate mitosis in leucocytes<sup>3</sup>, alter the sensitivity of leucocytes to cytotoxic agents and radiation<sup>4,5</sup>, and stimulate leucocytes *in vitro* to produce gamma globulin<sup>6</sup>. A commercially prepared extract of *Phaseolus vulgaris*, derived according to the method of Rigas and Osgood<sup>7</sup>, is available as phytohaemagglutinin (PHA-M, Difco Laboratories, Detroit,

Michigan). This preparation, which is apparently a mixture of proteins<sup>8</sup>, is frequently used as a mitogenic agent for *in vitro* leucocyte cultures. Some of its antigenic properties have already been reported<sup>9</sup>.

Sensitivity to other antigens, such as PPD, diphtheria toxoid, pertussis vaccine, or penicillin, is attended by this mitogenic response of cultured leucocytes to addition of the appropriate antigen<sup>10,11</sup>. Antibodies may stimulate mitosis; a leucocyte antiserum produced an increased mitosis in a leucocyte culture<sup>12</sup>. Extracts of pokeweed (*Phytolacca americana*) have been used as a mitogenic agent<sup>13</sup>; however, none of these agents produces the high mitotic rates that are consistently obtained with PHA.

The mechanism of action of the leucocyte mitogenic factor in PHA is not known, but various suggestions have been made. Beckman proposes that it acts by precipitating a mitogenic inhibitor in serum<sup>2</sup> while Gräsbeck indicates that it attaches to the leucocyte, thereby initiating mitosis<sup>12</sup>. Nowell suggested that the mitogenic factor acted on the cell surface to permit the entrance of materials needed for cell replication<sup>3</sup>. Hastings *et al.* concluded that the mitogenic action depended on the agglutination of the leucocytes<sup>14</sup>.

The objective of the present investigation was the production in rabbits of an antiserum to PHA-M that could be used to study the mechanism of action of PHA-M in leucocyte cultures.

Four adult white female rabbits were injected intravenously with 0.5–1.0 ml. of reconstituted Difco PHA-M three to five times each week initially for a period of 4 weeks. Throughout the experiment, litter mates from the same hutch were used as controls and were not given injections of PHA-M. Before, during, and after the immunizing phase of this experiment, blood samples were removed from the ear veins of all rabbits. The serum and cells were rapidly separated and the former promptly refrigerated. In a system of immunoelectrophoresis designed to detect the presence of anti-PHA-M antibodies in the serum of the injected rabbits, distinct precipitin bands were produced by the serum withdrawn from the injected rabbits 28 days after the commencement of PHA-M injections. When the antiserum was placed in the central well and the PHA-M in the lateral troughs, three definite precipitin bands were formed in the globulin region of the agar coated slide. By reversing the positions of the antiserum and PHA-M, six major precipitin bands were produced (Fig. 1). No precipitin bands were formed by the sera of control rabbits at any time, nor were precipitin bands formed by the sera of the injected rabbits during the control phase of the experiment.

The globulin fractions of the rabbit sera which contained antibody were precipitated by mixing equal volumes of antisera and of a 27.2 per cent solution of sodium sulphate. After standing for 10 min in a water bath at 37° C, the mixture was centrifuged for 10 min at 3,500 r.p.m. After discarding the supernatant, the precipitant

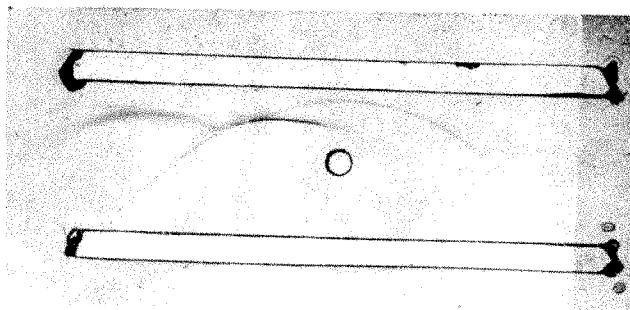


Fig. 1. Photograph of immunoelectrophoretic slide. Centre well contained PHA-M. Lateral trough without precipitin bands contained control rabbit globulin extract. Lateral trough showing precipitin bands contained rabbit anti-PHA-M globulin extract.



was reconstituted to the original volume with water. This procedure did not reduce the effectiveness of the antibody.

To determine the effect of this newly derived antibody on the mitogenic factor in phytohaemagglutinin, seven series of human peripheral leucocyte cultures from four donors (each series from a common donor) were initiated according to the method devised by Moorhead *et al.*<sup>15</sup>. The standard cultures contained 2 ml. of normal plasma with 5,000–10,000 leucocytes/mm<sup>2</sup>; 8 ml. of medium 199; 0.3 ml. of PHA-M; 1,000 u of penicillin and 5 mg of streptomycin in 0.1 ml. of saline. The constituents of the experimental cultures were identical except for the mitogenic factors. They were prepared by incubating 0.3 ml. of PHA-M for 3 h at 37° C with (1) saline, (2) graded volumes of control rabbit serum, (3) graded volumes of rabbit anti-PHA-M serum, and (4) graded volumes of the rabbit anti-PHA-M serum globulin extract. After incubation, these mixtures were centrifuged at 3,500 r.p.m. for 10 min, and the resulting supernatant solutions were used as source of carriers of mitogenic factors in place of direct addition of 0.3 ml. PHA-M. These four forms of mitogen were the only variables introduced into this series of cultures. After the cultures were incubated for 72 h, colchicine was added to a final concentration of 10<sup>-6</sup> moles/l. and the incubation continued for a further 4 h. The cells were then separated by centrifugation and resuspended in hypotonic (0.7 per cent) sodium citrate for 40 min as a preliminary to fixing in acetic acid, orcein staining, and squashing.

Both the injected rabbit antiserum and the globulin fraction extracted from it suppressed the mitogenic activity of the PHA-M in leucocyte cultures (Table 1). Addition of the supernatant from the incubation of larger amounts of the antiserum with PHA-M completely suppressed mitosis in the experimental cultures. When the supernatant from the incubation of the control rabbit serum and PHA-M was added the mitotic rate was as high as that in the saline control cultures. Addition of the antiserum to control cultures after culture initiation, however, did not completely inhibit leucocyte mitosis. The haemagglutinating factor was still present in the supernatant of the antiserum-PHA-M mixture even though the mitogenic factor had been completely inactivated.

Table 1. EFFECT OF RABBIT ANTISERUM TO PHYTOHAEMAGGLUTININ ON THE MITOGENIC FACTOR AS DEMONSTRATED IN HUMAN PERIPHERAL LEUCOCYTE CULTURES\*

Experiment No.	Treatment of phytohaemagglutinin			Mitotic activity	
	Rabbit serum	Rabbit serum added (ml.)	Globulin extract added (ml.)†	Cells counted	Mitoses
1	A	0.3		1,000	49
		0.5		1,000	7
		0.8		1,000	0
2	Control§	0.8		1,000	68
		0.3		1,000	49
		0.5		1,000	5
		0.8		1,000	0
		0.8		1,000	76
3	Saline			1,000	96
		0.5		1,000	0
		0.8		1,000	87
4	Control§	0.8		1,000	96
		0.8		1,000	0
		0.8		1,000	74
5	Saline			1,000	110
		0.8		400	0
		0.8		400	6
		0.8		400	6
		0.8		400	5
6	Control§	0.8		500	39
			0.5	1,000	0
			0.8	1,000	0
7	Control§		0.8	1,000	87
			0.8	500	36
			0.8	450	4
			0.3	1,000	15
			0.5	1,000	8
			0.8	600	7
			0.8	1,000	82

\* The standard control culture consisted of 2 ml. plasma with suspended leucocytes, 8 ml. media 199, penicillin, streptomycin, and 0.2 ml. PHA-M.

† The globulin extract was reconstituted to initial rabbit serum volume with distilled water.

‡ This animal developed severe otitis and did not receive PHA-M injections for 1 month before serum was taken for this experiment.

§ Non-immunized litter mate from the same hatch.

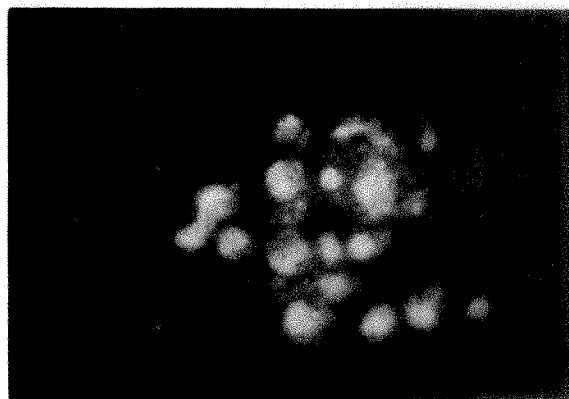


Fig. 2. Photomicrograph showing fluorescence of leucocytes exposed to PHA-M and anti-PHA-M globulin extract tagged with fluorescein isothiocyanate

In an effort to trace the distribution of PHA-M in cultured cells throughout the proliferative phase, fluorescein tagged antiserum was prepared according to a modification of the method of Riggs<sup>16</sup>. Six milligrams of fluorescein isothiocyanate in 1.5 ml. acetone was added to a suspension of 4 ml. rabbit antiserum to PHA-M, 16 ml. saline, 3 ml. carbonate-bicarbonate buffer (0.5 molar, pH 9), and 2 ml. acetone. After stirring in the cold for 18 h, this preparation was dialysed for 24 h against 0.1 molar sodium chloride buffered with sodium phosphate (pH 7.2), with at least three changes of dialysate buffer. The resulting conjugated antiserum was quickly frozen and stored for later use; control rabbit sera were also conjugated in the same manner.

Cultures were again set according to the method of Moorhead using leucocytes from a common donor and dispensing equal volumes of well mixed leucocyte rich plasma into each culture bottle. In this series, 0.2 ml. of PHA-M was used as the mitogenic agent in all cultures except for several control cultures which contained no phytohaemagglutinin. Cultures were interrupted at predetermined times throughout the 72 h period of growth. The cells were collected by centrifugation, smeared on microscope slides (in the manner of peripheral blood smears), fixed for 45 sec in cold methanol-formalin (90 ml. absolute methanol, 10 ml. 40 per cent formalin) leucocyte fixative, rinsed in cold saline and dried in air. Slides selected from each time interval were then placed in a Coplin staining jar containing fluorescein tagged anti-PHA-M serum; identical slides were placed in a Coplin jar containing tagged control rabbit serum. The slides were left in the Coplin jars overnight at a temperature of 6° C, then were removed and rinsed three times in normal saline, mounted in buffered glycerine and kept refrigerated until they were observed under an ultra-violet microscope. Eight to twelve per cent of the cells from an experimental culture (containing PHA-M) interrupted after incubation for 1 h fluoresced a characteristic apple green colour (Fig. 2) if they were exposed to the tagged anti-PHA-M serum. If identically cultured cells were exposed to the tagged control serum, there was no fluorescence. No fluorescence occurred when the cells placed in a control culture (containing no PHA-M) were exposed to the tagged anti-PHA-M serum. The percentage of tagged cells slowly increased until incubation reached 72 h at which time 45–68 per cent of the cells tagged.

In a previous investigation, which used a directly conjugated phytohaemagglutinin as the mitogenic agent, Michalowski *et al.*<sup>17</sup> reported that a majority of the cells fluoresced as early as 4–9 h. That procedure, however, did not rule out the possibility of the living lymphocytes preferentially phagocytosing the fluorescein-conjugated PHA. It would seem that our technique is more definitive because the fluorescein-conjugated antiserum is not introduced until after the cells have been cultured and fixed.

From the preceding data, it appears that the mitogenic factor which is present in phytohaemagglutinin does have antigenic properties because its antiserum does have an inhibitory effect on the mitotic rate of the cultured cells. Inhibition of cell growth as measured by the mitotic rate was found to be directly proportional to the amount of anti-PHA-M incubated with phytohaemagglutinin. Although the procedure for tagging with fluorescein does not permit localization of the primary site of action of phytohaemagglutinin, this procedure does permit enumeration of the cells altered by the presence of phytohaemagglutinin.

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## Identification of Host and Host Antibodies from Mosquito Blood Meals

INVESTIGATION of insect vectors of disease requires accurate information about their feeding habits and preferences. Mosquitoes, for example, serve as vectors for malaria, yellow fever, and the encephalitides. Each species or sub-species shows a varying degree of preference for man or other animals, and so it is most important to identify the host. Those Culicidae that do not favour man as host are not likely to be human disease vectors.

Different techniques have been employed to identify the source of blood meals, and some have met with a measure of success<sup>1,2</sup>. Even the best methods necessitate

the use of highly purified antisera for cross-reaction with diluted, extracted contents of mosquito stomach as antigen. We have recently worked out a method of micro-immunological analysis by means of a system based on a micromanipulator<sup>3</sup>. This report presents our findings so far on the identification of blood meals from *Culex* and *Anopheles* mosquitoes. Using micro-double diffusion in agar and micro-complement fixation tests on the blood from each mosquito stomach, we have successfully identified the host as well as the antibodies carried by the host, and within a general range have identified the antibody titre.

For double diffusion in agar, microtubes were prepared with short columns of 1 per cent agar. To make these microtubes, a length of borosilicate tubing (outer diameter 0.85 mm) was used to draw up a column of agar. A 10 mm microtube was cut off from this length of tubing to incorporate a 2.0 mm column of agar, which was positioned midway in the tube with a steel wire. A number of these agar-filled microtubes were mounted on a specially constructed carrier frame. The tests were then carried out using a microscope (magnification of  $\times 20-35$ ) and a micromanipulator. The details of preparation and filling of similarly sized microtubes for the micro-complement fixation tests are discussed elsewhere<sup>3</sup>.

Rabbits, dogs and humans were used as hosts. The rabbits and one of the dogs were sensitized to one of three antigens: bovine serum albumin, whole human serum, or Southern Bean mosaic virus (SBMV). *Culex pipiens* and *Anopheles quadrimaculatus* mosquitoes were trapped in isolated areas close to places of emergence to lessen the complication of previous blood meals, and were kept singly in small beakers covered with cheesecloth. They fed readily on the arm of human subjects or the ear of rabbits and dogs. After feeding, the mosquitoes were placed either in a refrigerator at 6°-9° C or at room temperature (25°-28° C) before experiments were run to define test stability under different conditions of time and temperature.

At periods ranging from 15 min to 12 days after a blood meal of approximately 1-2  $\mu$ l., each fed mosquito was killed on a glass slide and dissected. The blood meal in its peritrophic membrane was easily separated from the mid-gut region in all but the freshest samples. This unbroken sac was pulled up by light suction into a standard 1.3 mm microhaematocrit tube. The tube was sealed and centrifuged for 3-5 min on a standard microhaematocrit centrifuge. When the blood meal was old, the peritrophic membrane became rather hard and inelastic, containing clotted and lysed blood meal residue. Five to ten microlitres of 0.15 molar sodium chloride were added to the residue before centrifugation in these cases. Cross-reaction of a host sample with other than host-specific antiserum has been a great problem in other work on identification of blood meals<sup>4</sup>. This difficulty has not occurred in these microtests, but we do not know whether it is because of high test sensitivity or because more highly purified antisera are being used.

The micro-double diffusion test in agar takes only 2-4 h unless the blood meal samples are very old and have to be highly diluted; in the latter case, the test is left overnight. Five precipitin bands were present after 6 h in a test of whole human serum against 1:1 blood meal serum taken from a mosquito stomach. The mosquito took the blood

Table 1. MICRO-DOUBLE DIFFUSION IN AGAR ON MOSQUITO BLOOD MEALS

Test system	Mosquito cage temperature post feeding	Identification of host (I) and host antibody (II)															
		1 h		6 h		16 h		Time (post feeding) of killing mosquito and initiating test		24 h		36 h		72 h		96 h	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
<i>Culex</i> and <i>Anopheles</i> blood meals from rabbits with antibody to SBMV* or human serum†	6°-9° C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	25°-28° C	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+

\* Antibody titre by micro-complement fixation test on 1 h blood meal sample, 1:500.

† Antibody titre, same method, 1:100.

*Culex* only



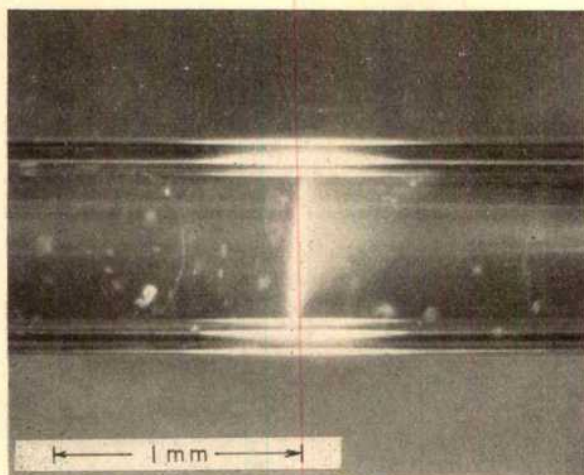


Fig. 1. Micro-double diffusion in agar. Mosquito blood meal versus Southern Bean mosaic virus (SBMV) suspension. The rabbit host carried antibodies to SBMV.

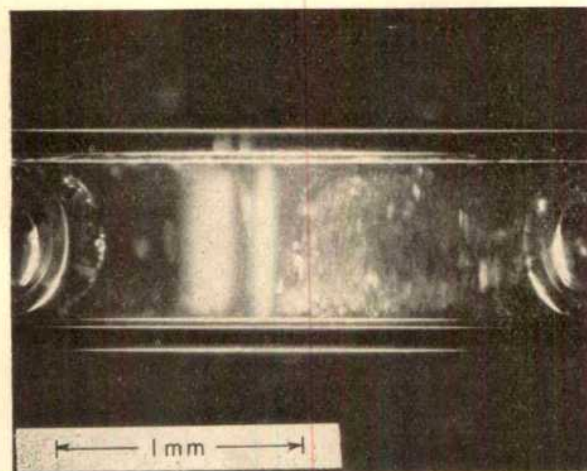


Fig. 2. The same mosquito blood meals as illustrated in Fig. 1, versus anti-rabbit goat serum (Pentex). This blood meal, like others, gave no precipitin reaction with either of the controls used, tobacco mosaic virus suspension and anti-human serum.

meal from a rabbit carrying a high antibody titre to human serum. No reaction was present with rat, duck, or dog serum. Photos show micro-double diffusion in agar (magnification,  $3.5 \times 6$ ) of host antibody reaction (Fig. 1), and host identification precipitin bands (Fig. 2).

Table 1 shows that *Culex* blood meals can be identified as to host and host antibodies after as long as 12 days if the mosquito is refrigerated. The *Anopheles* were quicker to digest blood meals, and no longer gave satisfactory tests after one week at  $1^\circ\text{C}$ , one day at  $28^\circ\text{C}$ .

In old blood meals (Table 1) the host antiserum can be identified several hours longer than the host can be identified. Perhaps this reflects the protein digestion pattern of the mosquito (the mosquito may digest globulin fractions containing antibody last). Preliminary results on human blood meals indicate that human serum albumen disappears quickly during digestion of blood meals.

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## BIOCHEMISTRY

### Specificity of Cleavage of Chitotriose by Lysozyme

AFTER the recent determination of the structure of lysozyme in the crystalline state<sup>1</sup> we have become interested in chemical and kinetic approaches to the study of its mechanism of action in solution.

The region of the crystalline enzyme to which inhibitors such as *N*-acetyl-D-glucosamine and chitobiose bind has been identified by X-ray analysis methods<sup>2</sup>, and the smallest known substrate for lysozyme, chitotriose<sup>3</sup>, has been shown to bind to the same cleft on the enzyme surface<sup>4</sup>. We have investigated the binding of chitotriose to lysozyme in solution and have shown<sup>5</sup> (unpublished work) that association with the trisaccharide affects tryptophanyl and carboxyl groups on the enzyme surface, in agreement with the results obtained from X-ray analysis<sup>2,4</sup>.

As chitotriose serves as a substrate for lysozyme<sup>3,6</sup> it was interesting to determine whether the enzyme selectively or preferentially cleaves one of the two  $\beta$ -(1-4) linked glycosidic bonds in the trisaccharide. Recent studies by Rupley<sup>7</sup> have shown that cleavage of the glycosidic bond farthest from the reducing end of the trisaccharide is indicated by carrying out the reaction in water labelled with oxygen-18. Cleavage of both glycosidic bonds with approximately equal frequency was, however, suggested by following transfer of trimer cleavage products to *N*-acetyl-D-glucosamine labelled with carbon-14. For this purpose we synthesized chitotriose in which the acetyl group of the sugar residue at the reducing end of the molecule was specifically labelled with carbon-14 (sugar residue A in Fig. 1) by methods related to those used by Leaback and Walker<sup>8</sup> to obtain *N*-acetyl-D-glucosamine from 1,3,4,6-tetra-*O*-acetyl, D-glucosamine hydrochloride. Peracetylated chitotriose was obtained by the method of Zechmeister and Toth<sup>9</sup> and was converted to chloro, decaacetyl-chitotriose using acetic anhydride and dry hydrochloric acid. Treatment of this compound with nitromethane containing 0.1 per cent 0.1 normal hydrochloric acid resulted in the formation of decaacetyl-chitotriose, *N*<sup>1</sup>-monohydrochloride. Acetylation with acetic anhydride labelled with carbon-14 gave undecaacetyl-chitotriose-*N*<sup>1</sup> labelled with carbon-14 which on de-*O*-acetylation gave the labelled trisaccharide shown in Fig. 1.

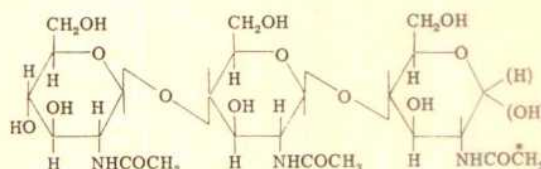


Fig. 1. Structure of chitotriose with the acetyl group of the hexosamine ring at the reducing end of the trisaccharide labelled with carbon-14.

Incubation of this labelled trisaccharide ( $10^{-2}$  molar) at pH 5.0 (acetate,  $\mu = 0.1$ , containing 0.01 molar sodium chloride) at  $40^\circ\text{C}$  for 6 h followed by fractionation of the resulting oligosaccharide mixture by gel filtration resulted in the pattern shown in Fig. 2B. Paper chromatography<sup>10</sup> confirmed the identity of the materials in each chromatographic peak. From the decrease in chitotriose concentration it was estimated that 35 per cent of the trisaccharide had been degraded by the enzyme. It was evident that in addition to hydrolysis of chitotriose to yield chitobiose and *N*-acetyl-D-glucosamine transglycosylation had also occurred to yield some chitotetraose. The specific activities of the oligosaccharides compared with the original chitotriose (18,700 c.p.m./ $\mu\text{mole}$ ) were 12,600

c.p.m./ $\mu$ mole for chitobiose and 6,400 c.p.m./ $\mu$ mole for *N*-acetylglucosamine. Because of the low yield of tetrasaccharide obtained it was not possible to estimate its specific activity with confidence. The results show that neither of the two glycosidic bonds are split with complete selectivity by the enzyme but that preferential cleavage of the bond farthest from the reducing end of the trisaccharide occurs.

Estimation of the relative amounts of chitobiose and *N*-acetyl-D-glucosamine produced revealed that the disaccharide was in excess of the monosaccharide by a factor of approximately 2:1 on a molar basis. This result, coupled with the observed formation of tetrasaccharide, is consistent with a major pathway for cleavage of the trisaccharide depicted schematically in Fig. 3. According to this scheme an enzyme-substrate complex is first formed, which then gives rise to a complex of free disaccharide and enzyme-monomer. The enzyme-monomer complex can react either with water to yield free enzyme and free monosaccharide or with trisaccharide to yield free enzyme and tetrasaccharide. Cleavage of the glycosidic bond nearest the reducing end of the trisaccharide substrate could, by a similar scheme, result in formation of chitopentaose. We have not observed any

of this oligosaccharide, but this does not exclude its formation, as the relative rates of cleavage of chitotriose, chitotetraose and chitopentaose have been shown to be 1:7:300 (ref. 11).

At present it is not possible to decide whether the substrate or products are at any stage of the reaction covalently attached to the enzyme. Further experiments to elucidate the mechanism of action of lysozyme are in progress.

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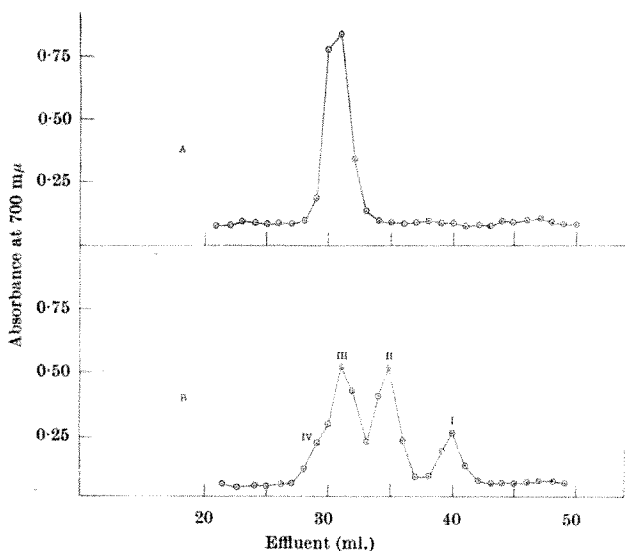


Fig. 2. A, Separation of chitotriose on a column (0.6  $\times$  100 cm) of bio-gel p-2. B, Separation of the products of lysozyme action on chitotriose (6 h at 40° C, pH 5.0) on a column (0.6  $\times$  100 cm) of bio-gel p-2. Numerals I, II, III, IV represent *N*-acetyl-D-glucosamine, chitobiose, chitotriose, and chitotetraose, respectively.

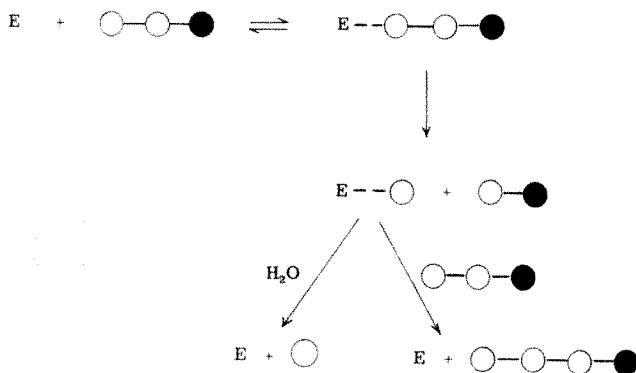


Fig. 3. Scheme depicting the major pathway for cleavage of chitotriose by lysozyme (E). Circles represent *N*-acetylhexosamine rings of *N*-acetylglucosamine units. The black circles show the *N*-acetylhexosamine rings in which the *N*-acetyl group is labelled with carbon-14. Solid lines represent covalent bonds while dotted lines depict other types of interaction.

### Succinylated Meromyosins

It has been found that on succinylation myosin releases a component of low molecular weight that can be isolated by fractionation with sulphate<sup>1</sup>. We now wish to report that this component is located in the heavy meromyosin part of the myosin molecule.

Myosin was prepared from rabbit skeletal muscle by extracting the mince for 15 min in a cold room with three volumes of a solution containing 0.3 molar potassium chloride, 0.15 molar phosphate buffer, pH 6.6, and 0.5 mmolar ATP. The suspension was diluted with an equal volume of cold distilled water and was centrifuged; the myosin in the supernatant was precipitated by dilution with 6.5 volumes of distilled water at 0° C. Any remaining *F*-actin was removed from the myosin according to the procedure of Portzehl, Schramm and Weber<sup>2</sup>; the myosin preparation so obtained was reprecipitated twice and dissolved in 0.5 molar potassium chloride, 0.05 molar phosphate buffer, pH 6.2.

Heavy meromyosin was prepared from this myosin by digestion with trypsin, at pH 6.2, for 5 min at 25° C. The weight ratio of myosin to trypsin was 200:1, and the reaction was stopped with trypsin inhibitor, twice the weight of the trypsin. The reaction mixture was dialysed against 7 mmolar phosphate buffer, pH 7.0, to precipitate the light meromyosin and undigested myosin. The heavy meromyosin was isolated from the supernatant after centrifugation at high speed. Light meromyosin fraction 1 was prepared from the precipitate according to the method of A. G. Szent-Györgyi, Cohen and Philpott<sup>3</sup>.

To prepare light meromyosin, myosin was digested for 40 min at 25° C, pH 6.2, with trypsin at a weight ratio of 100:1. The digestion mixture was dialysed against 7 mmolar phosphate buffer, pH 7.0; the light meromyosin precipitate was purified by twofold reprecipitation and dissolved in 0.5 molar potassium chloride and 0.05 molar phosphate buffer, pH 7.0.

The meromyosins were succinylated with 1.4 mg of succinic anhydride per mg of protein at room temperature while the pH was kept constant at 7.0 by addition of base. The reaction mixtures were afterwards dialysed against large volumes of a solution containing 0.5 molar potassium chloride, 0.05 molar phosphate buffer, pH 7.0.



Fig. 1 shows the ultracentrifugal patterns of light meromyosin and succinylated light meromyosin, at a protein concentration of 5 mg/ml. Both the original and the modified light meromyosin sediment as a single peak for as long as 4 h with a sedimentation coefficient of 2.2 *S*. Similar results were obtained when light meromyosin fraction 1 and succinylated light meromyosin fraction 1 were compared; the sedimentation behaviour of both was identical. Furthermore, determinations of molecular weight with light scattering showed no difference between the untreated and the succinylated light meromyosin fraction 1, and gave a value of 160,000–170,000 for both.

In contrast to light meromyosin, the gross structure of which is unchanged by succinylation, heavy meromyosin is split into two components. This phenomenon is demonstrated in the upper row of Fig. 2, which shows the ultracentrifuge pictures of heavy meromyosin and succinylated heavy meromyosin. Whereas heavy meromyosin is homogeneous (sedimentation coefficient, 5.6 *S*), succinylated heavy meromyosin gives two peaks (sedimentation coefficients, 6.4 *S* and 1.3 *S*, respectively). We were able to isolate the two fractions from the succinylated heavy meromyosin with the aid of ammonium sulphate. Fraction 1 was precipitated in a cold room from the mixture at 55 per cent saturation, washed with 55 per cent ammonium sulphate, then dissolved, and dialysed against a solution containing 0.5 molar potassium chloride and 0.05 molar phosphate, pH 7.0. To obtain fraction 2, the supernatant, obtained from the mixture at 55 per cent ammonium sulphate saturation, was dialysed against distilled water to remove salts and then freeze-dried; it was then dissolved and dialysed against 0.5 molar potassium chloride and 0.05 molar phosphate, pH 7.0.

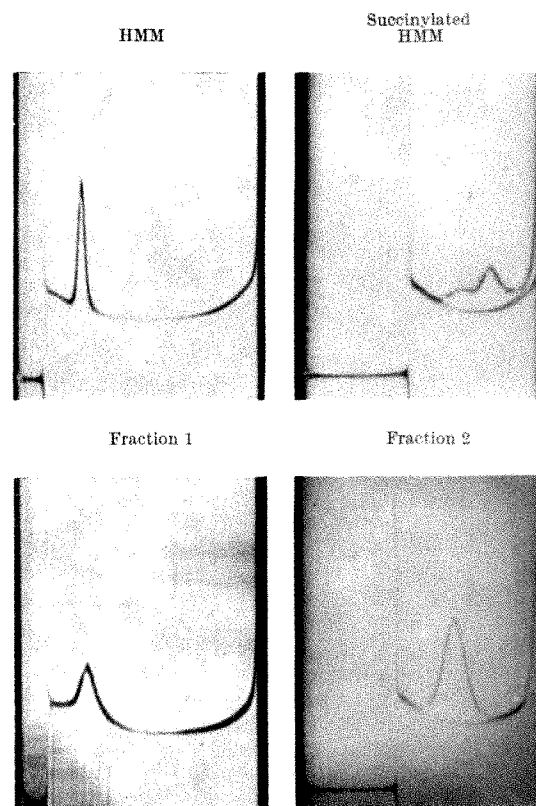


Fig. 2. Ultracentrifugal patterns of heavy meromyosin (HMM), succinylated heavy meromyosin, fraction 1 and fraction 2 isolated from succinylated heavy meromyosin. Protein concentration, 3.3 mg/ml.; speed, 59,780 r.p.m.; temperature, 20.0° C; pictures taken after 24 min. Sedimentation proceeds from left to right.

The sedimentation patterns of the isolated fractions 1 and 2 from the succinylated heavy meromyosin are shown in the lower row of Fig. 2. Both these fractions sediment as a single peak with a sedimentation coefficient of 6.2 *S* and 1.3 *S*, respectively.

These data indicate that succinylation produces no detectable alteration of the light meromyosin, while under identical conditions the heavy meromyosin is split into two components. We therefore conclude that the component of low molecular weight released on succinylation of myosin originates solely from the heavy meromyosin part of the myosin molecule.

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### Relative Distribution of Enzymes and Prosthetic Groups

THE *Enzyme Classification*<sup>1</sup>, published in 1961, is a convenient source of authoritative data on the numbers and types of enzymes. The recently revised version of the list<sup>2</sup> has extended the available data and made possible an assessment of the changes produced by 5 years of increasingly fruitful work in the field of enzymology. The figures quoted in this communication are based on my own examination of the two editions of the *Enzyme*

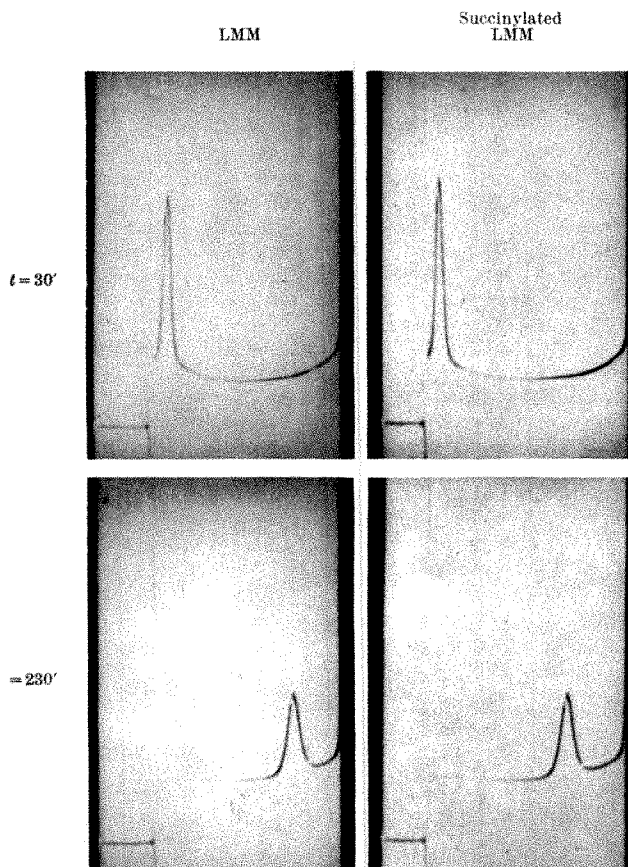


Fig. 1. Ultracentrifugal patterns of light meromyosin (LMM) and succinylated light meromyosin. Protein concentration, 5 mg/ml.; speed, 59,780 r.p.m.; temperature, 20.0° C.



**Classification.** In spite of any numerical errors which may have resulted, I think that the findings are quite clear and of general interest.

In the first place, the total number of enzymes recognized in the classification has risen from 703 in 1961 to 884 in 1965, an average of about 40 per year. The alterations within the six principal enzyme groups as a result of this substantial increase are shown in Table 1, which records the total number of enzymes in each group and the corresponding percentage of the total which each represents. It is quite clear that there is virtually no change in the relative proportions. Bearing in mind that each "enzyme" in the list is generally represented in several different forms of living organism, and that recent work has begun increasingly to embrace such comparative studies, it seems reasonable to presume that the figures in Table 1 are a close approximation to the overall distribution of enzyme types in living organisms generally.

The distribution of prosthetic groups can also be obtained from the classification, and is analysed in Table 2. The prosthetic groups indicated there represent all the groups now recognized as being able to form an integral and reactive portion of enzyme structure. The recent report of a previously unknown type of prosthetic group, possibly a quinone<sup>3</sup>, indicates that further additions to the table are in prospect. Some further provisos must be noted: (i) for the purposes of Table 2 I have not distinguished between cofactors and prosthetic groups, because in general it appears reasonably certain that in both cases the molecule is involved directly in the catalytic reaction; (ii) as an exception to the previous statement I have not felt justified in including enzymes which require metals except when the metal is definitely known to be firmly bound to the protein; as a result, the distribution of "metal enzymes" is certainly higher than shown here—how much higher can only be surmised until more evidence on reaction mechanisms is available; (iii) in a few cases where two distinct and different prosthetic groups occur in the same enzyme I have counted two enzymes for the purposes of Table 2.

Table 2 shows clearly that the total number of enzymes which have such auxiliary groups (at least 16 per cent of the total) is greater than the remarks of the Enzyme Commission (ref. 1, page 29; ref. 2, page 26) would lead one to imagine, but it is nevertheless equally clear that the great majority of enzymes carry out their catalytic activity in virtue of their constituent amino-acids alone. Pyridoxal phosphate is the commonest auxiliary group, both numerically and in its wide distribution.

The distribution of these groups is not now so sharply restricted within the principal groups of enzymes as was the case in the 1961 classification. One is tempted to make a comparison with the zoological classification and to suppose that the apparently aberrant enzymes which are now appearing in the classification are in some sense "missing

links" between the principal enzyme groups. It is also striking that a more detailed consideration of the enzyme sub-groups shows that auxiliary groups are very often even more restricted than Table 2 implies. This is more difficult to detail at present because information on reaction mechanisms becomes more important at this level. Also, one does not know to what extent the present classification will be revised as further knowledge accumulates. Obvious examples, however, are the transferases of sub-group 2.6.1.x, mainly catalysing amino-acid reactions, in which sixteen out of the twenty are pyridoxal phosphate enzymes (out of eighteen such enzymes in the group as a whole); and the ligases of sub-group 6.4.1.x, catalysing the formation of carbon-carbon bonds, where all four enzymes contain biotin (out of five such enzymes in the whole classification). One can also note that Group I enzymes contain all but one of the known flavin enzymes, and that all but two of these are in sub-groups 1 to 10. Within sub-groups 1 to 10, however, there is at present a clear distinction between enzymes requiring NAD or NADP and the rest. None of the former contain flavin, whereas the latter may (along with various other prosthetic groups, or none at all). One may say that all the dehydrogenases classed in sub-groups 1.(1-10).1.x use NAD or NADP exclusively as acceptor cofactor and do not contain flavin, whereas dehydrogenases in the remainder of this section [1.(1-10).(2-99).x] do not use NAD or NADP, but may contain flavin. These and other relationships of the Group I enzymes are very conveniently brought out by plotting a table with sub-groups reading down the page against the type of acceptor reading across. This gives a series of columns showing the acceptor with which the various enzymes react. In such a table the enzymes requiring NAD or NADP comprise all the entries in the first column. Other characteristics, such as the type of prosthetic group, are readily displayed in the table. Unfortunately, this treatment is not applicable to any of the other enzyme groups.

The results of general considerations as presented earlier, and of more detailed examinations as in the matrix just mentioned, suggest possible relationships between enzymes and enzyme groups which are not immediately apparent in the printed version. For example, contrary to what was stated previously, Katzen and Buchanan<sup>4</sup> have recently reported that methylene tetrahydrofolate reductase, 1.5.1.5, is an enzyme containing FAD. On the other hand, they also found that it did not require either NAD or NADP. On both grounds, therefore, it seems probable that either these authors were not studying enzyme 1.5.1.5 or else that enzyme 1.5.1.5 has been incorrectly classified. The ability to point out discrepancies such as this strengthens confidence both in the type of analysis given here and in the classification on which it is based. It appears that in an encouragingly high proportion of cases the classification based on overall reaction is turning out to be the same as that which would be based on the true reaction mechanism. One may hope to recognize further similarities based on the reaction mechanism, some of which are already emerging, as, for example, the lysine and serine enzymes.

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Table 1. PERCENTAGE DISTRIBUTION OF ENZYMES FROM THE ENZYME CLASSIFICATION

Enzyme group	1961		1965	
	Number	Percentage of total	Number	Percentage of total
I	182	26	222	25
II	173	24.5	238	27
III	183	26	213	24
IV	87	12.5	117	13
V	37	5	47	5
VI	41	5	47	5
	703	99	884	99

Table 2. DISTRIBUTION OF ENZYME PROSTHETIC GROUPS FROM THE ENZYME CLASSIFICATION (1965)

Enzyme group	Number of enzymes	Prosthetic group						
		Pyridoxal-P	Flavin	Metal	Haem	Thiamine-P-P	Biotin	B <sub>12</sub>
I	222	1	34	24	9	2		
II	238	18				1	1	
III	213	1		4				
IV	117	29	1	1		4		1
V	47	4						1
VI	47						4	
	884	53	35	29	9	7	5	2

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### Inhibition of Alcohol Dehydrogenase by Chloroquine

CHLOROQUINE is an anti-malarial drug which is also used in the treatment of rheumatoid arthritis. It has been shown to inhibit various thiol-containing enzymes such

as succinic dehydrogenase<sup>1</sup>, glutamic dehydrogenase<sup>2</sup> and the  $\alpha$ -ketoglutarate oxidizing system<sup>3</sup>. The prolonged administration of large doses of chloroquine causes the development of an irreversible retinopathy<sup>4</sup>. Chloroquine accumulates in the retinal pigment epithelium<sup>5</sup> and it also inhibits NADH-monodehydroascorbic acid transhydrogenase, a thiol-containing enzyme present in retinal microsomes<sup>6</sup>. Alcohol dehydrogenase is an important enzyme in the visual cycle, being involved in the retinol-retinal conversion, and in this communication it has been established that this thiol-containing enzyme is competitively inhibited by chloroquine.

Yeast alcohol dehydrogenase was obtained from Boehringer G.m.b.H., and the stock suspension (30 mg/ml.) was diluted 500-fold with water before use. The enzyme was assayed at 37° C by measuring the rate of oxidation of NADH at 368 m $\mu$  in a Unicam 'SP 700' recording spectrophotometer. Chloroquine absorbs strongly at 340 m $\mu$ , the wavelength at which NADH is usually measured, but at 368 m $\mu$  it has zero absorption while NADH still has 50 per cent of its maximum absorption.

The assay mixture contained 0.1 ml. of enzyme suspension, ethanol (0.36 molar), NADH and phosphate buffer, pH 8.0, up to a final volume of 3 ml. The final concentration of NADH varied from 0.034 mmolar to 0.35 mmolar and that of chloroquine from 1  $\mu$ molar to 1 mmolar. The reaction was started by the addition of the enzyme. NADH was omitted from the blank cell.

2,3-Dimercaptopropanol (BAL) reverses the inhibitory effect of chloroquine on the  $\alpha$ -ketoglutarate oxidizing system<sup>3</sup>. The protective effect of BAL on the chloroquine inhibition of alcohol dehydrogenase could not be investigated, because BAL, at a final concentration of 2 mmolar, itself caused inhibition by reaction with the zinc moiety of the alcohol dehydrogenase. Reduced glutathione was found not to inhibit alcohol dehydrogenase and reversal of chloroquine inhibition was attempted with this compound. The reduced glutathione (2 mmolar, final concentration) was added to the reaction mixture before the addition of 1 mmolar chloroquine; the NADH concentration was 0.25 mmolar.

Chloroquine inhibited alcohol dehydrogenase at all concentrations of NADH used (see Table 1); the maximum inhibition observed was at chloroquine and NADH concentrations of 1 mmolar and 0.034 mmolar, respectively. The inhibitory effect became less pronounced with decrease in chloroquine concentration or increase in NADH concentration. Chloroquine, at a final concentration of 1  $\mu$ molar, did not exert any inhibition on the reaction.

The results obtained with chloroquine at a fixed concentration of 1 mmolar and varying NADH concentrations were plotted according to the method of Lineweaver and Burke; the reciprocal of the velocity of the reaction (change in extinction at 368 m $\mu$ /min) was plotted against the reciprocal of the NADH concentration (Fig. 1). The plot obtained was characteristic of that given by competitive inhibitors, and both lines crossed the vertical axis at the same point. In the presence of glutathione (2 mmolar, final concentration) the inhibitory effect of 1 mmolar chloroquine was unaltered.

These results demonstrate that alcohol dehydrogenase, in common with several other thiol-containing enzymes, is inhibited by chloroquine, and the inhibition is of a competitive type, as is that of glutamic dehydrogenase<sup>2</sup>.

Histopathological investigations of chloroquine retinopathy have shown that it has some features in common

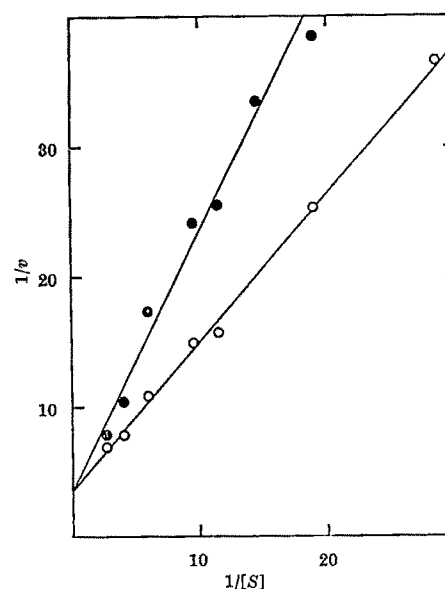


Fig. 1. Inhibition of alcohol dehydrogenase by chloroquine: data plotted according to the method of Lineweaver and Burke.  $1/v$  is the reciprocal of the velocity of the reaction (change in extinction at 368 m $\mu$ /min) and  $1/[S]$  is the reciprocal of the NADH concentration. ○, No chloroquine; ●, plus 1 mmolar chloroquine.

with that caused by iodoacetate<sup>7</sup>, another inhibitor of thiol-containing enzymes, and it is probably through an effect on enzyme thiol groups that chloroquine produces a retinopathy.

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### Enzyme Hydrolysis of 6-Benzylamino-9B D-Ribofuranosylpurine and 6-Hexylamino-9B D-Ribofuranosylpurine

THE N-6 substituted adenines with a lipophilic radical show a biological activity of the kinetin type<sup>1,2</sup>. The ribosides of substituted adenine present an activity at least identical to that of the free bases<sup>2,3</sup>; this could be possibly attributed to their hydrolysis and the subsequent formation of the free bases. To test this hypothesis, the action of nucleoside phosphorylase from *Escherichia coli* was analysed on the ribosides of two kinins: 6-benzylaminopurine and 6-hexylaminopurine. The method used for the enzyme preparation was that of Paegle and Schlenk<sup>4</sup>; the fraction of protein precipitated with ammonium sulphate between 60 and 80 per cent of saturation was retained and then dialysed. Preliminary tests on the hydrolysis of nucleosides were effected with inosine and adenosine.

The kinetics of the reaction were followed either spectrophotometrically<sup>5</sup> or by separating the ribosides from the liberated bases resulting from the hydrolysis, with thin-layer (cellulose) chromatography. For the spectrophotometrical analysis the reaction mixture was 0.2  $\mu$ moles of nucleoside; 0.2 ml. of catalase solution (2 mg/

Table 1. INHIBITION OF ALCOHOL DEHYDROGENASE BY CHLOROQUINE AT TWO CONCENTRATIONS OF NADH

Chloroquine concentration	Inhibition of enzyme activity (per cent)	
	NADH concentration 0.034 mmolar	NADH concentration 0.25 mmolar
1 mmolar	49	35
100 $\mu$ molar	31	26
10 $\mu$ molar	13	11
1 $\mu$ molar	0	0

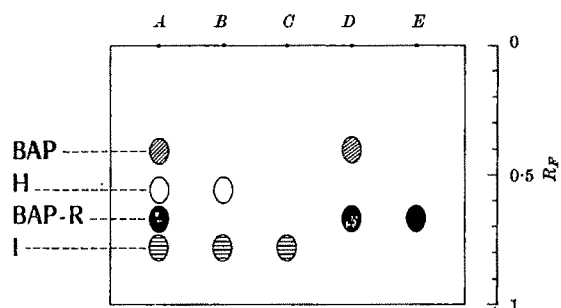


Fig. 1. Thin-layer chromatographic determination of the enzyme hydrolysis of some ribosides. Control (A). Hydrolysis of inosine by active nucleoside phosphorylase (B). Boiled nucleoside phosphorylase (C). Hydrolysis of 6-benzylaminopurine riboside by active nucleoside phosphorylase (D). Boiled nucleoside phosphorylase (E). BAP, 6-benzylaminopurine; H, hypoxanthine; BAP-R, 6-benzylaminopurine riboside; I, inosine.

ml.); 0.5 ml. of xanthine-oxidase solution (0.04 mg/ml.) for inosine hydrolysis or 0.2 ml. of this solution (2 mg/ml.) for adenine and substituted adenine nucleosides; 0.02 ml. of bacterial extract. The total volume was adjusted to 4 ml. with a phosphate buffer pH 8 and 0.03 molar.

For the chromatographic analysis the reaction mixture was 0.5  $\mu$ moles of nucleoside; 0.05 ml. or 0.1 ml. of bacterial extract. The total volume was adjusted to 1 ml. with the same buffer.

Table 1. SPECTROPHOTOMETRICAL DETERMINATION OF ENZYME HYDROLYSIS OF VARIOUS PURINE RIBOSIDES BY NUCLEOSIDE PHOSPHORYLASE

	Inosine $\Delta$ O.D. (290 $\mu$ )	Adenosine $\Delta$ O.D. (305 $\mu$ )	6-Benzyl- aminopurine $\Delta$ O.D. (305 $\mu$ )	6-Hexyl- aminopurine $\Delta$ O.D. (305 $\mu$ )
A	0.41	0.24	0.17	0.45
B	0.01	0.00	0.00	0.01
C	0.00	0.00	0.00	0.00
D	0.01	0.00	0.00	0.00
E	0.56	0.12	0.10	1.11

In Table 1 are shown variations of the optical density (in the wavelength corresponding to the maximum absorption of the oxidated bases) after 48 min of hydrolysis for different reaction mixtures. A—complete reaction mixture; B—without nucleosides; C—without xanthine oxidase; D—without nucleoside phosphorylase; E—nucleosides replaced by the free bases.

It is clear that the oxidation of nucleosides by xanthine oxidase is negligible compared with the oxidation of the free bases (D and E). With nucleoside phosphorylase, the situation is reversed, that is, the four ribosides (and especially the ribosides of 6-benzylaminopurine and 6-hexylaminopurine) are hydrolysed, and the liberated bases are then oxidated by xanthine oxidase (A). The presence of nucleosides (B), nucleoside phosphorylase (D) and xanthine oxidase (C) is necessary to make the reaction possible.

The separation of the free bases from the non-hydrolysed ribosides was possible by thin-layer chromatography of an aliquot fraction of hydrolysis products using distilled water as a solvent. In Fig. 1 it can be seen that the bases hypoxanthine and 6-benzylaminopurine effectively appeared during hydrolysis of their corresponding ribosides.

Consequently, nucleoside phosphorylase from *E. coli* is able to hydrolyse ribosides of the N-6 substituted adenine 6-benzyl- and 6-hexylaminopurine which present a kinetin activity.

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## Effect of Growth Hormone on the Isolated Pancreatic Islets of Rat *in vitro*

ISLETS of Langerhans were isolated by the technique of Kostianovsky and Lacy<sup>1</sup> from the pancreases of intact rats, as well as from rats hypophysectomized 4 weeks previously, some of which were treated for the last 3 days with 2 mg bovine growth hormone/day. Following an equilibration period of 20 min in cold Krebs-Ringer bicarbonate buffer, pH 7.2, with 0.6 mg/ml. glucose, five or six islets were placed in each incubation flask with 0.5 ml. of the same buffer, and incubated at 37° C with continuous gassing, using a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. After incubation for 15 min, the islets were transferred to a second incubation medium with either 0.6 or 3.0 mg/ml. glucose plus tritiated D,L-leucine (0.01 mc.,  $1.27 \times 10^{-5}$  mmolar) and incubated for a further period of 15 min. At the end of the incubation, the islets were removed, washed with cold buffer containing 1 mg/ml. unlabelled D,L-leucine, and homogenized with 0.5 ml. of 10 per cent trichloroacetic acid in a tissue micro homogenizer. Insulin extraction from the islets homogenized with TCA was carried out according to the procedure described by Taylor<sup>2</sup>. Portions of the acid-alcohol extract were used for insulin determination and for counting the incorporation of tritium in the insulin-anti-insulin precipitate. The precipitate that remained after extraction with acid-alcohol was treated with chloroform-methanol-ether in order to remove the fat, and the protein content was determined by the micro-method of Lowry<sup>3</sup>. Release of insulin into the incubation media, and the insulin content in the extracted islets, were determined by the immunoassay method of Herbert<sup>4</sup>, in which the separation of free insulin and insulin bound to antibody is achieved by "instant dialysis" with 'Dextran 80' coated charcoal. Some twenty islets from each animal were studied; two sets were incubated with 0.6 mg/ml. glucose in the two cycles (baseline), while another two were submitted to glucose stimulation (3.0 mg/ml.) in the second cycle.

The results are summarized in Table 1. In all cases the amounts of insulin are expressed in relation to protein content.

The present results show that a high concentration of glucose significantly stimulates release of insulin and incorporation of tritiated leucine by the isolated islets from normal rats. The increased incorporation of leucine, as an indication of increased insulin synthesis, may help to explain the finding of an unchanged insulin content of the stimulated islets by indicating that synthesis and release may have been equally enhanced.

Islets from the hypophysectomized rats had a significantly reduced insulin content, a lower insulin output, and a diminished insulin synthesis compared with the normal animals, either at a low or a high glucose concentration. Furthermore, the glucose concentration of 3.0 mg/ml. failed to stimulate the release or synthesis of insulin to values higher than basal.

Treatment for 3 days with bovine growth hormone corrected most of those parameters. The islets again responded to stimulation with glucose. The release, content and synthesis of insulin were all significantly higher than in islets from hypophysectomized untreated rats, and did not differ from the same factors in normal islets stimulated by glucose.

The present *in vitro* procedure has the advantage that it is possible to study islets directly in a pure preparation. In the particular case of the action of growth hormone on islets, the method eliminates the effect of the hormone on acinar tissue, a problem that has handicapped most of previous preparations of pancreas *in vitro*. The incorporation of labelled amino-acids into the insulin fraction, as an indication of its synthesis, is more meaningful, as the possibility of contamination by other non-islet proteins is reduced.

Table 1. INCUBATION OF ISOLATED ISLETS OF LANGERHANS OF RATS

	Insulin release ( $\mu$ U insulin/ $\mu$ g protein)		Islets insulin content ( $\mu$ U insulin/ $\mu$ g protein)		Tritiated leucine incorporation (c.p.m./mU insulin)	
	Baseline	Stimulation	Baseline	Stimulation	Baseline	Stimulation
Normal (18)	18.40 $\pm$ 3.81†	46.70 $\pm$ 15.05*	4,778 $\pm$ 873.11	3,978 $\pm$ 435.22	4.30 $\pm$ 0.81	11.57 $\pm$ 2.10*
Hypophysectomized (14)	6.80 $\pm$ 2.50	16.32 $\pm$ 5.48	1,714 $\pm$ 388.25	2,071 $\pm$ 405.30	5.98 $\pm$ 1.81	6.64 $\pm$ 1.81
Hypophysectomized + bovine growth hormone (8)	10.51 $\pm$ 2.86	29.00 $\pm$ 0.71*	1,400 $\pm$ 234.50	3,700 $\pm$ 822.50*	8.86 $\pm$ 1.78	15.63 $\pm$ 2.34*

\* The difference between response to glucose stimulation and baseline is significant ( $P < 0.05$ ). Number of experiments in parentheses.

† Mean  $\pm$  standard error of the mean.

The results reported here indicate that growth hormone increases the rate of synthesis and of release of insulin by the islets. These effects are more evident when they are stimulated by a high concentration of glucose, which suggests that the hormone may act by enhancing the activating mechanism of this sugar on beta cells.

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### C-Terminal Sequence of Pig Growth Hormone

THE wide interest in the comparative biochemistry of pituitary growth hormone prompted this investigation of the nature of the C-terminal region of pig growth hormone. Recent reports on the C-terminal octapeptide of ox growth hormone<sup>1,2</sup>, and on the entire amino-acid sequence of human growth hormone<sup>3</sup>, provide an interesting basis for comparison.

The purified pig growth hormone used in this work was kindly provided by Professor A. E. Wilhelmi. It had a biological potency of 2.3 IU/mg. Before digestion with carboxypeptidase, the hormone was reduced with  $\beta$ -mercaptoethanol in 8 molar urea<sup>4</sup>, and the sulphhydryl groups formed were reacted with iodoacetamide. This preparation of reduced and carbamidomethylated (RCAM) protein was dissolved in 0.1 per cent sodium bicarbonate containing 1 per cent sodium dodecyl sulphate at a protein concentration of 1 per cent. Carboxypeptidase A (Sigma, DFP treated) was added to make an enzyme: substrate ratio of 1:100. The mixture was incubated at room temperature, and aliquots were removed at 15 min, 1 h and 20 h. The liberated amino-acids were analysed by paper electrophoresis<sup>5</sup>. The results (Fig. 1) indicate that phenylalanine is C-terminal, followed by alanine and half-cystine. These results are calculated from an assumed molecular weight of 22,000 (refs. 6-8).

The procedure used by Wallis<sup>1</sup> to isolate the C-terminal peptide of ox growth hormone was followed exactly to isolate the corresponding peptide of pig growth hormone. Thus, a tryptic digest of the performic oxidized hormone was run on paper electrophoresis at pH 2.1, and the peptide which hardly moved from the origin toward the cathode was purified by electrophoresis at pH 6.5. The amino-acid composition of this peptide, determined on a Technicon 'AutoAnalyzer', was Phe (1.8), Ser (1.9), Glu (1.0), Ala (1.2), Val (0.9), CySO<sub>3</sub>H (0.9). Treatment of the peptide with carboxypeptidase showed that phenylalanine is C-terminal, and Edman-Dansyl degradation<sup>9</sup> revealed the N-terminal sequence Phe-Val-Glu-. Diges-

tion of the peptide with pronase released free phenylalanine and valine, and two tripeptides, Pr<sub>1</sub> [Glu (1.0), Ser (1.8)] and Pr<sub>2</sub> [(CySO<sub>3</sub>H (1.1), Ala (1.0), Phe (1.0)]. Analysis by the dansyl method<sup>10</sup> showed glutamic acid to be the N-terminal residue of Pr<sub>1</sub>, and cysteic acid of Pr<sub>2</sub>. These data are sufficient to deduce the structure shown in Fig. 2a.

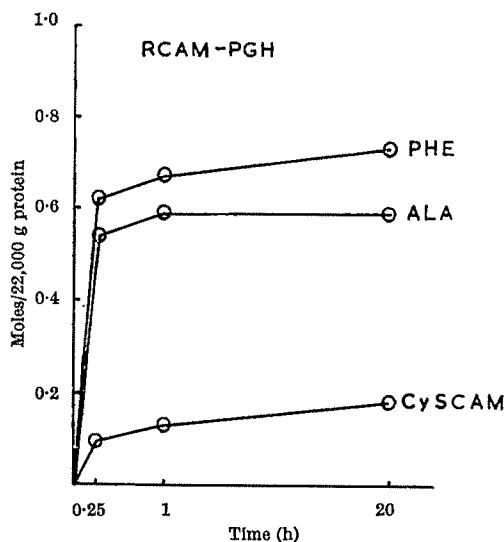


Fig. 1. Amino-acids released from reduced and carbamidomethylated pig growth hormone (RCAM-PGH) by carboxypeptidase A. The abbreviation CySCAM refers to S-carbamidomethylcysteine, which represents half-cystine in the native hormone.

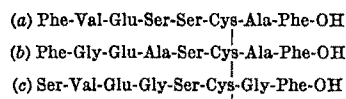


Fig. 2. The C-terminal amino-acid sequences of (a) pig growth hormone; (b) ox growth hormone<sup>1</sup>, and (c) human growth hormone<sup>3</sup>.

The sequence reported here is rather different from the sequence, -(Ser, Leu)-Phe-Ala-Phe, reported earlier<sup>11</sup>, but a comparison with the C-terminal sequences of ox growth hormone<sup>1</sup> (Fig. 2b) and human growth hormone<sup>3</sup> (Fig. 2c) shows several points of similarity. These results, together with recent investigations of the molecular weights<sup>6-8</sup> and immunological properties<sup>12</sup> of several species of growth hormone, indicate that the growth hormone molecules of various species could be much more similar than was earlier believed.

It is of interest to note that the amino-acid substitutions in the structures in Fig. 2 all involve single base changes in the genetic code<sup>13</sup>. The theory<sup>14</sup> that this is a requirement for amino-acid substitution, however, does not necessarily apply in this comparison of several species, since it was devised to explain point mutations within a given species.

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### Effect of Various Hormones on the<sup>3</sup>Milk Ejection Response of Tissue isolated from the Rat Mammary Gland

A SENSITIVE *in vitro* bioassay for oxytocin has been developed which makes use of the milk-ejection response of tissue from the mammary gland of lactating rats<sup>1</sup>. The object of the present experiments was to determine the specificity of this assay. The specificity of the response of mammary gland tissue to oxytocin, *in vitro*, has been a matter of controversy ever since Mendez-Bauer *et al.*<sup>2</sup> used the response of this tissue as a quantitative assay for oxytocin. These workers reported that the contractions of rabbit mammary strips were highly specific for oxytocin. Subsequently other investigators found that mammary strips from lactating rats contracted in response to a variety of substances, for example, arginine-vasopressin<sup>3,4</sup>, acetylcholine, epinephrine, norepinephrine, histamine and 5-hydroxytryptamine<sup>4</sup>. Mammary strips from lactating rabbits contracted when exposed to arginine-vasopressin and to acetylcholine<sup>5</sup>.

Mammary tissue contains two types of contractile cells, smooth muscle and myoepithelium<sup>6,7</sup>. There is no direct experimental evidence to indicate which of the two elements is responsible for milk ejection and which is concerned with the contractions of a strip of mammary tissue. It is conceivable that both the myoepithelial and smooth muscle cells participate in the contractions, while the myoepithelial cells are solely responsible for milk ejection. Conclusions concerning the oxytocin specificity of mammary gland tissue based on the contractions of strips may not therefore be relevant for the milk ejection response.

In the present experiments, milk ejection was measured by the technique of Van Dongen and Hays<sup>1</sup>. The time was determined between the application of the test substance to a small piece of mammary gland tissue and the ejection of milk. This interval, the latency, is quantitatively related to the concentration of the test substance. The following compounds were tested: syntocinon, bradykinin, 8-lysine-vasopressin, 8-arginine-vasopressin, pitocin, pitressin, adrenaline chloride, *l*-norepinephrine bitartrate monohydrate, histamine diphosphate, serotonin creatine sulphate, and acetylcholine chloride.

Lactating, primiparous, white rats with eight or more young and between the fifteenth and seventeenth days of lactation were used. The young were removed from the mother 5–8 h before the start of the experiment. The mother was anaesthetized with sodium pentobarbital (26 mg/kg body weight) and a portion of the fourth mammary gland was excised. Pieces approximately 2 mm<sup>3</sup>

were cut from the gland and stored in tissue culture medium containing 116.43 mmolar sodium chloride, 5.36 mmolar potassium chloride, 9.06 mmolar sodium dihydrogen phosphate, 0.81 mmolar magnesium sulphate, 1.80 mmolar calcium chloride, 27.74 mmolar sodium bicarbonate, and 5.55 mmolar glucose. Horse serum was added to this solution (10 per cent final concentration). The pH was adjusted to 7.4 with 0.1 normal hydrochloric acid.

A total of six experiments was carried out. All the compounds were tested in every experiment, the sequence of testing being rotated from one experiment to another. The latencies were measured and analysed according to established statistical procedures<sup>8</sup>. In order to check all compounds in every experiment, it was necessary for the tissue sensitivity to remain constant for at least 4 h, and for spontaneous contractions to be minimal. For these reasons the experiments were done at 5° C.

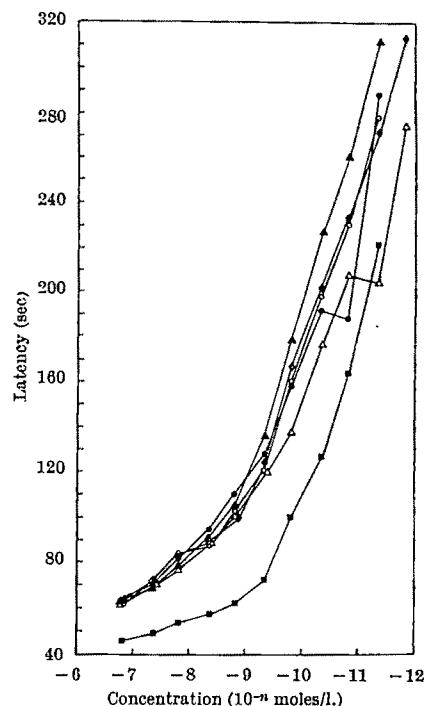


Fig. 1. Relationship between hormone concentration and the time from exposure of the mammary tissue to hormone until the ejection of milk (latency). Isolated segments of rat mammary gland, 5° C. Results from six experiments. Each point represents mean of sixty determinations (over the range of concentrations  $4.4 \times 10^{-7}$  to  $4.4 \times 10^{-10}$  moles/l.); and ten to sixty determinations (between  $4.4 \times 10^{-10}$  and  $8.8 \times 10^{-12}$  moles/l.). ●, Syntocinon; ○, pitocin; ◆, pitressin; ▲, 8-lysine-vasopressin; △, 8-arginine-vasopressin; ■, acetylcholine.

Bradykinin, adrenaline chloride, *l*-norepinephrine bitartrate monohydrate, histamine diphosphate and serotonin creatine sulphate, at concentrations ranging from  $4.4 \times 10^{-7}$  to  $4.4 \times 10^{-12}$  moles/l., did not cause any ejection of milk. Syntocinon, pitocin, pitressin, 8-lysine vasopressin, 8-arginine vasopressin, and acetylcholine chloride, at concentrations ranging from  $4.4 \times 10^{-7}$  to  $8.8 \times 10^{-12}$  moles/l., did produce ejection of milk. Fig. 1 shows dose-response curves for these compounds. Each point on every curve (in the range of concentrations from  $4.4 \times 10^{-7}$  to  $4.4 \times 10^{-10}$  moles/l.) is the mean of sixty latencies. Below  $4.4 \times 10^{-10}$  moles/l. the milk ejection ability of all compounds became erratic, and it was not possible to obtain equal numbers of latencies for every compound. From  $4.4 \times 10^{-10}$  to  $8.8 \times 10^{-12}$  moles/l. each point on the curves is a mean of between sixty and ten latencies.

There were no significant differences among the dose-response curves for the various polypeptides. The results



from an analysis of variance on these data appear in Table 1. The results from an analysis of variance on the data from polypeptides and acetylcholine are given in Table 2. The dose-response curve for acetylcholine differed significantly from those for the polypeptides (compare Tables 1 and 2).

Table 1. ANALYSIS OF VARIANCE OF THE LATENCIES FOR SYNTOCINON, PITOCIN, PITRESSIN, LYSINE-VASOPRESSIN, ARGinine-VASOPRESSIN

Data obtained from experiments shown in Fig. 1 over the concentration range  $4.4 \times 10^{-7}$  to  $4.4 \times 10^{-10}$  moles/l.

Source	DF	MS	F <sub>cal</sub>	F <sub>0.001</sub>
Total Samples	2,099			
Between animals	5	108,087	8.27*	4.10
Within animals	204	13,075		
Between compounds	4	4,577	0.35†	4.62
Within compounds	200	13,245		
Between concentrations	6	355,057	132.83*	3.74
Linear	1	1,970,785	737.29*	10.83
Non-linear	5	81,915	11.94*	4.10
Within concentrations	194	2,673		
Error	1,890	153		

DF, Degrees of freedom; MS, mean square.

\* Significant at the 0.1 per cent level of probability.

† Non-significant.

Table 2. ANALYSIS OF VARIANCE OF THE LATENCIES FOR SYNTOCINON, PITOCIN, PITRESSIN, LYSINE-VASOPRESSIN, ARGinine-VASOPRESSIN AND ACETYLCHOLINE

Data obtained from experiments shown in Fig. 1 over the concentration range  $4.4 \times 10^{-7}$  to  $4.4 \times 10^{-10}$  moles/l.

Source	DF	MS	F <sub>cal</sub>	F <sub>0.001</sub>
Total Samples	2,519			
Between animals	5	104,351	7.71*	4.10
Within animals	246	13,535		
Between compounds	5	90,773	8.20*	4.10
Within compounds	241	11,808		
Between concentrations	6	363,056	127.84*	3.74
Linear	1	1,996,848	703.12*	10.83
Non-linear	5	36,297	12.78*	4.10
Within concentrations	235	2,540		
Error	2,268	135		

\* Significant at the 0.1 per cent level of probability.

Under the conditions of our experiments, rat mammary tissue *in vitro* is stimulated by other neurohypophyseal hormones in addition to oxytocin and by acetylcholine, and hence is not specific for oxytocin. The discrepancy between our results and those of Rydén and Sjöholm, who found that adrenaline, noradrenaline, histamine and 5-hydroxytryptamine caused contractions of mammary gland strips at 38° C, may be explained by the fact that our experiments were carried out at 5° C. At this temperature the myoepithelial cells may be insensitive to these hormones. The *in vivo* bioassay, using the milk ejection response in lactating guinea-pigs as studied by Folley and Knaggs<sup>5</sup>, is highly specific for oxytocin; arginine or lysine vasopressin being only about one fifth as effective as oxytocin and acetylcholine several hundred times less effective. In terms of specificity therefore the *in vivo* assay seems to be the preferable method.

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## On the Site of Action of Luteinizing Hormone

THE site of action of luteinizing hormone in stimulating synthesis and secretion of ovarian steroid hormones has been the subject of some controversy. Armstrong *et al.*<sup>1,2</sup> have concluded that the primary action of this gonadotropin in rat lutein and rabbit ovarian interstitial cells is to facilitate conversion of stored cholesterol to progesterone, and suggested that increased incorporation of <sup>14</sup>C-acetate into steroids, when it occurs, is a consequence rather than a cause of this stimulation. Savard *et al.*<sup>3</sup> have proposed that LH increases steroidogenesis at least in part by a primary stimulation of the rate of *de novo* synthesis of sterols and steroids from small molecular precursors. In the experiments reported here, resolution of this controversy has been achieved for the rabbit ovarian interstitial gland by preventing cholesterol biosynthesis with *trans*-1,4-bis (2-chlorobenzyl-aminomethyl) cyclohexane dihydrochloride (AY9944), a compound which inhibits 7 dehydrocholesterol  $\Delta^7$ -reductase<sup>4</sup>.

Slices of ovarian interstitial tissue from mature New Zealand rabbits have been incubated in Krebs-Ringer bicarbonate buffer in the presence or absence of AY9944 (1 or  $2 \times 10^{-6}$  molar), and without or with luteinizing hormone (NIH-LH-B3, 1  $\mu$ g/ml.) in the incubation media. Net *in vitro* synthesis of progesterone and 20 $\alpha$ -hydroxy-pregn-4-en-3-one has been determined as previously described<sup>1</sup>, the mass being measured on the basis of the ability of these steroids to absorb ultra-violet light at 240 m $\mu$ . Estimates of relative rates of *de novo* synthesis of these steroids, as well as of cholesterol (both free and esterified), were made by measuring the carbon-14 content of these compounds after purification by thin-layer chromatography followed by enzyme reduction<sup>1</sup> and/or recrystallization of progesterone; acetylation and rechromatography of 20 $\alpha$ -hydroxy-pregn-4-en-3-one; and bromination, rechromatography, and debromination of free and ester cholesterol<sup>5</sup>.

The ability of luteinizing hormone to stimulate the *in vitro* synthesis of progesterone and 20 $\alpha$ -hydroxy-pregn-4-en-3-one equally well in the presence of AY9944 as in its absence is shown in Table 1. Specific radioactivities obtained in the same experiments (Table 2) revealed that AY9944 very markedly decreased the *de novo* synthesis of these steroids from acetate-1-<sup>14</sup>C.

The ability of the inhibitor to decrease the rate of cholesterol synthesis is illustrated in Table 3. This effect, which applies both to free and to esterified cholesterol, did not apply to lipids as a whole. Fatty acid specific activities of all ovarian lipids except sterol esters were essentially unchanged by AY9944 and luteinizing hormone.

Of considerable interest was the observation that while the incorporation of acetate carbon into the steroid hormones analysed was increased by luteinizing hormone, this incorporation frequently did not keep pace with the increased net synthesis (mass) of these steroids. Furthermore, the incorporation of acetate carbon into cholesterol, especially the esterified fraction, was decreased by luteinizing hormone. It is difficult to reconcile these results with the hypothesis of Savard *et al.*<sup>3</sup> that luteinizing hormone brings about increased steroidogenesis to a significant extent by increasing the rate of synthesis of cholesterol. The observations could be more readily interpreted as favouring the alternative proposal of Mason and Savard<sup>6</sup> that luteinizing hormone "acts primarily to stimulate the formation of steroid from precursors which are earlier (than cholesterol) in the biosynthetic scheme". By-passing cholesterol, were it not for the present finding that the inhibitor AY9944 greatly decreased the incorporation of acetate carbon into the progestins synthesized in response to luteinizing hormone without affecting the mass synthesized.

These results indicate that in the rabbit ovarian interstitial gland, luteinizing hormone stimulates steroidogenesis (progesterone and 20 $\alpha$ -hydroxy-pregn-4-en-3-one)

Table 1. EFFECT OF LUTEINIZING HORMONE AND OF AY9944 ON NET *in vitro* SYNTHESIS OF PROGESTERONE AND 20 $\alpha$ -HYDROXY-PREGN-4-EN-3-ONE BY RABBIT INTERSTITIAL TISSUE SLICES

Steroid	No. of experiments	Inhibitor	$\mu$ g Steroid synthesized/g tissue		Hormone	P-values	
			Control	Luteinizing hormone		Inhibitor	Interaction
Progesterone	4	None	23.2	137.6	<0.005	n.s.*	n.s.
		AY9944	34.3	161.6			
20 $\alpha$ -hydroxy-pregn-4-en-3-one	6	None	5.3	97.9	<0.005	n.s.	n.s.
		AY9944	11.3	86.8			

\* Not significant ( $P > 0.10$ ).Table 2. EFFECT OF LUTEINIZING HORMONE AND OF AY9944 ON SPECIFIC ACTIVITIES OF PROGESTERONE AND 20 $\alpha$ -HYDROXY-PREGN-4-EN-3-ONE SYNTHESIZED *in vitro* FROM ACETATE-1- $^{14}$ C

Steroid	No. of experiments	Inhibitor	$^{14}$ C Specific activity d.p.m. ( $\times 10^3/\mu$ mole)		Hormone	P-values	
			Control	Luteinizing hormone		Inhibitor	Interaction
Progesterone	3	None	115.7	219.0	n.s.	<0.10	n.s.
		AY9944	16.3	35.3			
20 $\alpha$ -hydroxy-pregn-4-en-3-one	5	None	279.4	111.5	<0.01	<0.005	n.s.
		AY9944	93.4	40.8			

Table 3. EFFECT OF LUTEINIZING HORMONE AND OF AY 9944 ON SPECIFIC ACTIVITY OF CHOLESTEROL SYNTHESIZED *in vitro* FROM ACETATE-1- $^{14}$ C

Sterol fraction	No. of experiments	Inhibitor	$^{14}$ C Specific activity d.p.m. ( $\times 10^3/\mu$ mole)		Hormone	P-values	
			Control	Luteinizing hormone		Inhibitor	Interaction
Free cholesterol*	3	None	25.3	9.0	<0.10	<0.05	n.s.
		AY9944	6.8	1.9			
Ester cholesterol†	4	None	7.2	0.7	<0.005	<0.005	0.025
		AY9944	1.3	0.3			

\* Specific activity determined after two-dimensional thin-layer chromatography (TLC) on silica gel, bromination, rechromatography, and debromination. (TLC systems for free sterols, before bromination—first dimension, hexane: ether: acetic acid, 90:10:1, followed by same solvents, 75:25:2; second dimension, methylene chloride: ether, 5:2. TLC system for isolation of 5,6-dibromo-cholesterol, as well as for cholesterol regenerated from debromination of 5,6-dibromocholesterol, benzene: ethyl acetate, 4:1.)

† Specific activities determined after single dimensional TLC on silica gel\*, followed by saponification and purification of the resulting cholesterol by the methods for free cholesterol.

*in vitro* equally well whether *de novo* cholesterol synthesis occurs or not.

In the presence of inhibitor the incorporation of acetate carbon into these steroids and into cholesterol is diminished: this indicates that cholesterol is an obligatory intermediate in the biosynthesis of ovarian progestins, as has been demonstrated previously for adrenal<sup>7</sup> and testicular<sup>8</sup> steroids. The evidence as a whole shows that the principal (if not the sole) action of luteinizing hormone in acutely increasing the synthesis of progestin in rabbit ovarian tissue is exerted at a point subsequent to the biosynthesis of cholesterol.

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### Glucose and RNA Synthesis in Mammalian Islets of Langerhans

It is well known that glucose stimulates the release of insulin from the mammalian pancreas. It has recently been shown, *in vitro*, that glucose and mannose, but not a non-metabolized sugar such as galactose, will also

stimulate insulin synthesis<sup>1</sup>. We have investigated the effects of glucose on the putative preceding stage, namely, the formation of new messenger RNA, using isolated islets of Langerhans from the duct-tied pancreas of the rat<sup>2,3</sup>. As the amount of tissue available for a given experiment is small (approximately 100  $\mu$ g dry weight), we have investigated the incorporation of 6- $^{14}$ C-uracil into total nucleic acids of the islets.

Islets were incubated in 0.25 ml. Krebs-Ringer bicarbonate buffer<sup>4</sup> containing varying concentrations of glucose, crystalline bovine albumin (2 mg/ml.) and 6- $^{14}$ C-uracil (15  $\mu$ Ci/ml.), with a gas phase of 95 per cent oxygen/5 per cent carbon dioxide. Incubations were carried out in a shaking water bath, at 37° C, for 4 h. At the end of the incubation the islets were transferred to homogenizing tubes, containing 1 ml. of ice-cold 15 per cent trichloroacetic acid. After homogenization, 25 mg crystalline bovine albumin and 3.0 mg of crystalline RNA were added and the whole extracted by the method of Wool and Moyer<sup>5</sup>. Preliminary experiments showed that this method gave recoveries of added RNA of 90–100 per cent. The final product, sodium ribo- and deoxyribo-nucleates, was suspended in phosphor (PPO 4 per cent, POPOP 0.01 per cent in toluene) and counted in an automatic liquid scintillation counter.

Table 1. EFFECT OF GLUCOSE CONCENTRATION ON THE INCORPORATION OF 6- $^{14}$ C-URACIL INTO TOTAL NUCLEIC ACIDS OF ISOLATED ISLETS OF LANGERHANS

Glucose concentration (mg/ml.)	Counts per 20 min minus background in total nucleic acids		
	1	2	3
3.0	2,964	3,119	1,074
1.0		2,236	925
0.15	286	1,295	391
0			540
No. of islets per incubation	25	26	21

Table 1 shows the results of three separate experiments in which incorporation of labelled uracil into nucleic acid was compared at various concentrations of glucose. Clearly, raising the glucose concentration from 0.15 mg/ml. to 1.0 or 3.0 mg/ml. greatly increases the amount of incorporation. Whether there is a real increase when the glucose concentration is raised from 1.0 to 3.0 mg/ml. is not apparent from this small number of experiments. It is, however, clear that increased incorporation is related to the concentration of glucose and not simply to its provision as an energy source. Stimulation of

nucleic acid synthesis by glucose has previously been demonstrated only in thyroid slices, *in vitro*<sup>6</sup>. In these experiments, the effect of glucose was "all or none" and not related to concentration. In our control experiments, using small pieces of the fibro-fatty tissue, which replaces the pancreatic acinar tissue in animals with ligatured pancreatic ducts, and also with rat hemidiaphragms, glucose had no effect on the incorporation of labelled orotic acid into nucleic acids.

The specificity of these findings was checked in the following experiment. Two batches of forty-five islets were incubated at a glucose concentration of 3 mg/ml., one of them in the presence of actinomycin D (5 µg/ml.). The extracted nucleates from each were divided into two aliquots and taken up in 1.0 ml. of *tris* buffer (pH 7.5). 0.1 µg of pancreatic ribonuclease (RNase) was added to one each of the paired aliquots and all four incubated in a shaking water bath at 37° C for 1 h. At the end of the incubation the nucleates were re-precipitated with ethanol and counted. The results are shown in Table 2. In the presence of actinomycin D, no incorporation of orotic acid was detected. Incubation for 1 h with RNase lowered the recovered radioactivity by more than 80 per cent. These two findings strongly support the thesis that the product being measured is, in fact, labelled RNA, as might reasonably be expected when using labelled orotic acid as a precursor.

Table 2. EFFECT OF ACTINOMYCIN D (5 µg/ML.) ON THE INCORPORATION OF 6-<sup>14</sup>C-OROTIC ACID INTO TOTAL NUCLEIC ACIDS OF ISOLATED ISLETS OF LANGERHANS AND THE EFFECT OF RNase ON THE RECOVERY OF RADIOACTIVITY FROM THE PRODUCT

Medium	Glucose 3 mg/ml. Counts per 20 min	Glucose 3 mg/ml. and actinomycin 5 µg/ml. minus background
Without RNase	1,415	0
With RNase	255	0

Whether other carbohydrates can provide the stimulus to RNA synthesis and whether the effect of glucose can be reproduced by simpler energy sources is at present under investigation.

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## RADIOBIOLOGY

### Enhancement of the Post-irradiation Protective Action of TAB by an $\alpha$ -Adrenergic Blocking Agent

GRAM-NEGATIVE endotoxins<sup>1</sup> or typhoid-paratyphoid A and B vaccine (TAB)<sup>2</sup> can have a favourable influence on the acute radiation syndrome when they are administered after total-body irradiation. By stimulating excessively the release of adrenergic neurohormones<sup>3,4</sup> or by increasing sensitivity to catecholamines<sup>7,8</sup>, however, these substances can cause reduced blood flow to the kidneys, liver and intestines<sup>5</sup> leading to ischaemia and irreversible tissue damage. Such changes may result from the vasoconstrictor action of catecholamines.

The vasoconstrictor action of the adrenergic neurohormones results from their interaction with the  $\alpha$ -receptors of the adrenergic nervous system<sup>9</sup>. Assuming that the full benefits of TAB treatment might not be obtained because of side effects, presumably related to reduced tissue perfusion, this investigation was designed to determine whether the radioprotective action of TAB could be enhanced through treatment with a pharmacological agent the principal action of which was  $\alpha$ -adrenergic blockade.

Female  $C_3H \times AKR$  mice (9–11 weeks old) were exposed to total-body doses of radiation (300 kVp X-rays; half value layer 1.7 mm copper; 19.5 amp current; 110 rads/min). The mice were distributed among three experimental groups. Group I received X-irradiation only. One hour after irradiation, Group II received an intraperitoneal injection of TAB containing  $3.3 \times 10^8$  killed organisms of *Salmonella typhi* and  $1.7 \times 10^8$  organisms each of *Salmonella paratyphi A* and *Salmonella paratyphi B*. Group III received X-irradiation, TAB and, starting 1 h after TAB, the reversible  $\alpha$ -adrenergic blocking agent phentolamine methane sulphonate (PMS), 5 mg per kg body weight, subcutaneously. PMS was given twice daily for 3.5 days; the twice daily injections were separated by about 8 h. The mice in Groups I and II received comparable volumes of pyrogen-free physiological saline.

At the end of a 30 day observation period, a chi-squared ( $\chi^2$ ) test for the difference between proportions was used to determine the significance of differences between the proportion of animals dying in the various groups. Probability values less than 0.05 were taken to represent significant differences. The  $\chi^2$  test was also used in determining the effect of PMS on the time-pattern of death of mice previously exposed to 750 rads total body irradiation and treated with TAB vaccine.

Treatment with TAB had immediate effects on the mice. For about 15–30 min after injection they showed excitation and became very aggressive towards one another. Some animals showed evidence of lacrimation. This phase of excitation and lacrimation was followed by several hours during which the mice remained almost motionless and with their eyelids closed. Some animals had episodes of defecation which almost approached diarrhoea. Within 12–24 h, the mice appeared to recover to normal behaviour. All mice, however, showed a 10–20 per cent loss of weight at 24 h after treatment with TAB. Thereafter, no other symptoms were observed that could be attributed directly to treatment with TAB.

Table 1 shows the mortality following the various treatments. At all radiation doses tested, TAB vaccine alone significantly reduced mortality below that of control mice ( $P < 0.001$ ). PMS significantly reduced mortality below that of mice treated with TAB ( $P < 0.05$  at the 700 rad level, and  $P < 0.005$  at the 750 rad level). Table 2 shows the influence of PMS on the time of death of mice exposed to 750 rad and treated with TAB. The effect of PMS in reducing mortality was most significant during the third week after irradiation. During this time, the death rate of animals treated with TAB was reduced by PMS from 67 to 32 per cent ( $P < 0.01$ ). No significant

Table 1. THIRTY-DAY MORTALITY OF  $C_3H \times AKR$  MICE

Radiation dose (rad)	Control	Treatment TAB	TAB + PMS
600	*16/40 (40)	—	—
650	38/56 (68)	1/33 (2.8)	—
700	62/64 (97)	22/40 (55)	11/40 (28)
750	67/67 (100)	50/60 (83)	34/60 (57)

\* Number dead/number treated (percentage mortality).

Table 2. EFFECT OF PMS ON TIME PATTERN OF RADIATION DEATH  
Weeks after 750 rads total-body irradiation

Treatment	First	Second	Third	Fourth
TAB	*0/60 (0)	30/60 (50)	20/30 (67)	0/10 (0)
TAB + PMS	0/60 (0)	20/60 (33)	13/40 (32)	1/27 (3.5)

\* Number dead during period/number entering period (percentage mortality).

differences in percentage mortality were observed during the first, second and fourth weeks after irradiation.

These results show that the protective action of TAB vaccine after irradiation may be enhanced by an agent the principal pharmacological action of which is  $\alpha$ -adrenergic blockade. Although it is not yet possible to exclude other pharmacological effects of PMS as being responsible for this enhancement, it is tentatively postulated that PMS exerts this action by blocking the effect on  $\alpha$ -adrenergic receptors of catecholamines released by TAB vaccine. The protective action of PMS was most apparent during the third week after irradiation, although PMS was administered for 3.5 days immediately following TAB. It appears that changes may be produced by TAB immediately after its administration which contribute to radiation mortality several weeks later, and that these changes (presumably related to tissue perfusion) are modified favourably by PMS.

These results may also suggest a possible mechanism for the radioprotective action of TAB and Gram-negative endotoxin. Shock, whether of haemorrhagic or bacteriaemic origin, is the main cause of death after exposure to potentially lethal doses of ionizing radiations. Catecholamines released in excessive quantities are thought to play a principal part in the mediation of bacteriaemic and haemorrhagic shock<sup>8,10</sup>. As catecholamines may only be slowly replaced in tissues after their release<sup>11-13</sup>, sub-lethal doses of endotoxin or TAB may deplete tissues of their catecholamines so that the quantities of free catecholamines required to produce irreversible shock may not be available during the haemorrhagic and/or bacteriaemic phases of the acute radiation syndrome. This hypothesis for the mechanism of action of TAB vaccine and Gram-negative endotoxins, including the postulated role of PMS, is illustrated in Fig. 1. As other substances are known to release catecholamines from tissues and as methods are available for the estimation of catecholamines in tissues<sup>14</sup>, the validity of this hypothesis can be tested. Should this mechanism prove to be a chief component of the action of TAB or endotoxin, then the depletion or partial reduction of catecholamines (or other potential shock substances) from tissues in the presence of  $\alpha$ -adrenergic blockade may represent an important preparation of irradiated subjects for entry into those phases of the acute radiation syndrome which lead easily to irreversible shock.

A more rapid recovery of haemopoietic tissues is often noted after total-body irradiation when this follows treatment with either TAB vaccine or Gram-negative endotoxins<sup>3,4</sup>. This effect need not be due to a direct action of these substances on organs which form blood cells. Any treatment which eventually reduces the

occurrence of either reversible or irreversible shock may make possible a more complete repair of tissues damaged by radiation.

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### Inhibition of Stromal Enzymes by X-Radiation

HIGH doses of X-radiation appear to alter the function of the erythrocyte membrane without producing much change in the internal metabolism of cells<sup>1</sup>. In order to define the changes which occur in the erythrocyte membrane more precisely, we have investigated the effects of X-rays on various enzymes in isolated stroma. Marked differences in the radiosensitivity of these enzymes were observed (Table 1).

Dose-effect curves for the most radiosensitive of these enzymes, the acid phosphatase, are shown in Fig. 1. Measurable inhibition of this enzyme was observed with radiation doses as low as 4 krad but the inhibition tended to approach a maximum value of 65 per cent as the radiation dose was increased up to 400 krad. Storage of the irradiated stroma at 4° C did not result in recovery but rather in further degeneration of the enzyme activity (Fig. 1). Qualitatively similar results were observed with nucleoside phosphorylase and acetylcholinesterase but not with the four other enzymes tested.

The inhibition of enzyme by X-irradiation has frequently been attributed to the oxidation of sulphhydryl groups. The total sulphhydryl content of the stroma, measured with dithiobis-(2-nitrobenzoic acid) in the presence of sodium dodecyl sulphate<sup>2</sup>, was found to decrease slowly with increasing doses of X-radiation (Fig. 1). This decrease in sulphhydryl, however, could not account for the differential radiosensitivity of the stromal enzymes. When stromal suspensions were pre-incubated for 1 h at 37° C with 0.03-3 mmolar *p*-chloromercuribenzoate or *N*-ethylmaleimide to block the sulphhydryl groups, the activity of radio-resistant adenosine-tri-phosphatases (ATPases) was more strongly inhibited than that of a radiosensitive enzyme such as acid phosphatase. Oxidative reagents, that is, iodine and hydrogen peroxide, had a similar effect. It seems unlikely, therefore, that the effect of X-radiation on stromal enzymes (Table 1) could be attributed primarily to oxidative reactions.

Enzyme inhibition by reduction of -S-S bridges during irradiation has also been

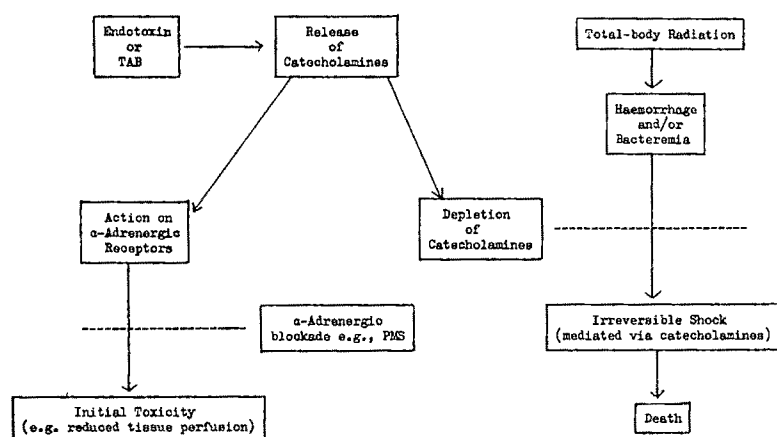


Fig. 1. Hypothesis for the radioprotective effect of TAB vaccine and Gram-negative endotoxins, and the postulated mechanism whereby PMS enhances their radioprotective action; explanation in text.

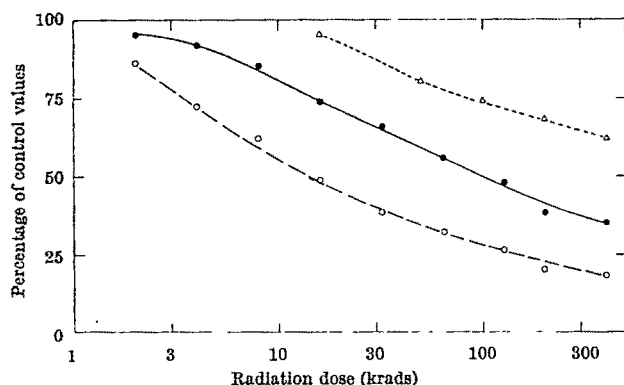


Fig. 1. Inhibition of the acid phosphatase activity of stromal suspensions following X-irradiation. Enzyme activities were measured immediately after X-irradiation (●) and after storing the same suspensions for 2 days at 4° C (○). The total sulphydryl content of the stromal protein (Δ) was also measured immediately after irradiation.

Table 1. EFFECT OF X-RADIATION ON STROMAL ENZYMES

	Enzyme activity (per cent control values)	
	64 krad	200 krad
Ouabain-sensitive ATPase <sup>a</sup>	109	102
Ouabain-resistant ATPase <sup>a</sup>	104	98
Diphosphoglycerate phosphatase <sup>b</sup>	120	—
Aldolase <sup>c</sup>	82	68
Nucleoside phosphorylase <sup>d</sup>	63	28
Acetylcholinesterase <sup>e</sup>	62	35
Acid phosphatase <sup>f</sup>	56	37

Erythrocyte stroma were recovered from a haemolysate of washed rat erythrocytes by centrifugation for 30 min at 3,000g and 4° C. The stroma were washed three times and finally suspended in 4 volumes of 1 mmolar ethylenediaminetetraacetate at pH 7.4. Samples of this suspension were irradiated (16 krad/min)<sup>g</sup> at 0° C in the presence of air and assayed for enzyme activity. The methods of assay are indicated by the references listed above.

reported<sup>9</sup>. In our experiments, the effects of X-rays on stromal enzymes could be partly reproduced with reducing agents, that is, sodium dithionite, mercaptoethylamine, and dithiothreitol. After incubation of stroma with 1 mmolar dithiothreitol<sup>10</sup> for 1 h at 37° C and pH 7.4, for example, the ouabain-sensitive ATPase, the ouabain-resistant ATPase and the acid phosphatase activities were 138, 103 and 54 per cent of the control values. The data thus suggest that part of the radiation effect on stromal enzymes may be caused by reduction of —S—S bridges and subsequent disruption of protein structure.

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with a deposit of manganese dioxide. Manganese-54 has been present in rain water in significant concentrations since the Soviet nuclear test series of 1961 (ref. 1) and samples of used filter bed material have been examined for their radiomanganese content. This particular material resulted from the filtration of a colourless moorland water at high pH and the process effectively removes the 0.25 p.p.m. of manganese present.

A preliminary examination of the material of the filter bed showed that the sand particles had been coated with manganese dioxide containing smaller amounts of aluminium, calcium, iron and magnesium. The coated grains were almost spherical and of uniform size. The manganese-54 content was 458 pc./g corrected for radioactive decay to the time of sampling (17.9.65). It was found possible to remove successive layers from the grains by abrasion. The manganese content of each layer was sensibly constant (60 per cent as manganese dioxide), but the specific activity of the manganese-54 decreased towards the centre of the coated grains. Twenty-two layers representing about 40 per cent of the coating were removed. Fig. 1 shows the reduction of the specific activity of the manganese in the successive layers.

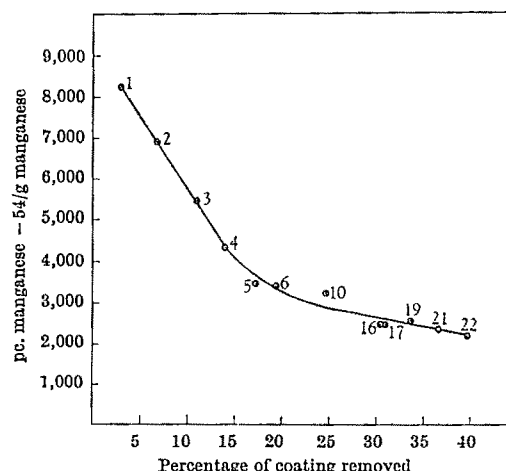


Fig. 1. Distribution of manganese-54 within the coated filter bed material. (The numbers on the graph relate to the layer removed.)

The initial part of the curve is straight. The subsequent curvature could be accounted for by a period of enhanced deposition of manganese-54 in fall-out such as occurred after the Soviet tests of 1961. Although it was known that the sand filter had been in use for about 10 years it was not found possible to relate a specific layer to a specific date.

Because manganese dioxide is known to absorb radionuclides derived from fall-out from water<sup>2</sup>, the filter bed material was examined for such radionuclides. The results are shown in Table 1. Because no atmospheric tests had taken place for 20 months before sampling, no short-lived radionuclides were expected or found. The radionuclides of highest activity were those of cerium, promethium and ruthenium, which would be expected to be coprecipitated with manganese. The concentration of caesium-137 was low and that of strontium-90 intermediate. Neither of the

## APPLIED SCIENCE

### Accumulation of Radionuclides in Water Works Filter Bed Material

THE presence of manganese in certain water supplies is an inconvenience which can lead to the formation of deposits and, in extreme cases, to blockage of the mains. Several water undertakings introduce a special precipitation and sand filtration process to remove it. The sand of the filter beds at such undertakings becomes coated

Table 1. FALL-OUT RADIONUCLIDES IN COATED FILTER BED SAND

Radionuclide	Activity* pc./g of coated sand
Strontium-90	13.8
Yttrium-90	13.8
Yttrium-91	N.S. < 0.1
Zirconium-95	N.S. < 0.1
Ruthenium-106/rhodium-106	27.4
Tellurium (total)	0.2
Antimony-125	< 0.4
Caesium-137	2.8
Cerium-144	17.0
Praseodymium-144	17.0
Promethium-147	29.1

N.S., Not significant.

\* Activities corrected for decay to 17.9.65.



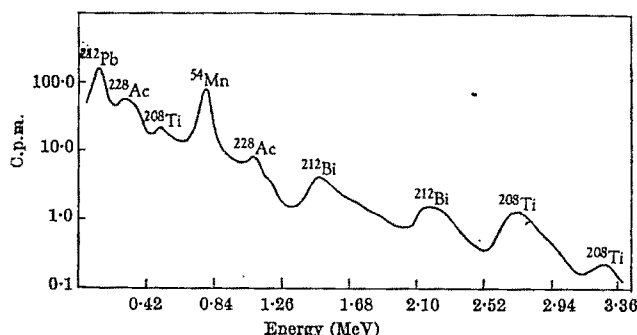


Fig. 2. Gamma-spectrum of coated filter bed material.

two latter radionuclides was likely to have been co-precipitated as hydroxides with the manganese and their presence in the material was probably due to their sorption from the water.

At this stage, total  $\alpha$ - and  $\beta$ -determinations on samples of the coated sand revealed that a major part of the radioactivity was still unaccounted for—in fact, three-quarters of the total  $\beta$ -activity of 490 pc./g. In view of this, and of the considerable total  $\alpha$ -activity present (840 pc./g) we considered the probable presence of naturally occurring radionuclides. A qualitative  $\gamma$ -spectrometric analysis (Fig. 2) confirmed this.

By estimating certain radionuclides it was found possible to build up a fairly complete picture of the concentrations of each of the natural radioactivities in the coated sand. It was assumed that because the time elapsed between the collection of the sample and its examination for natural radioactivity was more than 1 year, any radioactive daughters in either the thorium or uranium series of half-life 50 days or less would be in radiochemical equilibrium with their parents. Thus, protoactinium-234 and thorium-234 would have been in radiochemical equilibrium with uranium-238, radium-224 with thorium-228 and radium-224 and -226 likewise with their respective daughter products if the assumption can be made that the gaseous thoron (radon-220) and radon (radon-222), respectively, were held by the coating. This was shown to be substantially the case by an experiment in which air was passed continuously through some coated sand for 16 h and a reduction of only 20 per cent in the concentration of radium daughters took place. Considerably less thoron and radon would be expected to be released under normal conditions of storage. Table 2 lists the concentrations of those naturally occurring radionuclides in the coating determined experimentally.

A qualitative  $\alpha$ -spectrometric analysis, using a solid state silicon detector and a multichannel analyser, on a chemically separated thorium fraction shows that thorium-232 and -230 were absent. Fig. 3 shows the  $\alpha$ -spectrum of this thorium fraction approximately 2 days after separation from a sample of the coating. The daughters of thorium-228 were beginning to build up. Thorium-232 and -230 if present would have occurred in the neighbourhood of channels 14 and 22, respectively. Because thorium-232 was virtually absent, the thorium-228 concentration, calculated from that of its radium-224 daughter, must have arisen from its parent radium-228. Radiochemical equilibrium between radium-228 and thorium-228 would

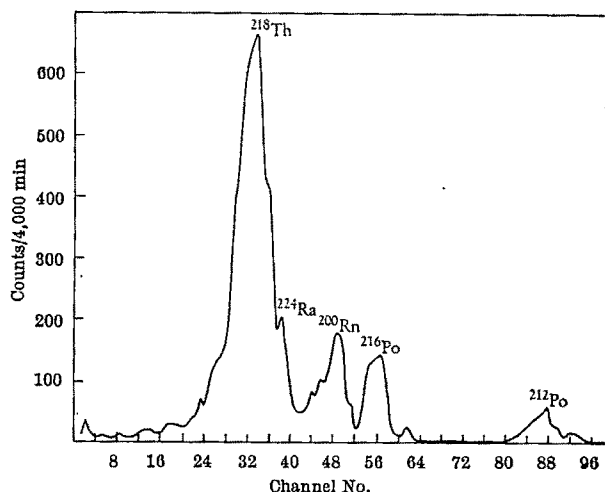


Fig. 3. Alpha-spectrum of thorium fraction separated from coated filter bed material.

take some years to become established, and as the figures in Table 2 show, this had only proceeded half way. Apart from the naturally occurring radionuclides, considerable radioactivity due to plutonium (-239 and -240), previously reported in fall-out<sup>3</sup>, was detected. The plutonium was presumably unfissioned material originating from nuclear devices exploded in the atmosphere. The presence of plutonium in the coating suggested that uranium-235, the other common fissionable material, might also be present in the coating. A radioassay on the chemically separated uranium, however, showed an equivalence with the colorimetric determination of the element which confirmed the presence of natural uranium with no additional content of uranium-235.

It can be inferred from the concentrations of the natural activities present in the coating that the main radionuclides present in the raw water were those of uranium, radium and plutonium, thorium being absent.

The activities of the various  $\alpha$ - and  $\beta$ -emitting daughter products not experimentally determined were deduced from the concentrations of the parent radionuclides in Table 2. From all these results, together with those from Table 1, separate inventories of the  $\alpha$ - and  $\beta$ -emitting radionuclides present in the coating were made. The inventory of all the  $\beta$ -emitters, both fall-out and naturally occurring, present in the coating gave a summed activity of 493 pc./g. This compared favourably with the measured total  $\beta$ -activity of 490 pc./g, both summed and total activities being calculated with respect to a strontium-90 standard at 17.9.65. This close agreement was perhaps fortuitous, but did serve to show that no principal  $\beta$ -emitters had been omitted from the inventory. The agreement between the total  $\alpha$ -activity found, 840 pc./g, and the sum of the individual  $\alpha$ -emitting radionuclides found, 775 pc./g, all activities being calculated with respect to a natural uranium standard at 17.9.65, was also considered satisfactory.

We thank Mr. A. B. Baldwin, general manager and engineer, Sheffield Corporation Waterworks, for providing the samples for this investigation and for suggesting that we study the deposition of manganese-54 within the filter bed sand coating. We also thank Dr. D. I. Coomber for his helpful advice.

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Table 2. EXPERIMENTALLY DETERMINED NATURALLY OCCURRING RADIONUCLIDES IN COATED FILTER BED SAND

Radionuclide	Activity* pc./g of coated sand
Potassium-40	0.14
Thorium-232	< 3.3
Radium-228 (actinium-228)	75.6
Radium-224 + 3 $\alpha$ -emitting daughters	149.6
Thorium-228†	37.4
Uranium	125.7
Radium-226 + 3 $\alpha$ -emitting daughters	190.4
Lead-210 (bismuth-210)	4.8
Polonium-210	2.1
Plutonium-239 + plutonium-240	123.0

\* At 17.9.65.

† Deduced from Ra-224 activity.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 13

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "The Impact of Integrated Circuits on the Role of the Circuit Designer", opened by Mr. A. G. I. Cressell and Mr. R. Towell.

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (Joint meeting with the Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Prof. A. E. Alexander: "The Role of the Surfactant in Heterogeneous Polymerization".

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 6 p.m.—Prof. A. Williams: "British Ordovician Shelly Faunas. III, Implications of Faunal Distributions".\*

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 6.15 p.m.—Dr. M. K. Towers: "Cardioversion and Control of Arrhythmias".\*

## Tuesday, February 14

INSTITUTE OF CHEMICAL ENGINEERS, SOUTH EASTERN BRANCH (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 11 a.m.—Symposium on "Chlorine in Industry".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTE OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Dr. B. J. Mason: "Recent Developments in Weather Forecasting and their Application to Industry" (James Forrest Lecture).

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. J. C. West, Dr. B. V. Jayawant and Mr. D. P. Rea: "Transition Characteristics of the Jump Phenomenon in Nonlinear Resonant Circuits".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. L. F. Boyd: "The Scientific Role of Man in Space" (Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on February 15, 21 and 22.)

UNIVERSITY OF ASTON IN BIRMINGHAM (in the Great Hall of the University, Gosta Green, Birmingham 4), at 5.30 p.m.—Prof. Gibson: "Universities and the Managerial Revolution" (Inaugural Lecture).\*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. B. R. Coles: "Solid State Physics—In Particular Metals" (Inaugural Lecture).\*

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. A. C. Allison: "Lysosomes". (Tenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (in the Anatomy Theatre, King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. H. Kellenberger (Geneva): "Studies on the Morphogenesis of the Head of Bacteriophage T4".\*

INSTITUTE OF THE RUBBER INDUSTRY, LONDON SECTION (at the Eccleston Hotel, Victoria, London, S.W.1), at 5.30 p.m.—"Technology and Uses of the Newer Types of Carbon Black" by a speaker from Cabot Carbon, Ltd. 7 p.m.—Mr. F. W. Gage: "Rubber to Metal Bonding".

## Wednesday, February 15

CHEMICAL SOCIETY (in the Chemistry Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 2.30 p.m.—Organic Chemistry Symposium. Prof. B. G. L. Weedon: "Recent Advances in the Carotenoid Field"; Prof. A. W. Johnson: "Macrocyclic Tetrapyrrole Compounds"; Prof. D. H. R. Barton: "A Region of Biosynthesis".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. F. H. Bushby and Mr. M. S. Timpon: "A Ten-Level Atmospheric Model and Frontal Rain"; Mr. R. F. Zobel: "Temperature and Humidity Changes in the Lowest Few Thousand Feet of Atmosphere".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Dr. V. D. Barnett: "Assessment of Low Temperature Probabilities throughout Great Britain".

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "Possible Alternatives to Copper" opened by Mr. E. H. Reynolds.

UNIVERSITY OF LONDON (at Senate House, London, W.C.1), at 5.30 p.m.—Prof. Suzanne Mansion: "Plato's Conception of the Intelligible. II. Mathematics and Dialectics".\*

INSTITUTE OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at 8-9 Bedford Square, London, W.C.1), at 6 p.m.—Mr. A. C. McKellar and Mr. M. J. Dilworth: "The Remote Control of Lighthouses and Beacons".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Arthur Southway: "British Printing Machinery".

SOCIETY FOR ANALYTICAL CHEMISTRY, RADIOCHEMICAL METHODS GROUP (at the Borough Polytechnic, Borough Road, London, S.E.1), at 6.30 p.m.—Dr. E. S. Williams: "The Basic Principles of Dilution Analysis, Activation Analysis, Derivative Analysis, Saturation Analysis and Immuno-assay in a Medical Context"; Dr. G. E. Harrison: "The Uses of Radioactive Markers in the Study of Mineral Metabolism with special reference to the Alkaline Earths".

INSTITUTE OF FUEL (at Croydon Technical College, Fairfield, Croydon, Surrey), at 7 p.m.—Mr. P. F. Corbett: "Natural Gas in Europe".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN AND THE BOTANICAL SOCIETY OF THE BRITISH ISLES (at 17 Bloomsbury Square, London, W.C.1), at 7 p.m.—Dr. T. D. Whitte: "Some Early Apothecary Botanists"; Mr. D. E. Allen: "The Origins of British Field Botany".

## Wednesday, February 15—Thursday, February 16

INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY, ELECTRON MICROSCOPY AND ANALYSIS GROUP, in association with the INSTITUTE OF METALS and the IRON AND STEEL INSTITUTE (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2)—Conference on "Recent Developments in the Theory, Technique and Application of Electron Probe Microanalysis".

## Thursday, February 16

SOCIETY OF INSTRUMENT TECHNOLOGY (at the Town Hall, Middlesbrough), at 9.30 a.m.—Symposium on "The Presentation of Data".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the Pharmaceutical Society, 17 Bloomsbury Square, London, W.C.1), at 2.30 p.m.—Meeting on "The Microbiological Determination of the Nutritive Value of Proteins". Speakers will include: Mr. J. E. Ford, Mr. W. S. Miller and Mr. J. A. Stott.

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 2.30 p.m.—Meeting on "Adhesives in Building". Mr. W. C. Wake: "The Types and Uses of Adhesives in Building"; Mr. R. T. Kelly and Mr. A. M. Berman: "Adhesives in Building".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. O. Hayward, Mr. D. A. King and Prof. F. C. Tompkins F.R.S.: "Sticking Probabilities, Redistribution Processes and Desorption Spectra of Nitrogen on Tungsten Films"; Mr. M. A. A. Clyne, Mr. D. B. Hartley and Mr. B. A. Thrush: "Direct Determination of the Rates of Elementary Processes in the Hydrogen-Oxygen Reaction".

UNIVERSITY OF LONDON (at King's College Hospital Medical School, Denmark Hill, London, S.E.5), at 4.45 p.m.—Prof. H. C. Killey: "Fractures of the Middle Third of the Facial Skeleton" (19th Thomas Percy Legg Memorial Lecture).\*

INSTITUTE OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. C. C. Dell and Dr. J. Sinha: "Experimental Study of the Behaviour of Flocculated Clays in Thickening"; Mr. K. J. Scott and Mr. J. L. Alderton: "Maximum Solids Handling Capacity of Continuous Thickeners"; Mr. E. Douglas and Mr. T. Walsh: "New Type of Dry, Heavy-Medium, Gravity Separator".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. G. S. Carter: "Polyphyletism and Vertebrate Evolution"; Dr. C. A. Wright: "The Molluscan Genus *Bulinus*; a Study in the Evolution of Taxonomic Methods".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. I. G. Macdonald: "Spherical Functions".

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, N.W.1), at 5 p.m.—Dr. J. M. Barnes: "Fungal Toxins with special reference to Aflatoxin".\*

UNIVERSITY OF LONDON (in the Eugenic Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. Charles Rousseau (Paris): "Scientific Progress and International Law".\*

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. J. van Dam: "Gas Production from the Producers' Viewpoint".

INSTITUTE OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal discussion on "Build Landour Systems and their Effect on Airport Design" introduced by Mr. O. B. St. John.

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Prof. George Dick: "Immunization Against some Virus Diseases". (Eleventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

INSTITUTE OF ELECTRICAL ENGINEERS (at the Central Hall, Westminster, London, S.W.1), at 6 p.m.—Mr. R. V. Moore: "Nuclear Power Today and Tomorrow" (Faraday Lecture).

OIL AND COLOUR CHEMISTS ASSOCIATION, LONDON SECTION (in the Physics Department, Imperial College, London, S.W.7), at 6.30 p.m.—Dr. A. C. Healey: "The Printing of Bank of England Notes".

UNIVERSITY OF LONDON (at Wye College, near Ashford, Kent), at 8.15 p.m.—Prof. H. Bondi, F.R.S.: "Science and Education".

## Friday, February 17

BIOCHEMICAL SOCIETY (at St. Mary's Hospital Medical School, Paddington, London, W.2)—468th Meeting. Programme will include a Colloquium on "Mechanisms of Biological Hydroxylation".

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Dr. A. Knowles: "Photochemistry of Flavins".

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "High-Pressure Gas Breakdown" opened by Dr. A. H. Cookson and Mr. G. F. Goldspink.

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Prof. Jean Frezal (Paris): "The Implication of Physiological Genetics in the Study of Inborn Errors in Man".\*

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN and the BRITISH ACADEMY OF FORENSIC SCIENCES (at the School of Pharmacy, University of London, Brunswick Square, London, W.C.1), at 6 p.m.—Mr. T. E. A. Carr, Mr. A. D. Oxford, Mr. J. Lynch and Mr. R. M. Mitchell: "Forged Prescriptions".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Lord Rothschild, G.M., F.R.S.: "Research in a Very Big Industry".

## Saturday, February 18

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. John F. Potter: "Fossils—Their Preservation and Occurrence".\*

## Monday, February 20

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 9.30 a.m.—Colloquium on "Message Switching".

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 2 p.m.—Colloquium on "Precision Resistance Standards".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Dr. K. R. Sealey: "The Siting and Development of British Airports".

UNIVERSITY OF LONDON (in the Engineering Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. G. Kendall, F.R.S.: "Delphic Semi-Groups".

Monday, February 20—Tuesday, February 21

INSTITUTION OF ELECTRICAL ENGINEERS (joint symposium with the I.C.E. and the I.Mech.E., at the Institution of Civil Engineers, Great George Street, London, S.W.1)—Symposium on "Area Control of Road Traffic".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER and an ASSISTANT LECTURER in PURE OR APPLIED MATHEMATICS (including numerical analysis and statistics)—The Registrar, The University, Manchester, 13, quoting Ref. 13/67 (February 18).

RESEARCH FELLOW (postdoctoral or equivalent) in the DEPARTMENT OF NATURAL HISTORY to join a team of ecologists studying rook populations—The Secretary, The University, Aberdeen (February 18).

LECTURER or ASSISTANT LECTURER (medically qualified or non-medically qualified) in the DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Leeds, 2 (February 20).

LECTURER or ASSISTANT LECTURER (preferably with teaching and research interests in genetics, especially the genetics of micro-organisms) in BIOLOGY—The Secretary, University of Stirling, Stirling, Scotland (February 20).

LECTURER or ASSISTANT LECTURER (with research interests in any branch of applied mathematics or mathematical physics) in APPLIED MATHEMATICS—The Registrar, University of York, Heslington, York (February 20).

LECTURER (preferably with an interest in statistics and operational research) in ENGINEERING MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (February 20).

LECTURER or ASSISTANT LECTURER (preferably with qualifications in algebra, topology or related subjects) in PURE MATHEMATICS—Head of the Mathematics Department, Imperial College of Science and Technology, London, S.W.7 (February 21).

SENIOR LECTURER (preferably with an interest in the industrial aspects of microbiology) in MICROBIOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (February 21).

LECTURER or ASSISTANT LECTURER in EXPERIMENTAL PHYSICS—The Registrar (Room 39, O.R.B.), The University, Reading (February 24).

UNIVERSITY TUTORS in PHYSIOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (February 24).

LECTURER or ASSISTANT LECTURER in APPLIED MATHEMATICS—The Registrar, The University, Hull (February 25).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Hull (February 25).

LECTURER or ASSISTANT LECTURER (with an interest in any field other than hard rock geology) in GEOLOGY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (February 27).

ASSISTANT LECTURER in ORGANIC CHEMISTRY—The Secretary, The Queen's University, Belfast, Northern Ireland (February 28).

ASSISTANT LECTURER in the DEPARTMENT OF ZOOLOGY—The Secretary, University College London, Gower Street, London, W.C.1 (February 28).

CHAIR OF SOCIOLOGY at the University of Ghana—The Assistant Registrar, Universities of Ghana Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (February 28).

LECTURER in PSYCHOLOGY in the DEPARTMENT OF PHILOSOPHY—The Registrar, University College of Wales, Aberystwyth (February 28).

LECTURER or ASSISTANT LECTURER in MATHEMATICAL PHYSICS—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (February 28).

ASSISTANT LECTURER or LECTURER (preferably with interests in locational studies) in the DEPARTMENT OF GEOGRAPHY in the field of economic geography—The Secretary, Faculty of Geography and Geology, Sedgwick Museum, The University, Cambridge (March 1).

LECTURER or ASSISTANT LECTURER (with a degree in physics, chemistry, metallurgy or engineering and research experience in one or more branches of materials science) in ENGINEERING MATERIALS—The Deputy Secretary, The University, Southampton (March 1).

SENIOR DEMONSTRATOR in CHEMISTRY—The Registrar, University of Durham, Old Shire Hall, Durham (March 1).

RESEARCH ASSISTANT (with a degree in botany or a related subject or substantial palynological experience) in PALYNOLOGY in the DEPARTMENT OF GEOGRAPHY in the Research School of Pacific Studies, Australian National University, to carry out the pollen analysis of Cenozoic (mainly Quaternary) sediments and similar work in collaboration with Dr. D. Walker—The Registrar, Institute of Advanced Studies, Australian National University, Box 4, P.O., Canberra, A.C.T., Australia, quoting Ref. No. 67007 (March 3).

SENIOR LECTURER in APPLIED MATHEMATICS in the DEPARTMENT OF APPLIED MATHEMATICS AND COMPUTER SCIENCE—The Registrar, The University, Sheffield (March 3).

LECTURER or ASSISTANT LECTURER in HISTOLOGY and/or NEUROANATOMY in the DEPARTMENT OF ANATOMY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (March 4).

CHAIR OF ARCHAEOLOGY—The Registrar, The University, Leicester (March 7).

PROFESSOR OF EDUCATION—The Registrar, University of Bradford, Bradford 7 (March 10).

READER in PSYCHOLOGY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 11).

PROFESSOR and HEAD of the DEPARTMENT OF GEOGRAPHY, Makerere University College, University of East Africa—The Inter-University Council, 83 Bedford Place, London, W.C.1 (March 13).

LECTURER (with special qualifications in animal housing and in one or more of the following fields: environmental control systems, environmental physiology and animal behaviour) in the DEPARTMENT OF AGRICULTURE—The Registrar (Room 39, O.R.B.), The University, Reading (March 15).

LECTURER/SENIOR LECTURER (qualified in any branch of psychology) in PSYCHOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 20).

CHAIR OF PURE MATHEMATICS—The Registrar, The University of Birmingham, P.O. Box 363, Birmingham 15 (May 31).

RESEARCH DEMONSTRATORS (2) (with a good degree in botany or horticulture) in the DEPARTMENT OF HORTICULTURE to assist with practical classes and carry out research in the physiology of horticultural plants or in plant-insect relations—Prof. O. V. S. Heath, F.R.S., University of Reading, Horticultural Research Laboratories, Shinfield Grange, Reading, Berkshire.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Royal Observatory Bulletins. No. 121: Photoelectric Magnitudes and Colours of Southern Stars, II. By A. W. J. Cousins, R. Lake and R. H. Stoy. Pp. E1-E56. 9s. net. No. 122: Fabry Photometry of Bright Southern Stars. By A. W. J. Cousins. Pp. E57-E124. 9s. net. (London: H.M. Stationery Office, 1966.) [1811]

Scientific and Industrial Measurement and Control: STRA's Contribution to British Industry. Pp. 20. (Chislehurst, Kent: The British Scientific Instrument Research Association, 1966.) [2111]

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1116, Vol. 261 (17 November, 1966): The Heats of Combustion of Graphite, Diamond and Some Non-Graphitic Carbons. By P. Hawtin, J. B. Lewis, N. Moul and R. H. Phillips. Pp. 67-95. 11s.; \$1.65. No. 1117, Vol. 261 (17 November, 1966): Cubic Equations or Additive Type. By Prof. H. Davenport, F.R.S., and D. J. Lewis. Pp. 97-136. 15s.; \$2.25. (London: The Royal Society, 1966.) [2111]

University of Oxford. Report of the Committee on University Libraries. Pp. 210. (Supplement No. 1 to the *University Gazette*, Vol. 97, November 1966.) (Oxford: The University, 1966.) 15s. [2211]

### Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1185-C: The Uraniferous Zirconium Deposits of the Pocos de Caldas Plateau, Brazil. By Gene E. Tolbert. Pp. iv + 28 + plates 1-3. \$0.70. Bulletin 1280-F: Mineral Resources of the Sycamore Canyon Primitive Area, Arizona. By Lyman C. Huff, Elmer Santos and R. G. Raabe. Pp. v + 19 + plate 1. \$0.50. Geophysical Abstracts, No. 237, October 1966. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel, and others. Pp. ii + 899-1002. \$0.35. Abstracts of North American Geology, September 1966. Pp. i + 917-1030. \$0.45. Professional Paper 514: Measurement of Hydraulic Diffusivity of Wedge-shaped Aquifers Drained by Streams. By R. W. Stallman and I. S. Papadopoulos. Pp. iii + 60 + plates 1-120. \$4.50. (Washington, D.C.: Government Printing Office, 1966.) [2111]

Rubber Research Institute of Malaya. Annual Report 1965. Pp. 118. (Kuala Lumpur: Rubber Research Institute of Malaya, 1966.) \$3. [2111]

Transactions of the American Philosophical Society, New Series, Vol. 56, Part 5: Mechanical Universe—The Astrarium of Giovanni de' Dondi. By Silvio A. Bedini and Francis R. Maddison. Pp. 69. (Philadelphia: The American Philosophical Society, 1966.) \$2.50. [2111]

Kungl. Svenska Vetenskapsakademien Handlingar. Fjärde Serien. Band 10, Nr. 3: *Protitanochthys* and some other Coccosteomorph Arthrodires from the Devonian of North America. By Roger S. Miles. Pp. 49 + 8 plates. 19.50 Kr. Band 10, Nr. 4: Attonde Jämförelsen Mellan Svenska Riksprototyperna för Metern och Kilogrammet och mynt- och Justeringsverkets Huvuddikare. Av Karl F. Laurell, L. E. Grönkvist och R. Luuk. Pp. 16. 3 Kr. (Stockholm: Almqvist and Wiksell, 1966.) [2111]

Energy R & D and National Progress: Findings and Conclusions. (An Interdepartmental Study.) Pp. vi + 18. (Washington, D.C.: Government Printing Office, 1966.) \$0.15. [2311]

Office of the Registrar General India, Ministry of Home Affairs. Census of India 1961. Monograph No. 1: Scientific and Technical Personnel. By K. Ray. Pp. ix + 88. (Delhi: Manager of Publications, 1966.) Rs. 1.50; 3s. 6d.; \$0.54. [2311]

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 144: Groundwater Resources of the Coastal Lowland and Adjacent Islands, Nanos Bay to Campbell River, East Coast, Vancouver Island. By B. C. Halstead and A. Treichel. Pp. 42. \$2. Paper 66-2: Report of Activities, November 1965 to April 1966. Edited by R. G. Blackadar. Pp. vi + 73. \$1. Paper 66-4: Abstracts of Publications in Scientific Journals by Officers of the Geological Survey of Canada, 1965. Compiled by S. E. Jenness. Pp. 56. \$0.50. Paper 66-32: Mont Laurier and Kempt Lake Map-Areas, Quebec. By H. R. Wynne-Edwards, A. F. Gregory, P. W. Hay, C. A. Giovannella and E. W. Reinhardt. (A preliminary report on the Grenville Project.) Pp. v + 82. \$1. Paper 66-46: Geochemical Prospecting Research in 1966, Cobalt Area, Ontario. By R. W. Boyle. Pp. 16. \$0.50. (Ottawa: Queen's Printer, 1966.) [2411]

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## NUCLEAR WEAPONS NO MORE ?

THE nuclear powers appear to be cheerfully and even unreasonably hopeful of an international agreement to prevent the spread of nuclear weapons. Mr. Kosygin, in London last week, referred on several occasions to the need for an international treaty, and the joint statement put out after his meeting with Mr. Harold Wilson, the British Prime Minister, expressed the hope that a non-proliferation treaty would "be concluded with the least possible delay". Meanwhile, it is clear that the United States and the Soviet Union have agreed on some kind of a draft for an international treaty, and the two countries have been discussing the draft with their military partners. The United States delegate to the North Atlantic Treaty Organization circulated the draft to the other members of the council on February 1, but it remains to be seen whether even this restricted group of nations will be able to agree within itself. Certainly the Federal German Republic has been sufficiently alarmed by some of the implications of the proposed treaty to send Mr. Willy Brandt, the Foreign Minister, to Washington for discussions. And as yet France has not said where she stands. Yet the nuclear powers apparently expect that it will be possible to make progress with the agreement at the next series of meetings of the Disarmament Committee of the United Nations, which meets at Geneva on February 21. Are they being realistic ?

First of all, it is welcome that the nuclear powers should now consider a further measure of arms control, backed up by international agreement, to be urgently desirable. That in itself is a step forward. It is also sensible that they should have singled out the potential proliferation of nuclear powers as an immediate problem, for so it is. Familiarity with nuclear technology is now so widely spread throughout the world that it is steadily becoming easier for nations to think of making weapons for themselves. At the same time, of course, there are natural restraints which limit the pace at which this process happens (see *Nature*, **210**, 1193; 1966). Although nuclear reactors are widely spread, plans for turning fissile material to military ends are not nearly as common. At least a part of the explanation is that the existing nuclear powers have consistently sought, usually with success, to see that technical assistance with nuclear energy does not help friends and allies to become nuclear powers on their own. The principal nuclear powers are now seeking to make explicit this previously tacit agreement. They have no doubt been moved to do so by the recognition that the collective influence of the United States and the Soviet Union, though still powerful, is no longer decisive.

What are the chances of success ? There are signs that the treaty to be put forward at Geneva will be a

simple document, binding nuclear powers not to help in proliferation and non-nuclear powers not to become recipients of military assistance with nuclear weapons, and not to manufacture them on their own account. But this is only a beginning. Steps will have to be taken to ensure that legitimate trade in nuclear materials is not turned to military ends, which implies inspection—at least for the non-nuclear powers. It is also likely that several nations will be unwilling to sign an international agreement until their own security can be assured, possibly by international guarantee; the difficulty here is that every nation at Geneva will have a different need.

But even if problems like these were entirely to be solved, it is unthinkable that the non-nuclear powers will willingly accept a situation in which the existing nuclear powers will be entitled to enjoy in perpetuity an instrument of military policy they deny to others. In other words, it will be good luck bordering on the miraculous if the nuclear powers are able to collect signatures at Geneva without making substantial concessions about their own freedom to accumulate nuclear explosives. Recent preoccupations about defensive missiles will not help but hinder.

There remains the problem of how it will be possible to safeguard the legitimate technological ambitions of industrialized societies within the framework of a non-proliferation treaty. Several sources in the Federal German Republic have been quick to point out, in the past few weeks, that signing the draft treaty would imply that German industry would be denied an opportunity to exploit the most advanced techniques in nuclear energy. Although these arguments have often been over-coloured by political considerations, they deserve to be considered seriously—especially by those who would like to see a treaty of some sort signed. There is a real conflict to be ironed out. Difficulties could, for example, arise from an attempt by a non-nuclear power to manufacture enriched uranium as a fuel for a fast reactor, or from an attempt to separate plutonium from spent uranium for the same purpose. The question is whether the building of a separation plant for fissile material is to be prohibited by the non-proliferation treaty, or whether the restrictions apply only to the production of that plant. And what will the treaty say about the natural uranium which is in any case the starting point ? The first point of view will expose the present nuclear powers to the charge that they are interfering unwarrantably in the affairs of others. The alternative, unfortunately, implies such detailed inspection of fissile material, not facilities, that this too may seem like unwarrantable intrusion. But a treaty on non-proliferation would be well worth having, which implies that the nuclear powers should not lose patience if they have to spend a year or more

at Geneva on this round of talks. They should also not be afraid of accepting some of the restraints they would impose on others.

## HOME FOR ARCHITECTS

THE Architectural Association in London is a most distinguished institution, with a long history of daring and innovation in the training of architects. It is also remarkable among British academic institutions in that it provides full-time courses for four hundred students or so without being a part of a university. To be sure, its qualifications count as if they were academic degrees, so that successful students are able to mix on equal terms with those from other architectural schools. (Indeed, given the great reputation of the association, they are sometimes thought to have an advantage.) But if academic standards are not a problem, survival is. In recent years the Architectural Association has found it increasingly difficult to keep going when its income is substantially no greater than the fees its students pay. Even though it has been possible to extract from them tuition fees of some hundreds of pounds—several times what students pay for tuition at British universities—the income from each student has been much less than the average cost per head of students at ordinary universities—something like £750 per head per year. No wonder, then, that the association must rub along with inadequate premises, and by paying its teachers less than the going rate so that it lies halfway between an academic institution and a kind of charity. No wonder, too, that the association has been looking for some place in the university system where its identity could be preserved but its exposure to the harsh winds of economic reality cushioned by the University Grants Committee.

Considerations like these have led the association to think of a marriage with the Imperial College of Science and Technology, and this by all accounts would be welcomed by the larger partner. The University Grants Committee is also, apparently, well disposed towards the match but not yet in a position to put its hand in its pocket and agree to add the Architectural Association to the payroll of the college. This is why a group of distinguished architects has now complained in public that the University Grants Committee must do something quickly, or the association will collapse before the marriage can be arranged. If this helps the University of London, Imperial College and the University Grants Committee to resolve their difficulties, it will be a useful step. But there are other problems to be tackled. If the Architectural Association is to be as valuable within Imperial College as it has been outside, it will need a handsome dowry—at least enough to build itself a building and to keep its traditional informal links with practising architects who serve as teachers. Ironically, of course, such a dowry would help to take the edge off present anxieties about the immediate future. The architects who have now complained should follow their protest

with an attempt to collect the sum of money which the association needs, one way or the other.

## TECHNOLOGY ON TOP

ON February 15 the Ministry of Aviation was formally merged with the Ministry of Technology, and the government department which the ministry itself has been calling "Mintech" became one of the largest, and potentially one of the most influential, in the British Government. Things have changed a lot in the two years since people were wondering just what the ministry had been created for—and how long it could last. The way in which the ministry has accumulated new responsibilities is in itself a kind of guarantee of survival. It is too important to be allowed to fail. But in the past six months the ministry seems also to have worked out for itself a framework within which it can function usefully and even imaginatively. Some of the unrealistic ambitions which were hung like albatrosses round the neck of the ministry when it was first set up have now been discarded. There is now much less to be heard, for example, of the "creation of new industries based on science". Instead, there is a much more realistic recognition that the immediate problem is to make all industries more productive. And there is much less talk of how it might be possible to make Britain less dependent on imports by deliberately developing substitutes. Instead, the ministry is turning out to be a source of encouragement to those who export—and a scourge to those who do not. No doubt the newcomers to the ministry—officials as well as ministers—have helped to make pragmatism respectable, but the ministry has also learned some useful lessons the hard way.

It does not follow that everything will now be plain sailing. It will be a long time, for example, before the old aviation research and development establishments can be welded into the structure of their new ministry and also given useful jobs to do. Many of the establishments have outgrown the problems with whose solution they were originally concerned, but attempts to make them more useful by turning them into free-lance research and development agencies for industry have not been successful. It would be far better if the ministry could find some way of placing some parts of its new empire in other environments. A deliberate dispersal of talent in commerce and industry is something real to aim at. Then the establishments should also be persuaded that it is no part of their business to build up a strong line in academic research, even if that does help in recruiting. Much better to form strong links with universities. And in the long run, the ministry should aim to use its establishments not as an independent machine for research and development but as a superior kind of management consultancy, able to turn its attention quickly to where it can be most useful. For even with its new strength, the ministry cannot hope to do everything by itself.



## NEWS AND VIEWS

### Loaves from Fishes

AFTER long hesitation, the United States Food and Drug Administration (FDA) has given its blessing to the protein concentrate with the unpalatable nickname of fish flour. Since 1961, congressmen from seafaring states have prayed for this event, sometimes gulping handfuls of fish flour in front of colleagues to prove that it is edible and does not taste of fish. But the FDA twice refused to rule the product safe, and thereby to open the way for its export to populations elsewhere starved of protein, because fish flour is made from the whole fish—entrails, stomach contents and all. To the FDA, this constituted filth—unacceptable in American food, and, by extension, in food shipped to poorer countries.

A little more than a year ago, however, a committee of the National Academy of Sciences turned the tide in favour of fish flour, declaring it to be not only safe but nutritionally valuable. The Bureau of Commercial Fisheries had meanwhile been busy at its laboratories in Beltsville, Maryland, trying to eliminate residues of the manufacturing process to which the FDA objected. (The bureau has found that isopropyl alcohol is satisfactory for extracting oil and water from the fish while keeping it palatable and stable.) And Congress became converted; last November it authorized a programme of research into fish protein concentrate—provided that the FDA gave its approval. That the agency has now done, just after President Johnson put a request for \$1.7 million for experimental FPC plants in his new budget.

True believers in FPC claim that it is the answer to protein deficient diets the world over. The product can be mixed with flour to make bread, noodles, cake or soup, or it can simply be sprinkled, as a protein additive, on food already prepared. It seems to offend neither taste nor taboo. And it is cheap—about 15 cents a pound for a substance which is 80 per cent protein. To make it, the Americans use hake, a fish rejected by their fishermen as a “trash fish”. The Fisheries Bureau estimates that about 12 billion pounds of this fish a year could be harvested, enough to supplement the diets of  $10^9$  people for 300 days at a cost of less than half a cent a day each. Once the manufacturing process has been refined, it seems likely that FPC could be made at many places around the globe—Sweden, Thailand and Morocco already produce it—and from other species than hake.

In the United States, FPC will be sold to begin with in packets of one pound or less; manufacturers are not to use it without giving evidence to the FDA that they are not deceiving the consumer. It must also be labelled with the advice that children under eight should not be given more than 20 grams a day, to guard against the mottling of their teeth from fluorides. This possibility will also be lessened by the insistence of the FDA that part of the fish bone be removed in processing to reduce fluoride residues.

### Artificial Kidneys

THE development of artificial kidney machines in Britain will not be accelerated by private donations or by an increased level of public spending. This at least is the verdict of the Ministry of Health, conveyed in a statement in the House of Commons by the Minister, Mr. Kenneth Robinson, on February 8. According to Mr. Robinson, there are three factors holding up development; the need to train staff, the time taken to plan and build accommodation for the units, and the risk of infection. At present, Mr. Robinson said, 116 patients are being treated in hospitals or at home, and the intention is to establish up to 20 main centres, each capable of treating 25 to 30 patients on twice weekly dialysis. There were, he thought, between 1,500 and 2,000 people who could be treated by renal dialysis; presumably the ministry is hoping that the difference between the number wanting treatment and the number of places available will be filled by those treating themselves at home.

The most serious factor is the risk of disease. The disease transmitted by the treatment seems to be serum hepatitis, a viral infection endemic in any part of the world. Patients with renal failure are likely to suffer because their disease involves them in a large number of blood transfusions; the Blood Transfusion Service refuses blood from donors who have had jaundice, but it is probable that there are a number of donors who have had sub-clinical attacks of serum hepatitis which were not diagnosed. The disease is spread from biological fluid to biological fluid, and can therefore be transmitted if a patient's blood is spilled on to abrasions on the hands of doctors and nurses; this may explain the serious outbreaks of jaundice at two renal dialysis units, at Manchester and Liverpool. Doctors regard the hazards as considerable, and are firmly resisting the pressure from the Ministry of Health to increase their work load.

Fortunately, the dangers to the health of the staff of renal dialysis units make home treatment even more attractive, or so some doctors believe. Dr. F. M. Parsons of the Renal Research Unit at Leeds believes that treatment at home may be better because fewer people are involved with the machines, and are likely to look after them better. Home treatment, of course, presupposes that the patient has the right sort of home, with a room that can be set aside for the purpose, and that the patient has some intelligence, and co-operation from his family. It is no quick and easy solution, however; patients require three months full time training in hospital to learn to use the machines.

### Reform at Oxford

THE University of Oxford shows signs of edging gingerly towards reforms recommended by the Commission of Inquiry under Lord Franks. On February 14, new statutes were read before Congregation which would alter the status both of Convocation and of Congregation itself. A debate on the new statutes will follow in two weeks.

The university is run by three bodies. Congregation, the supreme power, consists of almost everyone in a responsible position—the Chancellor, Vice-Chancellor,

Proctors, members of the faculties, members of committees, heads of colleges, and the like—and approves all legislation. The Hebdomadal Council looks after the running of the university, and consists at the moment of five ex-officio members and eighteen members elected by Congregation; it can submit legislation to Congregation. Convocation consists of all graduates of the university who hold a Master of Arts or higher degree; membership is therefore automatic to all graduates of the university if they apply for their Master's degree which is awarded after payment but without examination to all B.A.s 7 years after their matriculation. It is the powers of Convocation which will be most affected if the new statutes are passed; the power to elect the Professor of Poetry and the Public Orator, among others, will pass to Congregation, a loss which Fleet Street will probably feel more keenly than the university. Convocation will also lose the power to debate legislation which has not gained a two-thirds majority in Congregation, and will retain only the power to elect the Chancellor of the university by postal ballot. New statutes covering the Hebdomadal Council have also been published, and will be debated in the next few weeks.

The changes, if they are approved, will not greatly affect the administration of the university; in fact they give the impression of tinkering with inessentials while the need for a major overhaul is ignored. Those who feel that the stoutest defence of academic freedom in Oxford is provided by the general obscurity of the university's administration need feel no alarm about the new statutes. Two former Professors of Poetry have declared their opposition to the proposals. If this feeling is shared by more than one-third of Congregation, then presumably the new statutes will pass to Convocation for approval. Convocation will then have to give its opinion of proposals calculated to make its opinions irrelevant, a situation of some constitutional nicety.

## Scientific Advice

THE Scientific Branch of the Greater London Council advises the council on matters in which scientific judgment is valuable. The report of the Scientific Adviser for 1965 (G.L.C., 5s.) gives details of this work, and of the Scientific Branch, which has been divided into four main groups: Research and Materials, Analytical, Sewage Treatment, and Technical Support.

The Thames seems to be less polluted than in the past; the dangerous situation arising when there is no dissolved oxygen in the water has occurred less frequently. The air also seems to be fresher, with the sulphur dioxide content down by 14 per cent and the concentration of smoke down by 23 per cent. Clean fuels and efficient heating appliances have caused this encouraging improvement; there were no seriously polluted fogs during 1965. The branch has also been concerned with the examination of building materials, both as a means of quality control and to trace and eliminate defects; it is interested in replacing expensive materials with cheaper ones wherever possible. In addition, it has carried out the responsibilities of public analyst, ranging from dirty milk bottles (a common complaint) to the amount of meat in meat pies. Standards proposed for meat pies but not yet in force would require 25 per cent total meat, not on

the face of it an impossible requirement; pies with less than 25 per cent meat are to be described as cornish pasty.

## Feeding the 500 Million

It is difficult to say anything new about the problems of feeding the underdeveloped countries. *Science Reporter*, an Indian scientific journal published by the Council for Scientific and Industrial Research at New Delhi, has bravely devoted the whole of its latest issue to the problem. The magazine includes contributions from a number of distinguished men, including United States Secretary of Agriculture Mr. Orville Freeman, and United Kingdom Minister of Agriculture Mr. Frederick Peart, who briskly run through their respective paces without saying anything unexpected. Mr. Freeman believes that the war against hunger can be won, and details the amount of aid the United States has provided since 1954 (150 million tons of food, worth \$15.10<sup>9</sup>), while Mr. Peart applauds the growth rate of agriculture in Britain (6 per cent per year from 1960 to 1964) without saying how India can hope to do the same.

Perhaps the time for generalities is past, in any case. The contributors become more interesting as they become more specific. India has more than 4,600 km of coastline and one-fifth of the cattle in the world, but produces only 2.5 kg of fish per person per year, one of the lowest totals in the world, and only one cup of milk per person per day. Population control, the contributors agree, is essential; Dr. V. G. Panse of the Planning Commission in New Delhi believes that without it there is no chance of the Indian population reaching even the minimum nutritional level in the next 50 years. It also seems agreed that none of the present contraceptive methods is ideal; the loop is spreading, but if, as one writer suggests, its success depends on insertion by "trained lady doctors", progress is likely to be imperceptible. In 1961 there were about 4,200 trained lady doctors in India, for a population which has just passed 500 million. Perhaps the most hopeful avenue is the prevention of waste; Dr. S. V. Pingale of the Ministry of Food in New Delhi estimates that no less than 10 per cent of the grain in India is lost through indifferent handling and the depredations of insects and rats.

## John Doe, Scientist

THE National Register of Scientific Personnel, operated jointly by the National Science Foundation and the principal learned societies, has now been made to yield a detailed profile of the life and work of the scientific population in the United States (*American Science Manpower 1964*, Government Printing Office, \$1.25). In 1964, it seems, the median age of scientists on the register was 38, and 35 per cent of them had doctorates. 60 per cent of those on the books worked in the physical sciences, with life sciences claiming 24 per cent and other disciplines (including mathematics and some social sciences) the rest. Of all scientists, 38 per cent worked in industry and nearly as many—32 per cent—in educational institutions of all kinds, schools as well as universities. In practice, only just over a half of those employed in education are actually teachers: in 1964, 18 per cent of those on the register confessed

to teaching as their principal activity. Research and development occupied 35 per cent of all scientists, while administration and various managerial activities took 21 per cent.

The details of salary will probably command the closest attention—not only among those about to emigrate to the United States. In 1964 the median salary for scientists in the United States worked out at \$11,000, but there were significant variations from discipline to discipline, age to age, and even from place to place. The median salaries of economists, physicists and statisticians were \$1,000 above par. Chemists and mathematicians had median salaries of \$11,000, but other disciplines fell below par with agricultural scientists near the bottom of the list with median salaries of \$9,200. The possession of a Ph.D. degree seems to confer an advantage of \$2,000 in salary—the difference between the median salary of \$12,000 for doctors and \$10,000 for those with master and bachelor degrees. It is plain that the management and administration of research and development is more rewarding (median salary \$15,500) than research and development itself (median \$11,000), although this comparison is in part vitiated by the predominance of scientists with doctorates among the administrators. The median salary in industry or commerce worked out at \$12,000. The corresponding figure for educational institutions was \$9,600—a figure calculated on the basis of an academic year of nine or ten months for a good many teachers.

For all scientists, the upper and lower quartiles of the salary distribution lie at \$14,000 and \$8,600. Predictably, there is more uniformity among the lower quartile salaries earned in different specialities than among the upper quartiles, which range from \$11,200 (for agricultural scientists) to \$16,000 for economists. Economists are among the most highly paid of the scientists and social scientists on the national register, and 10 per cent of them earned more than \$20,000 in 1964. Among the pure scientists, straightforward biologists seem to have done slightly better than physicists and mathematicians, with an upper decile of \$19,000 (compared with \$18,700 for physics and \$18,500 for mathematics). Women, who account for 7.6 per cent of the scientists on the register, earn less than men. Their median salary works out at \$8,400, with the greatest rewards in the social sciences. Undoubtedly a part of this apparently unflattering comparison is to be explained by differences of age distribution and even of qualification between the sexes. Salaries increase with age up to the late fifties, with a median of \$7,000 between 20 and 24 and a plateau of about \$13,000 or just above which seems to be reached in the late forties.

## Mintech Marriage

THE Ministry of Aviation was formally merged within the Ministry of Technology on February 21, after some hectic days in the House of Commons when it seemed as if the enabling legislation would be defeated by procedural difficulties. In the new ministry Sir Richard Clarke will remain the administrative head. Sir Richard Melville, hitherto Permanent Secretary at the Ministry of Aviation, will become head of the Aviation Group. The direction of the scientific

establishments is to be shared between Dr. George MacFarlane and Mr. Ieuan Maddock. Dr. MacFarlane, at present director of the Royal Radar Establishment, will become Controller (research) with responsibility for the research stations already within the ministry, the Atomic Energy Authority, the National Research Development Corporation, the research associations and, eventually, the research establishments transferred from aviation. Mr. Maddock will be Controller (industrial technology), and will be responsible for the work of the ministry in promoting development within the engineering industry.

## Jobs for Biologists

BRITISH biologists who are looking for jobs will take heart from the results of a survey carried out by the Institute of Biology (*A Survey of the Employment of Graduates in Biological Posts in the United Kingdom: J. Inst. Biol.*, **13**, 131; 1966). The survey made use of published information on the specialization of biologists in the Civil Service, government research and education, and detailed questionnaires were sent to companies to obtain information about biologists in industry. The survey should put an end to the myth that biology graduates have poor prospects of employment. Of the eleven thousand graduate biologists employed in the principal British centres, nearly five thousand are teachers, chiefly in secondary schools. Another five thousand are about equally divided between state aided research and the universities, and the remaining fifteen hundred are employed in industry. There are 1,085 biochemists, 868 microbiologists, and 797 animal physiologists—the 4,143 general biologists are almost all engaged in teaching.

The form of the survey made it possible for the institute to estimate the rate of growth of the demand for biology graduates. Certain specialities, such as microbiology and toxicology, seem to be inadequately supplied with qualified people. The demand from industry so much exceeds the supply of such graduates that the additional requirement by June 1967 will be 25 per cent of the existing number of industrial microbiologists. Many of the available posts in such specialities are, of course, filled by scientists from related disciplines, but companies often find that it is expensive to retrain such people. The institute thinks there is a case for more specialized courses at the undergraduate level in subjects like industrial microbiology, and for establishing new departments where these do not already exist. Such a solution would solve the immediate problems for industry, but it would be at the expense of any attempts there might be to broaden the horizon of biology teaching in the universities. The proper place for specialized teaching of this sort is at the graduate level, and it is encouraging to see that many universities are now starting all their biology students on a general biology course.

Industry expects a rate of growth in the demand for biologists of 10–15 per cent each year. The universities are working on the assumption that they will require about 7 per cent more biology graduates each year. Applied biologists should benefit most from this labour shortage, but university and research biologists will also be assured of a place.

## Maps by Order

THE surveying and mapping of the British Isles are carried out by the Ordnance Survey. Despite an air of brisk efficiency, aided by a preponderance of army officers in senior positions, the survey has recently been the victim of some distinctly undisciplined shuffling in Whitehall. After 75 years in the care of the Ministry of Agriculture, the survey moved in 1965 to the Ministry of Land and Natural Resources, but had barely drawn breath before that ministry went into eclipse—it is at present in the process of dissolution. The survey now has its third Minister in two years—the Minister of Housing and Local Government, Mr. Anthony Greenwood.

The survey's work does not seem to have been disturbed by this uncertainty. It is still a long way from completing the basic survey of Great Britain, but is making progress. Three basic scales are used; 1 : 1,250, 1 : 2,500 and 1 : 10,560 (6 in. to 1 mile). So far, however, maps of the whole country are available only at scales of 1 in. to 1 mile (1 : 63,360) and 0.25 in. to 1 mile (1 : 250,000). Most urban areas are now mapped at scales of 1 : 1,250, but there is still much to do before the 1 : 2,500 plans of the counties are completed. Maps of mountains and moorland areas are also to be published, at the 1 : 10,560 scale, but here there is an enormous amount of work yet to be done. Another series has been undertaken during the past year, at 2.5 in. to 1 mile, and various archaeological and tourist maps are also available.

The survey is now more optimistic about finishing its task by 1980. During his tenure of office, Mr. Fred Willey, Minister of Land and Natural Resources, authorized a 20 per cent increase in the staff of the survey over 10 years. Without this, reports the Director-General of the survey, Major-General Edge, in the annual report for 1965-66 (H.M.S.O., 7s. 6d.), the job would hardly have been finished before the end of the century. Operations should also be easier when the survey moves to new headquarters in Southampton; this will bring together the work carried out in Esher and Chessington in Surrey and the drawing and reproduction department in Southampton.

If the move makes the work of the survey any less expensive, nobody will complain, as the budget is hardly chickenfeed. Expenditure last year was £5.5 million, against receipts of £1.7 million. The survey has a staff of 4,548, with 1,718 surveyors and 1,274 draughtsmen. The impression of a military machine is perhaps deceptive, as there are only 26 army officers on the staff, but most of them seem to be concentrated in the top executive positions.

## Qualified Success

THE fourth French earth satellite launched from North Africa on Wednesday, February 8, and known as *Diademe*, seems to have been only a partial success. The device, of mass about 15 kg, was found after launching to be in an orbit about the Earth with an apogee substantially lower (1,340 km) than that planned (1,843 km). It is therefore possible that the geodetic triangulation measurements which had been planned will now be difficult and even perhaps impossible. The original orbit was chosen so that the satellite would be simultaneously visible from three ground stations around the Mediterranean—at Colomb-Bechar

in the Sahara, Saint Michele-de-Provence in France and at a station on the Gulf of Corinth. Lasers at each of the stations, together with Doppler measurements, were to have been used for triangulation. No doubt it will be possible to make good use of observations from pairs of stations and also from sky photography from several observatories beneath the orbit, but some precision will undoubtedly be lost.

Those responsible for the rocket programme were hoping earlier this week to repeat the triangulation experiments by launching a second satellite (and the fourth in the present series) into a somewhat higher orbit. The launching was due to take place on February 15.

## Do Worms Learn?

THE controversy about the mechanism by which planarian worms appear to learn by the ingestion of nucleic acids from other worms remains unresolved despite the deliberations of a conference at Michigan State University, East Lansing, last year (1966). Several papers at the conference were devoted to the factors which may directly or indirectly affect the results observed. There appear to be marked differences between the properties of planarian worms of different species—and there are as many as forty species in the United States. Much of the evidence which supports the notion of a chemical basis of learning has been based on experiments which demonstrate that an acquired response can survive regeneration, and a French participant at the conference described how different species of planaria differ in a mechanism of tissue renewal. Some do not regenerate at all. Others regenerate only at the front and others regenerate at any point along the axis. This obviously affects the way in which acquired information can be retained during regeneration. Among other animals to which the chemical basis of learning may be important are what have been described as the "micrometazoa"—hydra and so on. The conference also heard of experiments with the isolated insect ganglion, and one participant produced evidence which indicates that a single isolated ganglion in a cockroach can acquire an avoidance response. There is also histochemical evidence that may enable identification of the muscles and axons that are involved in information storage.

## Control of Haemoglobin Function

from a correspondent in Molecular Biology

ONE of the most tantalizing problems in molecular biology concerns the nature of the haem-haem interactions in haemoglobin. It is well established that a conformational change occurs during oxygenation, but in spite of the detail in which the structure—at least of the oxygenated form—is known, how the change is controlled and transmitted remains obscure. The problem has the widest relevance to control mechanisms in enzyme systems.

Among the more promising approaches have been attempts to block selectively the coupling between the haems and the Bohr effect. Thus it has been found that normal human haemoglobin can under some circumstances be made to remain in the same confor-

mational state throughout the oxygenation reaction. This is the state of high affinity for oxygen, which physiologically is responsible for the high reactivity of the fourth haem after the other three have reacted: the rate of combination with oxygen in this step is known to be some forty times greater than in the earlier phase. It has been found that only the high-affinity state is observed in haemoglobin H, the abnormal human form which contains  $\beta$  but no  $\alpha$ -chains, in haemoglobin pre-treated with carboxypeptidase (which selectively removes residues from the C-termini), in isolated  $\alpha$  and  $\beta$ -chains and in a form of haemoglobins produced by photolytic removal of ligands in which the conformational change does not have time to occur. Another case was discovered recently, when it was observed that no haem-haem coupling (or Bohr effect) is present in haemoglobin complexed with haptoglobin, a specific haemoglobin scavenging plasma protein.

The initial observation has now been extended by Nagel and Gibson (*J. Mol. Biol.*, **22**, 249; 1966), who have measured the velocity of carbon monoxide binding to haemoglobin in such a complex. First, it is found that the velocity does not depend on  $pH$ —there is no Bohr effect; second, the rate for the complex is an order of magnitude greater than for free haemoglobin. In flash photolysis experiments, moreover, it is noted that, unlike free haemoglobin, the complex shows no biphasic rate curve, and the absence of haem-haem coupling is clearly inferred. There is also a markedly lower activation energy for the process in the complex. It therefore appears that haptoglobin associates with haemoglobin in its high-affinity form, and locks its conformation in that state. The experiments have been performed with two genetically different haptoglobins, one of which is in a higher polymerization state. Qualitatively, the complexes behave similarly in respect of carbon monoxide binding, but differ considerably in rate constants and activation energies.

A most remarkable observation on the regulation of haemoglobin function has been made by the Benesch (*Biochem. Biophys. Res. Commun.*, **26**, 162; 1967), and this will without doubt re-direct much of the current thinking in the field. The authors set out from the fifty-year-old observation that, in the absence of particulate salts, haemoglobin loses its sigmoid oxygen dissociation curve and behaves like myoglobin. Its oxygen affinity is then very high. Although this curve is not affected by sodium chloride or sulphate, the phosphate ion is shown to decrease oxygen affinity and to generate a perceptible haem-haem interaction. On the other hand, the compound 2,3-diphosphoglyceric acid, which exists in substantial concentration in the mammalian red cell, produces at millimolar concentrations a dramatic shift in the oxygen dissociation curve to precisely the position associated with a normal haemolysate or whole blood. Indeed, when the oxygen affinity or the exponent  $n$ , which measures the degree of haem-haem interaction, is determined as a function of diphosphoglyceric acid concentration, there is a very sharp rise and, particularly in the case of  $n$ , no further change with a more than tenfold increase in concentration. This suggests the presence in haemoglobin of specific binding sites of high affinity for diphosphoglyceric acid and its analogues (a number of which have also been studied), and indicates that it is this ligand which determines the allosteric properties of the protein.

## Parliament in Britain

IN a written answer in the House of Commons on February 13, the Secretary of State for Education and Science, Mr. A. Crosland, announced that the Government intended to set up a special fund which would enable grants to be made to students from overseas if they could show that the recent increase in tuition fees would involve them in hardship. The aim was to ensure that overseas students already here would not be prevented from finishing their courses because of hardship. It was too soon to give details of these arrangements, but the long term situation would be kept under review by the departments concerned.

IN a written answer on February 3, Mr. Crosland stated that in 1955–56 there were 9,290 overseas students at full-time and sandwich courses in universities, the estimated subsidy being £3.4 million; the figures for further education establishments were not known. In 1961–62 the corresponding figures were 13,020 and £6.9 million for universities and 13,770 and £3.2 million for further education establishments; and for 1965–66, 16,256 and £12.0 million, and 16,292 and £6 million, respectively. The subsidy in 1967–68 under the new arrangements was expected to be no less than in 1965–66.

IN a written reply to a question in the House of Commons on February 9 regarding research in Government establishments into fishermen's diseases during the past five years, the Minister of State, Department of Education and Science, Mr. G. Roberts, stated that a study was being made in the Department of Social Medicine, University of Aberdeen, of causes of death among a group of trawlermen.

IN reply for the Government on February 3 in an adjournment debate in the House of Commons on sonic boom problems in civil aviation, the Minister of Aviation, Mr. J. Stonehouse, agreed that it was important to have a proper evaluation of the position regarding the sonic boom as supersonic transport developed. Apart from the possibility of damage to structurally unsound buildings, he thought there was no real likelihood of damage or loss being caused on the ground as a result of sonic bangs such as were likely to be caused by the Concord aircraft. Mr. Stonehouse pointed out that it was also necessary to consider the economic viability of the Concord project if supersonic flight over land was disallowed. A complete ban on supersonic flights over land would reduce the market for the Concord to 55–65 per cent of that at present expected and to 75 per cent if the ban was partial.

REPLYING for the Government on February 6 in an adjournment debate in the House of Commons on the oil pollution of beaches, the joint parliamentary secretary to the Ministry of Housing and Local Government, Mr. J. MacColl, emphasized that dispersal at sea was not a Government responsibility: that responsibility was limited to detecting offenders and trying to bring them to book. The Warren Spring Laboratory had studied techniques for removing oil from beaches, including full-scale trials at Shoeburyness and Eastbourne, and a technical working group to find an efficient and economic method of combating oil pollution on beaches started work in October 1966.



## University News:

London

PROFESSOR J. LEWIS, professor of inorganic chemistry in the University of Manchester, has been appointed to the chair of chemistry tenable at University College, and Professor O. J. Lewis, professor of mammalian morphology at St. Bartholomew's Hospital Medical College, has been appointed to the chair of anatomy tenable at that college.

## Strathclyde

A DEPARTMENT of Computer Science has been created and an extensive programme of development in computer work, centred on a newly installed ICT 1905 machine with a 32K word core store and magnetic tapes, has been launched by the university. It is hoped that an undergraduate degree course in computer science will soon be introduced.

## Appointments

MR. R. B. ABEL, formerly assistant research co-ordinator, Office of Naval Research and executive secretary of the Interagency Committee on Oceanography, has been appointed head of the U.S. National Science Foundation's programme to implement the National Sea Grant College and Programme Act of 1966. The Act assigned to the foundation the task of mobilizing appropriate institutions to play a more significant part in the development of marine resources through education, research, and advisory services.

## Announcements

THE U.S. Atomic Energy Commission has appointed an advisory panel on high-energy physics to help foster "a vigorous and productive national high-energy physics programme". The chairman is Professor Victor F. Weisskopf of M.I.T. The panel includes Dr. Rodney L. Cool (Brookhaven National Laboratory), Professor Earle C. Fowler (Duke University), Professor Leon Lederaman (Columbia University), Dr. Edward J. Lofgren (Lawrence Radiation Laboratory), Dr. George E. Pake (Washington University), Professor W. K. F. Panofsky (Stanford University), Professor Robert G. Sachs (Argonne National Laboratory), Professor Keith R. Symon (University of Wisconsin), Professor Robert L. Walker (California Institute of Technology), Professor Robert R. Wilson (Cornell University) and Professor C. N. Yang (State University of New York).

A DELEGATION from the Royal Society, consisting of Professor H. W. Thompson, Dr. R. D. Keynes and Dr. D. C. Martin, is visiting Bulgaria during February 17-23 at the invitation of the Academy of Sciences of the People's Republic of Bulgaria. The purpose of the visit is to sign a direct agreement for scientific collaboration between the society and the Bulgarian Academy of Sciences.

THE Ford Foundation has made a grant of \$200,000 over a period of three years in support of the Royal Society's new European Programme, which is aimed at strengthening the European scientific community by promoting interchange between scientists. This grant will be used to support the European Programme of fellowships at postgraduate and postdoctoral levels, study visits of shorter duration and small European research conferences. The first closing date for applications for fellowships is February 28.

THE meeting of the Mass Spectroscopy Group on "Analysis of Inorganic Solids", which was to be held at the University of Manchester on April 5, has been cancelled.

**ERRATUM.** The statement on the Information Exchange Groups in *Nature* last week (213, 547; 1967) was wrongly attributed to the "Commission of Biological Editors". The statement was, in fact, prepared by the Commission of Editors of Biochemical Journals under the International Union of Biochemistry.

## CORRESPONDENCE

## Fire Risks in Space Capsules

SIR,—There are important weight and control system advantages to using a pure oxygen atmosphere for space vehicles. In such a system one purges the capsule with pure oxygen at a pressure slightly greater than atmospheric for some hours and then allows it to vent to a pressure of about 5.5 p.s.i. in the case of the "Apollo" and early MOL projects during the first minutes of lift-off and ascent. Thereafter the capsule atmosphere is maintained as pure oxygen at that pressure. During descent the environment of the crew is returned to atmospheric pressure by the inlet of air. During the purging procedure and in pressurized flight the crew breathe through the open visors of their pressure helmets.

The fire studies carried out at this institute some time ago suggested that a threshold existed for the appearance of flash fires in oxygen rich gas environments, and that this threshold was unlikely to be below 6.0 to 6.5 p.s.i. partial pressure of oxygen. It seems to us that this has important implications for the future of single gas space vehicle systems. If, as it suggests, the in-flight condition carries no risk of flash fire, one can think of two methods which eliminate that risk from the remainder of the flight envelope, and retain the advantage of single gas systems without structural or control system modifications. Both involve breathing, with the helmet visor closed, from a personal supply of pure oxygen until the respirable atmosphere of the final flight condition is achieved some minutes after lift-off.

The methods are: (a) Dilution of the cabin oxygen with a soluble gas which can be absorbed once the fire hazard has been removed by a fall in the total environmental pressure. (b) The use of a mixture of oxygen and an insoluble gas, which can later be voided to the external vacuum and replaced by pure oxygen.

Carbon dioxide would appear to be an obvious choice for the soluble gas, because absorbants are already required for removing the carbon dioxide produced by metabolism. It is estimated that an additional two pounds of lithium hydroxide would be needed for the cabin of a spacecraft with a volume of 150 cubic feet, an atmosphere consisting of 40 per cent of oxygen and 60 per cent of carbon dioxide and a total pressure of 6 p.s.i. A similar weight of oxygen (equivalent to the metabolic needs of one man for one day) would suffice to restore the atmosphere of the spacecraft to the desired pressure. Engineering problems associated with the control and handling of large quantities of carbon dioxide during the purging phase might, however, dictate the choice of some other soluble gas.

Air would be a satisfactory mixture for the second method, the major disadvantage of which is the necessity for a decompression of the capsule early in the flight. Five pounds of oxygen would be enough to replace the atmosphere of the cabin.

Clearly, these arguments hinge on the existence and level of a threshold for flash fires which we know to be somewhat dependent on the nature and history of the supporting surface. The present series of fire studies at this institute is intended to give a better understanding of this phenomenon. This threshold might be exceeded on re-entry until "splash-down".

Both the solutions require that the crew have their visors closed for some hours before orbital flight is attained. This appears to be a small price to pay.

Yours faithfully,

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# Fluorescence Microscopy with the Quartz-Iodine Lamp

by

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Many dyes fluoresce when they are irradiated with light of particular wavelengths. This is the basis of some specific microscopical staining techniques, especially in the field of immunology. Where activation depends on blue-violet light the quartz-iodine lamp is a useful and convenient source for fluorescence microscopy.

THE fluorescence microscope has developed from a specialized apparatus for research, where elaborate or inconvenient equipment may be acceptable, to the present stage when it is increasingly needed for routine use in biology and medicine. Convenience and simplicity of operation have become important considerations. Recently it was suggested that the cadmium lamp<sup>1</sup> is a feasible and highly efficient light source for immunofluorescence microscopy. The only cadmium source with adequate intrinsic intensity is the cadmium electrode spark; unfortunately this apparatus is expensive and inconvenient. It may therefore be useful at this time to report on experience gained in this laboratory with the quartz-iodine lamp, which merits consideration as a possible alternative to the mercury high-pressure lamps that have hitherto been regarded as the only adequate and practical light source for activation of fluorescence in microscopical specimens<sup>2</sup>; the carbon arc gives good results but is inconvenient in use.

Tungsten filament lamps as ordinarily used for light microscopy have occasionally been used for fluorescence microscopy<sup>3</sup>, but it is generally agreed that their efficiency is low. The ratio of useful blue-violet light to total energy output is diminished in practice by two factors: first, the proximity of the excited fluorescence to the activating maxima in the blue region necessitates the use of a primary filter with a sharp cut-off in transmission at 500 m $\mu$ ; second, there is an intense emission of visible light extending into the red region, which must be effectively absorbed by the primary filter. Inevitably the intensity of the required blue-violet emission falls below a useful level. If the lamp is over-run at high voltage it is possible to boost the output of useful blue-violet light; but the life of the lamp is then drastically reduced.

The 100 W quartz-iodine lamp is a very compact tungsten filament source, with a quartz bulb in which a small quantity of iodine is enclosed. The filament operates at a higher temperature than in conventional tungsten lamps, giving greater efficiency in the production of light. As a result of the "iodine cycle", in which evaporated tungsten redeposits on the filament, the quartz remains clear until burn-out. An important feature in relation to fluorescence activation is that the inclusion of iodine modifies to some extent the emission spectrum, by selective absorption in a broad band in the yellow-green region; violet emission below 430 m $\mu$  seems to be increased as the radiant energy from the filament passes through an atmosphere of iodine vapour<sup>4</sup>.

Compared with mercury lamps with an average life of 400 operating hours, the quartz-iodine source with 50 h appears to be at a disadvantage. In practice this is not necessarily so. Mercury-pressure lamps require a warming up period of several minutes or more before they reach full intensity, and also need to cool before the arc can again be switched on. Consequently many users leave the lamp burning for long periods even when it is

not actually in use. Quartz-iodine lamps can be switched on and off at will, and give full brightness at once. They withstand rapid voltage change, provided that the full wattage is not exceeded, and perform with only slight reduction in lumen efficiency when slightly under-run. Replacement costs are much less than for mercury-pressure lamps.

Comparison of the emission spectrum of the quartz-iodine lamp with that of mercury-pressure lamps shows that, whereas the latter emit light in the form of separated narrow bands or lines, with a strong line in the ultra-violet at 365 m $\mu$ , the quartz-iodine lamp gives a continuous spectrum (Fig. 1A). Ultra-violet output is negligible, but the intensity of the visible blue-violet emission compares well with that of mercury lamps<sup>2</sup>. Workers using the mercury-pressure lamp for fluorescence microscopy have often preferred to use the ultra-violet for the activation of fluorescein conjugates despite the fact that apart from a small peak at 325 m $\mu$  absorption by the

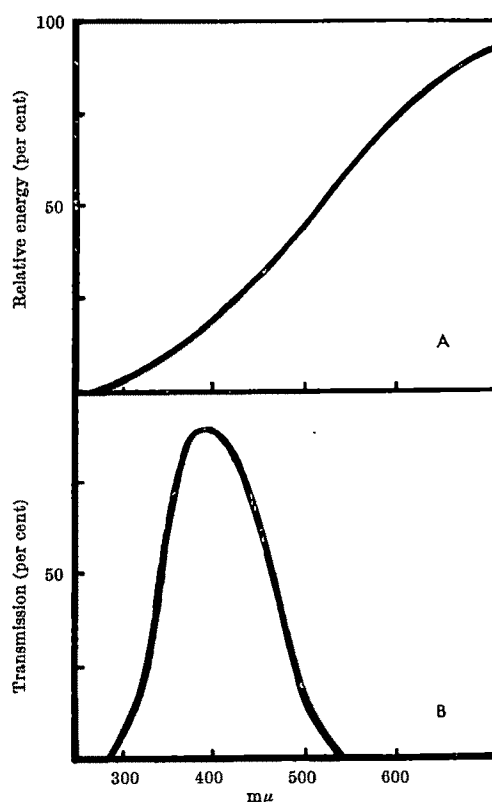


Fig. 1. A, Spectral emission curve for the Atlas 100 W quartz-iodine lamp, operated at 12 V; colour temperature 3,300° K (based on manufacturer's data). B, Spectral transmission curve for the Corning blue-violet glass filter No. 5850; thickness 4 mm.

dye between 300 m $\mu$  and 400 m $\mu$  is low<sup>5</sup>. A disadvantage of ultra-violet activation arises in some biological specimens, where the apple-green colour of specifically labelled areas may be modified or masked by autofluorescence of tissue components induced by ultra-violet light. Nairn<sup>6</sup> suggests that the strong absorption of fluorescein at 495 m $\mu$  is too close to its emission peak at 525 m $\mu$  for matched filters to function satisfactorily; because the blue-absorbing secondary filter cannot be exactly complementary to the primary filter, it may be difficult to distinguish between specific and non-specific coloration of the specimen. This is so if the specimen is to be illuminated with a bright field condenser; however, dark field condensers are now frequently used for fluorescence microscopy, and under these conditions a balanced filter system for blue-violet activation of fluorescein conjugates is readily made for any of the available light sources. It is frequently convenient, of course, if the secondary filter transmits a faint blue dark field image of the unstained elements of the tissue, facilitating localization of the specific fluorescence.

In view of these factors it is reasonable to expect the quartz-iodine lamp to function as a useful activating source, not only for work with fluorochrome dyes such as acridine orange, thioflavine, primulin or auramine, but also for immuno-fluorescent staining with fluorescein or rhodamine conjugates. Tests with a variety of biological specimens have been made in this laboratory to compare the performance of fluorescence microscope units incorporating the MED/250 high-pressure mercury lamp or the 100 W quartz-iodine lamp. The former was used in the horizontal optical bench apparatus previously described<sup>2</sup>; the quartz-iodine lamp formed part of a commercial fluorescence microscope (manufactured by Gillett and Sibert, Ltd.). The same type of cardioid dark-field condenser was used in each case. The primary filter in both was a Corning C.S. 7/59 No. 5850 blue-violet glass filter combination (see Fig. 1B); the secondary filter was an Ilford No. 107 gelatine film. Heat and red light from the quartz-iodine lamp were absorbed with a Chance ON.13 glass filter, and from the mercury lamp with a 10 per cent solution of cupric sulphate in a 1 cm thick cell. In one test, monolayer cultures of monkey kidney cells were inoculated with a B strain (Lee) of influenza virus and examined 24 h and 48 h later, together with control cultures, for the presence of viral antigenic materials. For immunofluorescent staining the cells were

fixed in alcohol and stored at  $-70^{\circ}\text{C}$ ; after treatment with guinea-pig antisera to the "S" or "V" viral antigens, they were stained with fluorescein-labelled rabbit anti-guinea-pig gamma-globulin serum. Changes in the pattern of intracellular nucleic acids were shown, after fixation in alcohol, by staining with acridine orange at pH 3.5. Newly formed virus at the cell surfaces, and the intranuclear localization of viral soluble ("S") antigen with its associated RNA, were observed with about equal clarity on the two microscopes. Photographic exposures indicated slightly higher intensity of fluorescence with the mercury lamp, but image contrast against the black background was better in the microscope fitted with a quartz-iodine source.

Our observations on these and other specimens indicate that when combined with a filter system properly matched to its emission spectrum, and provided that blue-violet activation is acceptable, the 100 W quartz-iodine lamp is a reasonable alternative to the mercury pressure lamp for general work on cell cultures or thin tissue sections. At the same time, more searching tests to compare the limits of sensitivity obtainable with different light sources may well show the mercury lamp to be superior. There are also cases where ultra-violet activation is essential; for example, in observations of autofluorescence from tissue components such as vitamin A, or for tracing reagents which absorb strongly only in the ultra-violet. Moreover, there are circumstances where the physical state of the specimens may preclude the use of blue-violet light; should the thickness or refractivity of the material exceed practical limits imposed by the method of dark-field illumination, for example in thick smears, excessive scatter of blue light may result and cause marked loss of contrast and definition in the fluorescent image.

It appears, in conclusion, that the quartz-iodine lamp in its present form has definite limitations, but is an adequate and very convenient light source for many biological applications of fluorescence microscopy.

We thank Atlas Lighting, Ltd., for permission to publish the technical information which they kindly supplied on the 100 W quartz-iodine lamp.

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## Protein Synthesis in the Absence of the Nucleus

by  
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Some living cells without their nuclei can continue to survive and develop, at least for a time. Do they use up stores of messenger RNA? Or are the organelles of the cytoplasm less passive than is often thought?

In recent years our embryological research has been directed towards a deeper understanding of the relationships during morphogenesis between the activities within the nucleus and those in the cytoplasm. The most suitable biological material for these investigations has been found to be the giant unicellular alga *Acetabularia mediterranea* and the eggs of the sea-urchins and amphibians. With each of these, it is possible to separate nucleate and anucleate cellular fragments. What follows describes

some of the findings which have emerged from our investigations.

### *Acetabularia mediterranea*

*Acetabularia mediterranea* (Fig. 1) measures 2–3 cm in length and can thus easily be cut into two parts, one containing the nucleus (which is located in the rhizoid), the other lacking it (compare Hämmerling<sup>1</sup>). Anucleate fragments can survive for several months, and can even

regenerate a reproductive organ—the cap. Except for its smaller size, the morphological aspect of the cap is typical of the species to which the nucleus belongs. This clearly implies gene expression in the absence of the nucleus. Analysis of interspecific nucleate and anucleate fragments of *Acetabularia* led Hämmerling<sup>2</sup> to the conclusion that the nucleus must produce "morphogenetic substances" which are specific to the species. Synthesized in the nucleus, they would migrate to the tip of the stalk and autonomously control the formation of the cap (Fig. 2).

We have been able to show<sup>3</sup> that net protein synthesis occurs in anucleate fragments. Within 3 weeks of nuclear ablation, the protein content increases by a factor of three. The proteins synthesized by the anucleate fragments include several enzymes the synthesis of which is usually considered to be controlled by genetic material—nuclear DNA.

The five isozymes of acid phosphatase are among the best known<sup>4,5</sup>. Their activity seems to be independently controlled even in anucleate cytoplasm. Two of them, which are associated with the chloroplast fraction, increase in activity when caps are formed by anucleate fragments. The activity of a third isozyme, not found in the chloroplasts, decreases with time.

Net synthesis of RNA can also occur in anucleate fragments, especially if the alga has previously been cultivated in the dark<sup>6</sup>. We have observed a two-fold increase in the RNA content of anucleate fragments over a period of 5–7 days. Earlier experiments by Naora *et al.*<sup>7</sup> have shown that this RNA synthesis primarily occurs in the chloroplasts.

When these facts are re-examined in the light of present knowledge, it seems probable that the morphogenetic substances are messenger RNAs which are stable and long-living. They would migrate from the site of synthesis on chromosomal DNA to the apex of the alga, and bring with them the information required for species specific cap formation. The duration of their life can be calculated from the length of time during which anucleate fragments maintain their capacity to form caps and synthesize proteins—about 2–3 weeks. Fig. 3 summarizes these ideas<sup>8</sup>.

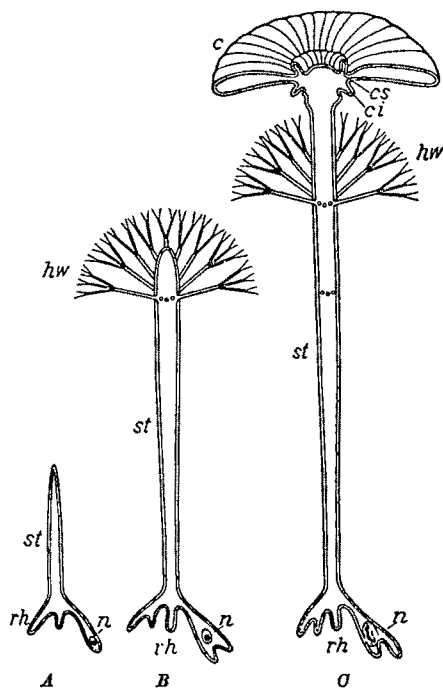


Fig. 1. Vegetative development of *Acetabularia*, diagrammatic. c, Cap; ci, corona inferior; cs, corona superior; hw, hair whorl; n, nucleus; st, stalk; rh, rhizoid.

There is a great deal of evidence to support this hypothesis. Just as in other cells, nuclear (and particularly nucleolar) RNA is labelled first after a pulse of several hours with uridine. When the algae are later transferred to unlabelled seawater, the previously labelled nuclear RNA migrates towards the apex of the stalk<sup>9</sup>. Certain proteins which are also synthesized in the nucleus accompany the migrating RNA<sup>10,11</sup>. Werz<sup>12</sup> has also shown by cytological techniques that RNA and basic proteins collect at the apex of the stalk. Our explanation also accounts for what happens when pre-existing RNA is broken down, or when the DNA dependent synthesis of RNA is inhibited. Anucleate fragments treated with RNase<sup>13</sup> or by ultra-violet light<sup>10</sup> entirely lose their ability to regenerate and to produce caps, because the stable mRNAs which have accumulated in their tips are degraded and cannot be replaced in the absence of the nucleus. Nucleate fragments, on the other hand, are capable of replacing the degraded RNA by renewed syntheses, shortly after the RNase is removed or the treatment with ultra-violet light stopped; but if the synthesis of RNA is blocked by actinomycin, only the regeneration of the nuclear fragments is inhibited; the anucleate fragments are able to begin cap formation in a normal manner, because they already contain the required stable mRNAs. The formation of the cap is, however, slow and imperfect<sup>14,15</sup>. Puromycin and cycloheximide, which block protein synthesis, prevent regeneration in both nucleate and anucleate fragments, reversibly in the former, irreversibly in the latter (Brachet, unpublished). When regeneration occurs in nucleate fragments it is often abnormal and results in bifid or trifid stalks. An ordered synthesis of proteins is thus required for the formation of a cap<sup>14</sup>.

The fact that the formation of the cap is imperfect in anucleate fragments treated with actinomycin despite normal initiation may indicate that the antibiotic combines with the DNA of the chloroplasts. There is much to suggest such an explanation. Radioactive actinomycin administered to the algae *in vivo* can afterwards be detected not only in the nucleus but also in the cytoplasm where it associates, in the main, with the chloroplasts<sup>11</sup>. The latter are known to contain enough DNA to code for several hundred proteins with a molecular weight of 20,000 (refs. 16 and 17). (This chloroplastic DNA has a base composition which is different from that of nuclear DNA (unpublished experiments).) Actinomycin causes definite morphological and physiological lesions of the chloroplasts (changes in ultrastructure<sup>18</sup>) and loss of photoperiodic rhythm, followed by loss of photosynthetic activity itself<sup>19</sup>. Chloroplasts isolated from anucleate fragments of *Acetabularia* are able to synthesize RNA and proteins<sup>20,21</sup>. Addition of actinomycin to isolated chloroplasts strongly inhibits such syntheses, which require light. The synthesis of chloroplastic proteins must therefore take place in the same way as in whole cells (transcription, translation).

Our colleague D. Shephard<sup>22</sup> has observed that the number of chloroplasts increases in the anucleate fragments. They are therefore capable of self-replication in the absence of a nucleus, but they do divide less frequently in anucleate fragments than in whole plants. This, and the fact that abnormal "dumb-bell" forms occur, suggests that the normal pattern of division is disturbed in the absence of the nucleus.

The increase in the number of chloroplasts in anucleate fragments may explain our recent paradoxical observations<sup>23</sup> that DNA can be synthesized in the absence of the cell nucleus. The DNA content of anucleate fragments of *Acetabularia* doubles in 5–10 days and this increase is blocked by a classical inhibitor of DNA synthesis, hydroxyurea. As we have already seen, this is also true for the DNA content. This simultaneous increase in the amount of both nucleic acids present must reflect the autonomous multiplication of the chloroplasts in anucleate cytoplasm.

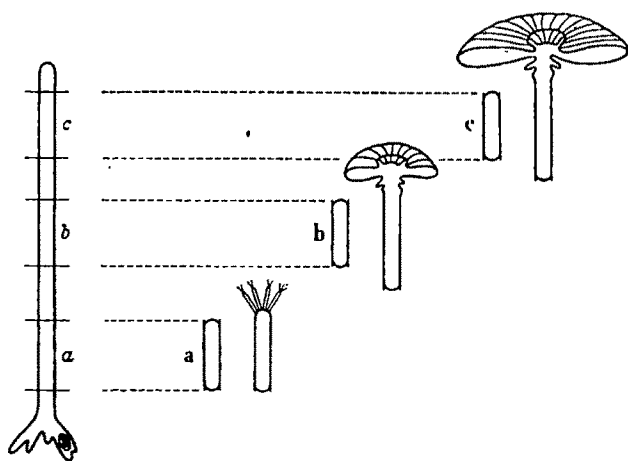


Fig. 2. Diagrammatic representation of the polar distribution of morphogenetic substances in *Acetabularia*.

I have already mentioned that the net synthesis of RNA which occurs in the anucleate fragments takes place mainly in the chloroplasts<sup>7</sup>. Using more refined biochemical methods, and notably the fractionation of algal RNA labelled with phosphorus-32 by column chromatography, Janowski<sup>24</sup> has confirmed the important part played by the chloroplasts in the synthesis of RNA by the anucleate fragments. Three distinct kinds of RNA were found to be labelled in the absence of the nucleus: *s*RNA (which is ubiquitous), as well as a ribosomal type of RNA, and a hybrid DNA-RNA, which are associated with the chloroplasts. These findings strengthen the conviction that chloroplastic DNA and RNA can be synthesized in the absence of the nucleus.

Less is known about the ribosomes which are not associated with the chloroplasts, and which I shall call "true" ribosomes: the ribosomal RNAs seem to disappear from the cytoplasm 2 days after enucleation, although ribosomes are still visible under the electron microscope (experiments are in progress). This would suggest a close nuclear control of ribosomes in *Acetabularia*, as in *Amoeba* (where the *r*RNA of anucleate fragments is reduced by two thirds after 1 week<sup>25</sup>). The base composition of the RNAs extracted from true ribosomes and chloroplasts is, however, very different.

Janowski's recent findings<sup>26,27</sup> that anucleate as well as nucleate fragments are capable of synthesizing polysomes are surprising. Polysome synthesis in anucleate fragments is greatly enhanced after these fragments have been subjected to the dark for 2 days, which suggests that the *m*RNAs involved are probably chloroplastic. According to other recent experiments in this laboratory<sup>28</sup>, nucleolar RNA resembles algal nuclear DNA in base composition (A equal to U, G equal to C), whereas in most other cells nucleolar RNA resembles *r*RNA (rich in G). This invites the speculation that, in *Acetabularia*, informational DNA-like RNA, rather than *r*RNA, accumulates in the nucleolus. This might be the informational RNA which migrates to the tip of the stalk.

In the dark (which interrupts photosynthesis and stops the growth and multiplication of the chloroplasts) the structure and chemical composition of the nucleus are considerably altered. It diminishes in volume, the nucleolus (normally sausage-shaped) becomes spherical and vacuolated, and the RNA content is reduced. All these changes, which result from a decrease in the production of energy by the cytoplasm, are reversed when the algae are brought back into the light<sup>29</sup>. Moreover, in another type of experiment, once the cap has been formed, the nucleus degenerates, the nucleolus disappears and the chromosomes repeatedly divide. After several months of interphase, the big vegetative nucleus enters a phase of intense mitotic activity. It gives rise to numerous daughter

nuclei which colonize the alga and ensure its reproduction. Although nothing is known of the stimulus which causes the destruction of the large vegetative nucleus, we do know that this can be prevented indefinitely simply by cutting the stalk each time the cap is about to form<sup>2</sup>.

The nucleus can thus be "rejuvenated" at will by an operation which only affects the cytoplasm. Some kind of control must therefore be exerted by the cytoplasm on the fate of the nucleus. A biochemical analysis of the nature of this control would be most interesting. It now seems certain that the morphogenetic substances described by Hämmerling<sup>1</sup> are in fact stable informational RNAs synthesized in the nucleus. Undoubtedly the chloroplasts also contribute to the astonishing regenerative capacity of the anucleate fragments. It was thus of interest to compare the situation in anucleate systems lacking chloroplasts, such as the eggs of sea urchins and amphibians.

## Sea-urchin Eggs

As Harvey<sup>30</sup> has shown, unfertilized sea-urchin eggs can be separated into two parts by centrifugation in a density gradient (Fig. 4). Parthenogenetic activation of the anucleate halves thus obtained is followed by repeated but irregular cleavage (Fig. 5). Pseudo-blastulae result, and never differentiate. The developmental capacity of anucleate sea-urchin eggs is thus much lower than that of *Acetabularia* stalks. No authentic morphogenesis occurs. All that can be observed is the repeated replication of the centrosomes, the formation of asters around them, and the laying down of cleavage furrows.

The fertilization of sea-urchin eggs and the concomitant stimulation of amino-acid incorporation and protein synthesis raise interesting problems which can only be summarized briefly here (the recent book by A. Monroy offers a full review<sup>31</sup>). The ribosomes of unfertilized sea-urchin eggs are much less active *in vitro* in the incorporation of amino-acids than those prepared from fertilized eggs<sup>32</sup>. The addition of synthetic messenger (polyuridylic acid, for example) to the ribosomes of unfertilized eggs strongly stimulates the incorporation of the corresponding amino-acid, phenylalanine<sup>33,34</sup>. It would therefore be thought that both fertilization and parthenogenetic activation stimulate the synthesis of *m*RNA by the nucleus, for it would then associate with the monosomes of the unfertilized egg, transform them into polysomes, and participate in the burst of protein synthesis. Such has not proved, however, to be the case. It has indeed been found impossible to demonstrate any synthesis of RNA before the 2-4 blastomere stage, either by autoradiography or by biochemical methods<sup>35</sup>. Moreover, actinomycin fails to inhibit protein synthesis or cleavage of fertilized sea-urchin eggs<sup>36,37</sup>. A study of protein synthesis in anucleate fragments<sup>35,38</sup> has further revealed that, whereas the anucleate fragments of ripe unfertilized eggs scarcely incorporate any amino-acids, their parthenogenetic activation causes a massive, almost instantaneous, burst of amino-acid incorporation into proteins. This stimulation actually proves to be more intense in the anucleate than in the nucleate halves. Thus it cannot result from new synthesis of *m*RNA by the nucleus and the facts require some other explanation.

It has not yet been proved that the proteins formed in the anucleate fragments are the same as those found in the whole fertilized egg, though all available information points to such an interpretation. The degree of incorporation of the various amino-acids varies. Yet the ratio between incorporation of different amino-acids in fertilized and unfertilized eggs is invariable<sup>39,40</sup>. Puromycin blocks the incorporation of amino-acid in both cases. Parthenogenetic stimulation alone therefore seems sufficient to induce the synthesis, by both anucleate fragments and intact eggs, of one or more specific proteins. The biological role of these proteins is unknown.



Monroy *et al.*<sup>41</sup> have shown that in fact unfertilized eggs contain in their cytoplasm a reserve of inert *m*RNA, which is formed during oogenesis. These stable messenger RNAs from unfertilized eggs are able to stimulate *in vitro* the incorporation of amino-acids into the proteins of bacterial ribosomes. But they have no stimulatory effect on the ribosomes of unfertilized sea-urchin eggs. The latter can, however, be activated by pretreatment with trypsin, in which event the addition of *m*RNA from unfertilized eggs is followed by stimulation of the incorporation of amino-acids. The main, if not the only, site of the block in protein synthesis in unfertilized sea-urchin eggs is thus the ribosome itself. The ribosomes cannot associate with the messenger RNAs present in the cytoplasm; they are probably coated with basic proteins which prevent the formation of active polyribosomes.

The idea of a control of protein synthesis at the ribosomal level which was first presented in 1962 (ref. 42) is also supported by experiments by Mano<sup>43</sup>, by Mano and Nagano<sup>44</sup> and by Bell and Reeder<sup>45</sup>.

The activation of protein synthesis in anucleate fragments stimulated by parthenogenetic treatment is not yet fully understood. We know, however, that the activated anucleate fragments contain small quantities of functional polyribosomes, actively engaged in the synthesis of proteins (Burny *et al.*<sup>46</sup>). Like whole fertilized eggs, they therefore possess messenger RNAs which are probably identical with those the existence of which has been demonstrated by Monroy *et al.*<sup>41</sup>. They are probably synthesized much earlier by the nucleus (germinal vesicle) of the growing oocyte. This may be concluded from the autoradiographic findings of Ficq<sup>47</sup> and of Das<sup>48</sup>. The various findings can therefore be summarized as follows: An initial period of intense RNA synthesis in the nucleus is followed, at the end of oogenesis, by a phase of repression. At maturation, the RNA synthesized by the nucleus migrates into the cytoplasm and is probably fixed in a stable form to the ribosomes or analogous particles. The ribosomes themselves are inactivated (or repressed) by their association with a protein, which can be digested by trypsin (probably a basic protein).

Fertilization would thus induce the activation or de-repression of the ribosomes, which would be transformed into functional polysomes. It is not without interest to recall that the fertilization of the sea-urchin egg is followed by a transitory, though intense, increase in proteolytic activity<sup>49,50,51</sup>: this increase might very well be responsible for the "activation" of the ribosomes.

Although it seems likely that the stable *m*RNAs present in the cytoplasm of unfertilized sea-urchin eggs originate in the nucleus, one cannot *a priori* exclude a further possible explanation: these RNAs might be synthesized directly in the cytoplasm, perhaps under the control of cytoplasmic DNA.

It has long been known that the unfertilized sea-urchin egg contains a large excess of DNA, as compared with the quantity of DNA contained by the nucleus<sup>51</sup>. We have shown<sup>40</sup> that this DNA is equally divided by centrifugation between nucleate and anucleate fragments. There is thus no doubt that the excess DNA is localized in the cytoplasm. Cytoplasmic DNA has the same density (and thus the same overall base composition) as nuclear DNA; it has a high molecular weight, it exists in a double stranded form<sup>52,53</sup>, and might possibly control the autonomous synthesis of *m*RNA in the cytoplasm. Such a hypothesis seems, however, to be contradicted by the following data: (1) early protein synthesis is insensitive to actinomycin and therefore does not depend on simultaneous DNA-dependent RNA synthesis; (2) it has so far not been possible to demonstrate any early synthesis of *m*RNA; (3) RNA polymerase cannot be detected in the anucleate fragments of unfertilized eggs<sup>40</sup>.

These arguments seem convincing, but need further investigation. Actinomycin might not combine *in vivo* with cytoplasmic DNA; the latter is probably bound

differently to proteins from nuclear DNA and might have less affinity than the latter for actinomycin. Radioactive actinomycin could be used to check this possibility.

We have observed<sup>40</sup> a slight incorporation of uridine, but partially sensitive to actinomycin, in the anucleate fragments of sea-urchin eggs. It would be interesting to find out whether this is indicative of newly synthesized RNA in anucleate fragments or merely evidence of RNA turnover. The origin and intracellular localization of cytoplasmic DNA in sea-urchin eggs also require investigation.

## Amphibian Eggs

The situation with amphibian eggs closely resembles that of sea-urchin eggs. Anucleate eggs show partial cleavage<sup>54</sup>. The replication of centrosomes, the formation of asters and cleavage furrows occur in the absence of the nucleus. Anucleate fragments can synthesize proteins immediately after activation<sup>55,56</sup>. Radioactive amino-acids are incorporated by anucleate activated fragments just as in normally fertilized eggs. Cleavage, unaffected by actinomycin, is interrupted almost immediately by inhibitors of protein synthesis like puromycin and cycloheximide<sup>57</sup>. Thus, cleavage is possible when transcription is prevented, and must be controlled at the translational level. The initiation of RNA synthesis (mainly *m*RNA and *r*RNA) occurs later in amphibian than in sea-urchin eggs; it only starts at gastrulation as compared with the 2-4 blastomere stage in sea-urchins. We do not as yet know whether parthenogenetic activation causes as explosive a burst of protein synthesis in frogs' eggs as in sea-urchins' eggs.

The localization, composition and biosynthesis of cytoplasmic DNA, however, have been more satisfactorily investigated in amphibians. They contain a greater reserve of DNA than sea-urchins (equivalent to several thousand diploid nuclei as compared with a hundred in sea-urchins). Most of this DNA is associated with slowly sedimenting particles, probably yolk platelets<sup>58,59</sup>.

In the past few years, we have tried to locate accurately the DNA present in the cytoplasm of amphibian oocytes<sup>60,61</sup>. Special methods were required to detect it, because it is destroyed by the strong acid hydrolysis used in the classical Feulgen reaction. This difficulty has been overcome by using 1 normal hydrochloric acid in absolute alcohol (instead of in water) to obtain more tempered hydrolysis, and also by using radioactive actinomycin as a cytological marker for the presence of DNA. The results so obtained have led us to the conclusion that amphibian oocytes probably contain at least two kinds of DNA. One is fairly resistant to acid hydrolysis; it accumulates in the perinuclear cytoplasm of the large oocytes and is found in the hyaloplasm after mild centrifugation; it might be bound to mitochondria. It probably originates in the germinal vesicle, as can be judged from experiments using radioactive precursors (it can be labelled with thymidine, uridine and formate).

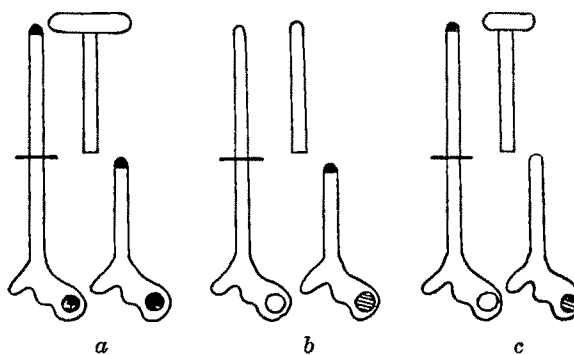


Fig. 3. Schematic representation of the synthesis of *m*RNA by the nucleus and their migration in *Acetabularia*: (a) normal algae; (b) algae treated by ribonuclease; (c) algae treated by actinomycin.

The nuclear sap of amphibian oocytes indeed contains an excess of DNA probably resembling that of the chromosomes<sup>62,63</sup>. This perinuclear DNA is probably of chromosomal origin, and is released—at least in part—into the cytoplasm when, at the end of oogenesis, the germinal vesicle attains its maximum and begins to regress. The other type of DNA seems to be associated with the yolk, is acidolabile and can only be detected with the aid of radioactive actinomycin. It cannot be labelled with thymidine, but accumulates <sup>14</sup>C-formate when this precursor is administered to oocytes. It may be partly exogenous in origin: the follicular cells and the blood plasma may constitute one of the main sources of yolk DNA. Indeed, the injection of heterologous radioactive DNA (extracted from *Escherichia coli* or phage) into the peritoneal cavity of amphibians is followed by the incorporation of radioactive material first into the cytoplasm and then into the nucleus of the oocyte. Almost intact molecules of DNA actually seem to be entering the oocyte (unpublished results of Y. Kong and E. Sempinska).

Perinuclear DNA, which apparently is itself derived from the nucleus, might have a genetic function. Yolk DNA, on the other hand, is more likely to constitute a simple reserve material of lower molecular weight, a supply of the precursors required for DNA synthesis before nuclear multiplication during development.

Dawid<sup>64</sup> has investigated the chemical and physical properties of DNA in amphibian eggs (frogs and *Xenopus*). The resemblance, as regards density and molecular weight, between the cytoplasmic DNA of amphibian eggs and the nuclear DNA of the liver of the same species seems to be fairly superficial. Only 0.1–5 per cent of hepatic DNA is homologous to cytoplasmic DNA. (According to Shmerling<sup>65</sup>, the cytoplasmic DNA of sturgeon eggs has a molecular weight of 800,000 and a high degree of homology with the DNA of spermatozoa.) The latter small fraction might correspond to the perinuclear (mitochondrial) DNA of the oocyte. But the homology found between a minute fraction of amphibian egg DNA and adult chromosomal DNA poses, on the other hand, the problem of the biological significance of the cytoplasmic DNA in eggs.

It is as yet impossible to decide whether the cytoplasmic DNA of these eggs can be synthesized autonomously in the absence of the cell nucleus: in the anucleate fragments of the sea-urchin, we have observed a slight incorporation of thymidine under conditions in which every possible precaution had been taken to reduce bacterial contamination to a minimum. A short note by Mezger-Freed<sup>66</sup> affirms that the DNA content of anucleate unfertilized frog eggs may double in a few hours. This work is now being repeated in our laboratory.

The complexity of the problems raised by the presence of cytoplasmic DNA in amphibian eggs appears even

greater when one takes into consideration the observations we recently made on the behaviour of DNA during the maturation of the oocyte.

The maturation of toad or of *Xenopus* oocytes can be obtained *in vitro* by placing the large oocytes in Ringer solution containing minced hypophyses<sup>67</sup>. This technique has the advantage of making it possible to follow the action of the hormone on an isolated oocyte. After treatment for 3–4 h, the nuclear membrane presents, on the vegetative side, many invaginations extremely rich in RNA. Simultaneously, the nuclear sap becomes basophilic: according to preliminary electron microscope studies, this basophilia seems to be caused by the synthesis of ribosomes, or an invasion of the nuclear sap by the latter. These changes probably correspond to real synthesis of RNA. Brown and Littna<sup>68,69</sup> have shown by biochemical techniques that appreciable quantities of heterogeneous RNA (presumably mRNAs) are synthesized during the maturation of *Xenopus* oocytes. Extensive protein synthesis also occurs during maturation<sup>70</sup>; it is inhibited by actinomycin and puromycin (ref. 71 and personal observations).

When the nuclear membrane begins to break down, a number of Feulgen-positive granules appear at the site of the disintegration of the nuclear membrane. DNA synthesis, which is evidenced by considerable incorporation of thymidine into the breaking down germinal vesicle (unpublished), thus follows very closely the synthesis of RNA.

After treatment with hormone for 7–8 h, the Feulgen-positive particles collect at the animal pole and usually assemble in the cortex to form two or three large inclusions which are intensely coloured with the Feulgen reaction. There is no doubt that these cortical grains contain much more DNA than the chromosomes.

The part played in development of the DNA which forms in the cytoplasm when, during maturation, the nuclear sap and the cytoplasm mix, after having been separated for 2 or 3 years by the nuclear membrane, is not yet known. The fact that this DNA is found in the cortical granules raises the question of whether it accumulates in the "grey crescent" after fertilization of the egg. The removal of the cortex of this grey crescent is known to stop normal morphogenesis (only cleavage is possible after this operation), while the grafting of a supplementary dorsal cortex into the ventral half of the fertilized egg results in the formation of double embryos (Siamese twins<sup>72</sup>). It seems likely that, as I have suggested<sup>73</sup>, the dorsal cortex of the fertilized egg controls derepression (that is to say, RNA synthesis) in the nuclei of the dorsal lip of the blastopores (organizer).

The existence in the dorsal cortex of amphibian eggs of particles containing DNA may explain the very curious experiments recently made by Curtis<sup>74</sup>; his results, which show that the cortex possesses its own heredity, are difficult to explain except by admitting the presence in the dorsal cortex of particles capable of self-replication. Perhaps these are the DNA particles which we saw appearing in the cytoplasm after interactions between the nucleus and the cytoplasm at the time of the maturation of the oocyte.

One may also ask whether the cytoplasmic DNA of nuclear origin plays a part in determining the fate of the germinative plasma; in fact, ultra-violet irradiation of the vegetative pole of amphibian eggs is known to provoke sterility in the irradiated embryos<sup>75</sup>.

There is therefore no lack of theories concerning the hypothetical role of cytoplasmic DNA in amphibian eggs. For the time being, however, there is a scarcity of conclusive facts; fortunately both biochemical and cytochemical methods now exist which may enable these problems to be solved.

It is worth drawing attention to some analogies between amphibian eggs and *Acetabularia*. The proportion of nuclear to cytoplasmic volume controls the entry of the

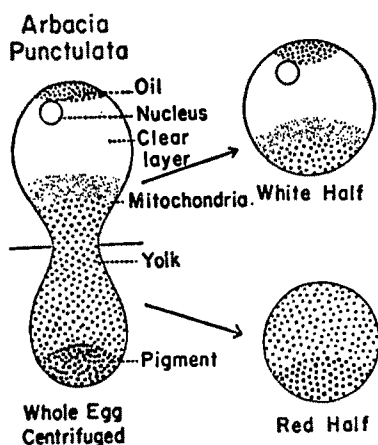


Fig. 4. Ultracentrifugation of unfertilized sea-urchin egg (E. B. Harvey, 1932).

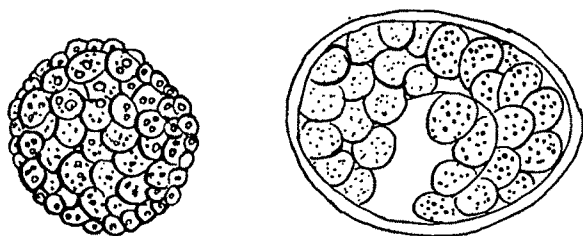


Fig. 5. 13 h non-nucleated morula and 3-day non-nucleated blastula (E. B. Harvey, 1936).

big *Acetabularia* nucleus into mitosis. When the cytoplasm reaches a certain volume, it seems to repress the synthesis of nuclear RNAs (transcription) in favour of DNA synthesis (replication). Treatment of amphibian oocytes with hypophyseal hormones interrupts, after a brief period of stimulation, the synthesis of nuclear RNA. The synthesis of extrachromosomal DNA begins at just that moment. The control mechanisms repressing the synthesis of RNA and inducing the replication of DNA thus come into force during maturation. They continue to function throughout cleavage, which is characterized by repeated multiplication of the chromosomes in the absence of any considerable synthesis of rRNA or mRNA<sup>67,76,77</sup>.

Experiments by Gurdon and Brown<sup>78</sup> and by Graham *et al.*<sup>79</sup> show that the cytoplasmic mechanisms regulating the synthesis of the two nucleic acids also come into force when adult nuclei are introduced into unfertilized, activated and enucleated eggs: if these nuclei were synthesizing rRNA, they would stop doing so after transplantation. If nuclei from adult organs no longer synthesizing DNA are injected into activated and enucleated unfertilized eggs, they immediately start to incorporate thymidine.

Thus, it is certain that the state of the cytoplasm of the eggs regulates the beginning of their development and the orientation which the synthesis of nucleic acids (DNA and RNA) is going to take. Repressors, which prevent the synthesis of RNA on the matrix of DNA, must exist in the cytoplasm of the egg. Their presence does not affect the synthesis of DNA or of proteins. In sum, during the preliminary stages of development, the transcription of new genetic messages does not take place; on the other hand, those which already exist are transcribed whereas the DNA replicates itself. Such repressors as those hypothesized here have yet to be identified. It is interesting, however, to note that Yamana *et al.*<sup>80</sup> have recently shown the presence, in blastulae of *Xenopus*, of a specific inhibitor of ribosomal RNA synthesis.

Embryology is undoubtedly moving toward new "molecular" horizons. Uncertainty as to the role of cytoplasmic DNA, stable messenger RNA and ribosomes will be dissipated in the coming years. The future will also confirm whether or not, as we suggested in 1962 (ref. 42), the ribosomes of the egg play a less passive part than that usually attributed to them, whether they intervene directly in the reading of the genetic message and whether one of the factors of embryological differentiation is implicit in a competition within a heterogeneous population of ribosomes. Whatever the answer to these questions, molecular embryology is clearly moving ahead with new impetus, and is now ready to solve the great problems raised by experimental embryology: preformation, epigenesis, regulation, induction, and so forth.

I thank Dr. P. Malpoix for her translation of this text into English. The experiments from my laboratory received financial support from Euratom and the Centro nazionale delle Ricerche.

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## BOOK REVIEWS

### HAPPENINGS ANALYSED

#### The Statistical Analysis of Series of Events

By D. R. Cox and P. A. W. Lewis. (Methuen's Monographs on Applied Probability and Statistics.) Pp. viii + 285. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1966.) 50s. net.

THE times of occurrence of accidents, of computer failures, and of the pulses in a nerve fibre are among the phenomena that give motives for the theory in this book and provide examples of its application. For the phenomena considered, stochastic models, based on the idea of point events occurring in a haphazard way in space or time, can be usefully employed. It is towards the problems of statistical inference about these stochastic point processes that the book is primarily directed.

Books about the probability theory of stochastic models have proliferated in recent years, but the subject of inference about these models has been much less widely covered. This is partly because in current research the building of the models has run ahead of the statistical analysis of the situations to which they may apply. Nevertheless, a good deal is now known and gratitude is due to the authors for making much of it available in a single volume. This volume is the largest so far to appear in Methuen's series of monographs, and the reason is that although the authors' primary purpose is to deal with statistical inference, they find it necessary to present substantial accounts of the probability theory of many of the processes as well. The completeness that this gives certainly justifies the extra size of the volume, and will make it a useful tool in the hands of anyone who has to deal with problems involving stochastic processes of point events.

The introduction of the book includes several problems from various fields of application, and this is followed by a consideration of the Poisson process, which illustrates a number of the points that have to be dealt with in handling more complex processes, and of course these often arise when one observes a departure from the Poisson characteristics of "pure randomness" in a series of point events. Regression analysis and other methods of dealing with a trend in the series are covered in Chapter 3, and the rest of the book deals with stationary processes. The chapter on stationary point processes gives general results including forward recurrence times and some consequences of the relation between counts of events and times between events. The estimation of second-order properties of stationary processes which follows includes spectral estimates, and estimates of first and second moments, for intervals between events, and counts of events. The sixth and seventh chapters cover renewal processes, some related significance tests and generalizations of renewal processes. Some models useful in textile technology, computer failure studies, and road traffic movement appear here. Superposition of processes concerns the pooled output of several series of simpler form, and an application to nerve pulse data. In the comparison of rates of occurrence in Chapter 9, applications to accident rates and failure of air-conditioning equipment and computers are dealt with. Finally, there are some generalizations, where a brief introduction to events in several dimensions and events of more than one type is given.

The book ends with several appendixes, including some data for models which have been discussed in the text, and a set of thirty-eight exercises and further results. It will be useful to workers in numerous fields of application, and essential to any student of stochastic processes and their statistics, for whom at present-day prices it is good value at 50s. W. A. O'N. WAUGH

### PROPULSION WITHOUT WHEELS

#### Propulsion Without Wheels

By E. R. Laithwaite. Pp. x + 273. (London: The English Universities Press, Ltd., 1966.) 63s. net.

To anyone previously unaware of the author's interests the title may be rather misleading. Only electromagnetic means of propulsion are considered, and, although the emphasis is very much on linear machines, propulsion is by no means the only, or even the major, topic. The main limitation on subject matter seems to have been that it should be both interesting and unusual, and the applications described range from levitation to impact extrusion.

The machines considered present some of the most difficult analytical problems that an electrical engineer could expect to encounter. Yet with a minimum of mathematics and with considerable use of simple sketches and diagrams, the material is generally presented so clearly and carefully that even readers with only a rudimentary knowledge of electricity and magnetism should be able to follow most of it. At the same time some of the phenomena described are so intriguing, and the author's approach so stimulating, that even the more knowledgeable reader will find the book interesting.

For many the most fascinating parts of the book will be those which deal with the working models that Professor Laithwaite has demonstrated on television. Not only are the various experiments described, but very full design and constructional details of the models are given at the end of each chapter. Professor Laithwaite states in the preface that his aim in writing the book was not only to inform about linear motors and their application, but also to help science teachers and amateur experimenters to design and build good working models of these. The book fulfils these aims admirably and must surely arouse yet more interest and enthusiasm for this subject which the author finds so fascinating. R. BROWN

### HYPERSONIC FLOW

#### Fundamental Phenomena in Hypersonic Flow

Edited by Gordon J. Hall. (Proceedings of the International Symposium sponsored by Cornell Aeronautical Laboratory.) Pp. viii + 354. (Ithaca, New York: Cornell University Press; London: Oxford University Press, 1966.) 100s. net.

As part of the centenary celebrations of Cornell University, the Cornell Aeronautical Laboratory sponsored a symposium on hypersonic flow in June 1964. The present volume contains the thirteen invited lectures as well as comments by appointed reviewers and discussions from the floor. The organizers are to be congratulated for bringing together such an array of talent in the field. The contributions are all of high standard and pleasantly combine critical review material with new results covering the major branches of the field of hypersonic research.

H. J. Allen reviews the aerodynamic heating problems of re-entry and shows how careful observations of meteors can provide information at entry speeds beyond those at present attainable in earth-bound experimental facilities. J. A. Fay reviews heat transfer theories in the velocity range 10–20 km/sec and extends them to obtain estimates at higher speeds. Van Dyke "revisits" the blunt-body problem and demonstrates that it is possible to improve the analytical methods and, thus, to use computers more efficiently. M. P. Guiraud contributes further to the controversy between himself and R. Vaglio-Laurin about asymptotic expansions for power law bodies.

Viscous flows are discussed by H. K. Cheng, who presents work on slip effects and shock structure of the bow wave and obtains flow patterns at low Reynolds numbers, and by N. C. Freeman, who looks at the diffusion of species

in hypersonic boundary layers, obtains a general solution as a Mellin transform and investigates special cases. Three papers are concerned with relaxation effects and chemical kinetics. A. G. Gaydon reviews methods of temperature measurement with emphasis on the sodium-line reversal method and presents new experimental results. H. W. Palmer reviews chemical kinetics problems which can be studied in the shock tube, and M. Camac presents a thorough investigation of the structure of shock waves in carbon dioxide in the Mach number range 5–25.

R. Goulard contributes to radiation gas dynamics by exploring the analogy between radiation transfer and the neutron-flux problem. In the field of rarefied gas dynamics S. F. Shen develops a method for handling flows with solid boundaries based on hydrodynamic equations in which the Navier–Stokes stress-strain relations and the Fourier heat conduction law are replaced by more general expressions. Y. S. Pan and R. F. Probst discuss the flow past a flat plate and the transition from ordinary continuum boundary-layer flow far downstream to almost free-molecule flow near the leading edge. In the last paper L. Lees and H. Gold study the instability of laminar wakes. Theoretical calculations are compared with experimental results on transition to turbulence.

Because of the time lapse between the symposium and the publication of the proceedings most of the new results presented will now be well known to active research workers who will nevertheless undoubtedly find the volume useful for reference. It should also serve almost as a text-book for newcomers, in particular those who prefer short stories to the full-length novel as contained in the ordinary text-book. The editor and Cornell University Press have done a splendid job. Rarely are the proceedings of a symposium presented as attractively as in this case.

N. H. JOHANNESSEN

## WELSH NATURALISTS

### The Snowdonia National Park

By W. M. Condry. (The New Naturalist: a Survey of British Natural History.) Pp. xvii+238+28 plates. (London: William Collins, Sons and Co., Ltd., 1966.) 30s. net.

THIS is the forty-seventh book issued in Collins's main "New Naturalist" series—the most impressive publishing feat in the biological field in modern times—and the sixth of the series to deal with a region. One of the previous five, indeed, was also about Snowdonia, so that it must be said at once that this is an entirely new book by a different author. It has all the merits to be expected from a book by a single author and is in many ways a model of how a regional natural history should be written.

The author lives just outside the southern boundary of the region, and is clearly thoroughly familiar with it. The boundaries are those appointed by the National Parks Commission, and run south from Conway to the River Dovey, and from the Berwyns in the east to Moel Hebog and the Snowdon massif in the west. Here, especially on either side of Llanberis Pass, is some of the classic ground of British natural history. The Merioneth part of the park is much less widely known, and this part of the book is therefore the more valuable.

The wildlife riches of Snowdonia make it the most important single region in Wales, and almost the most important upland region south of the Highland Line—but how can one in any useful sense grade in importance three such splendid mountain areas as the English Lakes, Upper Teesdale and Snowdonia? It has one unique British plant, *Lloydia serotina*, which is found nowhere else nearer than the Alps, a good many other alpine in their most southerly British sites, an endemic fish in the gwyniad *Coregonus pennantii*, choughs breeding inland and several

feral herds of goats. The fine dune systems of its Merioneth coastline are especially rich in plant life, and its hanging oakwoods are also of the greatest importance in maintaining the diversification of wildlife which is one of the chief fascinations of the British countryside.

A chapter on conservation deals faithfully with the Nature Conservancy and its fourteen national nature reserves (seven woodland, five mountain and two dune), but evidently must have been written before the foundation of the North Wales Naturalists' Trust, which is not mentioned. Important as the conservancy undoubtedly is, the task of preserving the rest of Snowdonia's important habitats will fall largely on the trust, which already has three small reserves in the area.

Three of the dozen national parks in Britain now have their New Naturalist volume—the others are Dartmoor and the Peak District—and it is much to be hoped that the editors of the series will succeed in producing volumes of equally high standard for the remainder. With such a guide in his hand, the naturalist will double or treble the pleasure and knowledge he gains from a visit to a national park.

R. S. R. FITTER

## POLISH PLANT LIFE

### The Vegetation of Poland

Edited by W. Szafer. (International Series of Monographs in Pure and Applied Biology, Division: Botany, Vol. 9.) Pp. xxiii+738. (London and New York: Pergamon Press, Ltd.; Warsaw: PWN—Polish Scientific Publishers, 1966.) 120s.

THIS book is a translation from the Polish of *Szata roślinna Polski*, and is a symposium produced by eight workers at Cracow edited by the professor of botany in the Jagellonian University. Two chapters of the original work, dealing with detailed descriptions of the Polish lowlands and mountains, are omitted from the translation as being of less general interest. This is a pity, because in a work specifically devoted to the vegetation of Poland one looks especially for Polish particularities.

For a summary of the contents one can hardly do better than quote from the preface: "A brief outline of the history of phytogeographic research in Poland is followed in natural sequence by chapters dealing with factors affecting the geographical distribution of plants and, separately, with the influence of man and his economic activities . . . and with the role played by other biotic factors on the vegetation and flora. Next we have information concerning the geographical and historical elements of the Polish flora and its statistics. Then there are two chapters . . . giving a survey of plant communities on land and in the Baltic Sea. The following chapters deal with the history of the origin and development of the Polish vegetation and, separately, with the history of the more important cultivated plants. The final chapter presents analyses of the vegetation of Poland, descriptively and cartographically."

It is one of the peculiarities of some of the eastern European countries, and perhaps of Poland in particular, that botanical investigation has become centred on phytogeography and ecology; in most countries in western Europe, largely because of an army of enthusiastic amateurs, preoccupation with taxonomy has produced a notable series of systematic floras which are largely lacking in Poland. The present work demonstrates that there are historical reasons for this, apart from the transitional place occupied by Poland between the varied topography and geology of western and southern Europe and the monotonous plains of the east and north-east—a fact which provides opportunities for many interesting studies in species with their easterly and westerly limits of distribution in Poland. This phytogeographical trend is continued and emphasized in the present work. A vast amount of information on Polish phytosociology, geology,



climate, soils and most relevant subjects is presented in a lucid and readable manner, freely illustrated with species and habitat photographs, maps (a schematic geological map is particularly useful), profile diagrams and all the necessary clarifying media. A large and detailed map of the geobotanical division of Poland is contained in a pocket in the back cover, and is printed with exemplary clarity. The translation is wholly admirable except in one or two instances where presumed literal translations of vernacular names occur. For example, on p. 580, *Pinus cembra* L., of which the universally employed English name is "arolla pine", is referred to as the "stone pine"—a name equally universally applied by English speakers to *P. pinea* L.

The space occupied by the various sections is somewhat uneven—for example, the review of terrestrial and freshwater communities, much of which will contain little new to most ecologists, occupies almost one third of the entire book; on the other hand, that of the vegetation of the Baltic is one of the smallest, even allowing for the obvious limitations here. Although there are some references to bryophytes, lichens and fungi, it is unfortunate that cryptogams do not feature more in this book, because in any community no one plant is more significant than any other. Perhaps this limitation is a function of a lack of Polish taxonomists! It is the more regrettable because some species, for example the moss *Tortula velenovskyi* Schiffn. as shown by Waclawska, have as interesting a "Central European" distribution as any flowering plant.

One of the most interesting chapters is that concerned with man's influence on plant life, dealing as it does with the retreat of many interesting species before his usually savage over-exploitation of the land and the influx of introduced, mainly weedy species in his wake. The development of crops is less satisfactory because, although the author of this section discusses the possible wild origins of various cereals, there is no mention of current ideas about the origin of *Triticum aestivum* L. emend. Fiori et Pal. (the correct name of *T. vulgare* Vill.) as a product of *Triticum monococcum* L., *Aegilops speltoides* Tausch and *A. tauschii* Coss., or of the very interesting discussion in recent years concerning the ancestry of the six-rowed barley.

This thoroughly commendable book closes with an exhaustive bibliography, frequent references to which considerably enrich the text. C. C. TOWNSEND

## LIMB ISCHAEMIA

### Pathology of Limb Ischaemia

By J. Henry Dible. (Pathological Monographs, No. 3.) Pp. vii+100. (Edinburgh and London: Oliver and Boyd, Ltd., 1966.) 57s. 6d. net.

A DISORDER of such commonplace occurrence as limb ischaemia is one which lends itself to exploration in depth and is thus an ideal subject for a monograph such as this. The other factor which contributes even more to the undoubted success of this book is the life-long enthusiasm of the author for his subject—an enthusiasm which he succeeds in communicating to the reader.

In his preface, Professor Dible relates how he devised an improved technique for injecting limb arteries with a radio-opaque mass, as a result of an abortive investigation into gas gangrene, planned during the Second World War. This technique he used in subsequent years to study spontaneous limb ischaemia caused by thrombosis, atherosclerosis and Buerger's disease, and this book sums up his observations. The author sets the stage with three opening chapters which deal with fundamental matters, such as arterial structure, arterial nutrition and the vasa vasorum, and age changes in arteries. Here the reader is quickly made aware of the many points of uncertainty in our basic knowledge, often glossed over in

pathology textbooks. There follows a detailed consideration of atherosclerotic occlusive disease and thromboangiitis obliterans. The numerous excellent illustrations include radiographs of injected limbs, photomicrographs and diagrams, which illustrate clearly the system of arterial arcades found in limb arteries. It is this anatomical arrangement which explains why gangrene is a late development, even where multiple arterial obstructions are present. The relative freedom from disease of the peroneal artery is one of the many interesting facts brought to light in these investigations.

By his painstaking studies, Professor Dible has done much to narrow the field of ignorance, and this book will be of interest to many besides pathologists and vascular surgeons. The book is well produced, but it is a pity that it is so highly priced. A. G. STANSFELD

## LIVES OF SCIENCE

### Asimov's Biographical Encyclopedia of Science and Technology

By Isaac Asimov. Pp. x+662. (London: George Allen and Unwin, Ltd., 1967.) 63s.

HERE is a chronological sequence of biographies of men of science from ancient Egypt to the space age. Some of the subjects seem to have been chosen less for their science than for their fame; the meaning of science is being stretched to include anything connected with the advance of knowledge and with technical achievement. One might expect to find the eighteenth century theatrical designers and the cloud machines here if there had not been so many of them. The subject of the first entry in the book is Imhotep, the Egyptian scholar, whose dates seem to be uncertain, but who flourished between 2980 and 2950 B.C. He was probably the architect of the earliest of the pyramids and was well known as a man of healing. By Ptolemaic times he had been deified, an honour to science which has not been repeated. The Greek and Roman philosopher-scientists and engineers receive ample mention; and Tsai Lun, the supposed Chinese inventor of paper, is celebrated as perhaps the only eunuch to claim a key position in the history of science. With the end of the classical era and the coming of the dark ages, learning was maintained by the monks, such as the Venerable Bede, who compiled his history of early Anglo-Saxon England largely from the writings of Pliny. Bede was also concerned in the controversy about the dating of Easter.

There is plenty more such detail in this book, which is intended, as the preface implies, as a history of science, told biographically. Its value seems to be limited—although writers of obituaries might glean some useful information from the later entries—when critical biographies of scientists abound. A disjointed collection of personal histories such as this is surely not the most interesting way of presenting a comprehensive history of science. MARY LINDLEY

## MAN AND INSECTS

### Insects and Hygiene

The Biology and Control of Insect Pests of Medical and Domestic Importance. By J. R. Busvine. Pp. xi+467. (London: Methuen and Co., Ltd., 1966.) 100s. net.

THE greater part of this book is a factual account of the biology and control of the insects and allied arthropods likely to be encountered in association with man—as parasites, for example, lice, bed-bugs, fleas and mites; as vectors of disease, for example, houseflies and mosquitoes; as pests of his food such as grain weevils; or in his home,

for example, furniture beetles and clothes moths. A smaller initial section serves to introduce the subject of entomology with the structure, classification, anatomy, physiology and ecology of insects all dealt with at some length. In between the two sections of the book are three important general chapters concerned with all the different aspects of preventive and control measures, including legal and commercial aspects.

The numerous insecticides available today are described in some detail with chemical formulae, formulations and methods of application all given prominence. Resistance to insecticides and concern about the widespread contamination of man's environment with stable toxic chemicals has led in recent times to consideration of other methods of control. Some of these involving genetical manipulations and the release of sterilized insects are referred to, as well as the use of parasites and predators.

This is the second edition of a work first published in 1951 and then aimed at those concerned with domestic hygiene in Great Britain. Now it is considered equally applicable to most of the temperate regions of the world. With only a little more elaboration it could have been made applicable to the tropics as well. Already many references are made to the importance in the tropical hygiene field of many of the pests dealt with, and it can be argued that insect pests are hardly of sufficient consequence in public health considerations in temperate climates to warrant a book as detailed and well presented as this one.

G. DAVIDSON

## FIBRE SCIENCE

### Man-Made Fibres

By R. W. Moncrieff. Fourth edition. Pp. ix + 742. (London: Heywood Books; New York: John Wiley and Sons, Inc., 1966.) 75s. net.

THIS impression of *Man-Made Fibres* carries on the general pattern of its preceding editions in standard and presentation. With the passing of time the information available about man-made fibres is increasing, and so is the number of man-made fibres available. Hence the volume of the publication is increasing, although this second impression contains only slight variations on the 1963 edition.

The great virtue of this publication is the uniformity of the standard of presentation. The work purports to be a general description of man-made fibre manufacture and properties, and the early chapters which deal with the fundamentals of "fibre science" are simply presented. X-ray structure is given an elementary treatment, as is viscosity of polymer solutions and also the general physical properties of fibres. The techniques of infra-red and electron microscope studies of fibres might have had some mention in a new edition, and this could well have applied also to chromatography and other new chemical and physical techniques.

It is unlikely that any normally used man-made or regenerated fibre is not mentioned and a greater or lesser description of its properties given. Allied groups of fibres are treated chapter by chapter, and in an appendix table the known trade-names of various yarns are given. At the end of each chapter an additional reading list is given which is probably of great value. As a work of reference the book suffers from the fact that no adequate reference system is introduced. The text is almost the sole source of information, but the information is voluminous. Having passed through numerous editions since its introduction, there is little doubt that it is finding increased use in textile circles, both industrial and academic. Provided, therefore, that the reader is not looking for extensive reference coverage, the book can be recommended to all sections of interest in man-made fibres.

F. H.

## ADVANCES IN THE ANALYTICAL SCIENCES

### Analytical Chemistry

Vol. 4. Edited by Carl E. Crouthamel, D. C. Stewart and H. A. Elion. (Progress in Nuclear Energy, Series 9.) Pp. 166. (London and New York: Pergamon Press, Ltd., 1966.) 70s. net.

THIS, the fourth volume in the *Analytical Chemistry* series of *Progress in Nuclear Energy*, contains four useful review articles, two of which might be said to be on the periphery of the subject and two nearer to the main area of interest.

The first article, on solid state charged particle detectors, by N. J. Hansen, is one of the peripheral type but nevertheless is valuable in that it gives a broadly based review of the solid state and nuclear physics as well as of the electronics theory behind the use of these detectors in nuclear spectroscopy. The applications are described only briefly and, as the title indicates, are limited to the detection of alpha particles, deuterons, protons, heavy ions, electrons and fission fragments.

Advances in neutron activation are discussed by V. P. Guinn in the second article, which though short (only fourteen pages) really does describe advances made in the period 1958-64 in this now widely used method. It discusses improvements in equipment, that is neutron sources, pulse-height analysers and counters; advances in technique, and new applications in a range of industry and science including scientific crime detection and space studies. Though very good in what it covers, the article was presented at the Geneva Conference in August and September 1964, and describes only those advances made in the United States. It seems a pity that what was quite properly presented as a national contribution to a United Nations conference is now given permanence two years later in a series which prides itself on its international and up to date coverage.

Slow electron interaction with adsorbed gases is discussed by D. Lichtman and R. B. McQuistan in the third, and the other "peripheral", review. It describes one approach to the problem of providing information about the surfaces of materials, especially metals, by bombardment with electrons (and possibly photons and ions) in conditions where the atoms, molecules and ions desorbed during the bombardment can be identified by mass spectrometric analysis. The examination of surfaces is a very important area of growth in the analytical sciences, and the article is very useful in giving information about one approach to the problems.

Use of neutron generators in activation analysis is the subject of the fourth review by J. E. Strain. This again is quite short (twenty pages) but is valuable and realistic. After describing the general requirements and how these are met, several examples of neutron generator application are given, by element, with some excellent practical information on sensitivity attainable, interferences, advantages and disadvantages. This article will be of much interest to the increasing number of industrial users of neutron generators, though unfortunately it does not cover the fairly recent sealed-tube generators where shielding can be so much easier.

Altogether a useful assembly of topics, the present volume continues to show the wide range of interests of modern analysis.

A. A. SMALES

**ERRATUM.** In the review by Dr. H. Lehmann last week (*Nature*, 213, 552; 1967) of Georges Sandoz's *Serum Proteins in Health and Disease*, lines 4 and 5 of the first paragraph should read "that there are few people left who—like the author—span the history from the past to the present day". In the printed version, "like me" was unfortunately substituted for "like the author".

## OBITUARIES

### Professor Sir Thomas M. Cherry

SIR THOMAS CHERRY, who died in Melbourne on November 21, 1966, at the age of 68, played a leading part in university affairs in Australia for more than thirty years. He was widely known and respected as the country's most distinguished mathematician and a man of keen insight and unusual force of character.

After taking his first degree at Melbourne he studied at Cambridge and obtained both his Ph.D. and a research fellowship at Trinity College in 1924. During the period 1922–28 he worked on the theory of ordinary differential equations, and gained a mastery of the subject which was later transmitted to generations of undergraduates. He made a number of significant contributions in particular to knowledge of the properties of Hamiltonian systems of differential equations and of their solution curves. This subject of research suited him well; it demanded precision of thought, skill with mathematical analysis of the kind which leads to general results, and a taste for the austere beauty of "rational mechanics", all of which he could supply. In 1929 Cherry was appointed professor and head of the Department of Mathematics at the University of Melbourne, and the central phase of his life began. For the subsequent thirty-four years he devoted himself unsparingly to the teaching work of the department. The modern means by which a head attempts to improve and extend the teaching provided by his department is to press for more staff; Cherry's way was to do much of the teaching himself and (unconsciously) to inspire his staff by his own high standards of thought and discipline. He gave lectures on many topics, in both pure and applied mathematics (which for him were not divided); epsilon-analysis, projective geometry, celestial mechanics, hydrodynamics, whatever was needed, and at all levels. He had a dry manner and lacked small talk, but the clarity of his exposition, the breadth of his knowledge of mathematics and mathematical physics, and his obvious integrity made an indelible impression on the many honours mathematics students who passed through Melbourne in this period. In accordance with his belief that teaching was his primary task, Cherry also served as chairman of the Mathematics Committee of the Schools Board for twenty-three years, when he was responsible for the mathematical syllabuses in secondary schools, and did much in support of the Mathematical Association.

During the war Cherry acted as a consultant to several government establishments, and his contact with the Aeronautical Research Laboratories generated an interest in gas dynamics which led to two further sequences of papers. One of these sequences dealt with asymptotic expansions for functions occurring in gas dynamics. The other was concerned with the use of the hodograph equation and a family of exact solutions of two-dimensional flow of which a part may be supersonic. Such problems of transonic flow are very difficult, but his remarkable mastery of analytical methods enabled him to make a number of decisive contributions. Cherry never sought recognition of his research, but these papers on gas dynamics were in the main stream of current work and he was elected to the Royal Society of London in 1954.

Further recognition of his high standing in Australian scholarship came with his appointment in 1956 as foundation President of the Australian Mathematical Society and in 1961 as President of the Australian Academy of Sciences,

and again in 1965 when he was knighted. Cherry retired at the end of 1963, and was appointed emeritus professor of applied mathematics. He was still exceptionally vigorous, and everyone expected that the years of retirement would yield much creative thinking and writing, in addition to active work on the council of the new Latrobe University, but they were cut short by heart trouble. The value of his professional work and his contributions to science cannot be measured by his list of publications. He knew much more than he ever wrote, and his influence was transmitted more by personal contact than by the printed word. That it was a unique and powerful influence any mathematician from Melbourne will testify.

G. K. BATCHELOR  
E. R. LOVE

### Professor A. D. Ross

ON December 14, 1966, at Albany, Western Australia, Professor Alexander David Ross died peacefully after a short illness at the age of 83.

Born in Glasgow in 1883, Ross had a distinguished career at the University of Glasgow, where in 1905 he was Thompson Research Fellow. In 1906 as Houldsworth Research Fellow he studied at the University of Göttingen, and in 1908 was appointed lecturer in natural philosophy at Glasgow. His research interests were in the fields of magnetism and spectroscopy. In recognition of his researches the University of Glasgow in 1914 awarded him the Kelvin Medal, of which he was the first recipient.

On the foundation of the University of Western Australia, Ross was appointed in 1912 to the chair of mathematics and physics. He arrived in Perth to take up duties early in 1913, and in that year classes commenced. When in 1929 mathematics and physics were separated he took over the chair of physics until his retirement in 1952. Except for a visit to Europe during the First World War and again in 1951, Ross gave uninterrupted service as teacher, scientific investigator, consultant and administrator over a period of nearly forty years. He was the last in office of the eight foundation professors. By his students he will be remembered particularly as a teacher, clear in exposition, thorough in treatment and considerate to all who were zealous in their aspiration to academic distinction.

Professor Ross played a distinguished part in building up the university tradition in Western Australia as professor of mathematics and as professor of physics, and in the numerous posts he has held in scientific and other organizations within and without the university. He and other professors, coming from abroad in the early days of Australian universities, had the distinction of being agents for the transmission of higher educational standards and sound cultural traditions to the state of Western Australia.

The wide range of his scientific interests in Australia is indicated by the many posts which he held at various times during the occupancy of his chair at the University of Western Australia. These posts included terms as president of the Australian Branch of the Institute of Physics and the Royal Society of Western Australia; he was local secretary of the Australian and New Zealand Association for the Advancement of Science, and chairman of the Western Australian Division of the National Research Council. Ross always lived an active life, and carried over into retirement some of his responsibilities, particularly his association with the Pan-Indian Ocean Science Association, of which he was founder and honorary secretary, and with the National Association of Testing Authorities. During the Second World War, Ross was a member of the Commonwealth Munitions Panel, deputy director of camouflage for Western Australia and consulting physicist to the Royal Australian Navy. He was made a C.B.E. in 1949.

JOHN SHEARER

# Palmer Ridge: a Section through the Upper Part of the Ocean Crust?

by

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Palmer Ridge, part of the Kings Trough feature, N.E. Atlantic, consists of metamorphic and igneous basement with a cover of Tertiary sediments uplifted and intruded by a mass of serpentinite. The basement was formed about 60 million years ago at the mid-ocean ridge crest, and the uplift of the ridge and opening of Kings Trough occurred about 27 million years ago.

FOURTEEN successful dredge hauls were made in 1965 and 1966 from R.R.S. *Discovery* of the National Institute of Oceanography in a small area ( $18 \times 18$  km) of Palmer Ridge, an element of the Kings Trough feature in the N.E. Atlantic. This article gives a preliminary account of the rocks recovered and a possible explanation of the geological structure of Palmer Ridge.

Kings Trough<sup>1</sup> is a feature north of the Azores about 200 miles long running approximately N.W.-S.E. It consists for the most part of a central depression flanked by ridges on either side: the seamount Ant-Altair forms part of the southern ridge. At the S.E. end, around  $42^\circ$  N.,

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$20^\circ$  W., two deeps, Peake and Freen Deep, are found, flanked by small ridges to the north and south and separated by a ridge rising to about 1,300 fathoms which is Palmer Ridge (see Fig. 1).

Fig. 2 shows the results of a detailed bathymetric survey of the small area of Palmer Ridge and the dredge stations. The ridge here is an asymmetric feature dropping steeply to Freen Deep in the south and much more gently to the deeper Peake Deep to the north. In the middle of the survey area the crest of the ridge is very nearly horizontal, remaining within 20 fathoms of 1,700 fathoms ( $3,110 \pm 34$  m) for about 7 km. Near the crest, particularly on the north side, are a series of nearly horizontal terraces not well shown on the map which range in width up to 2 km

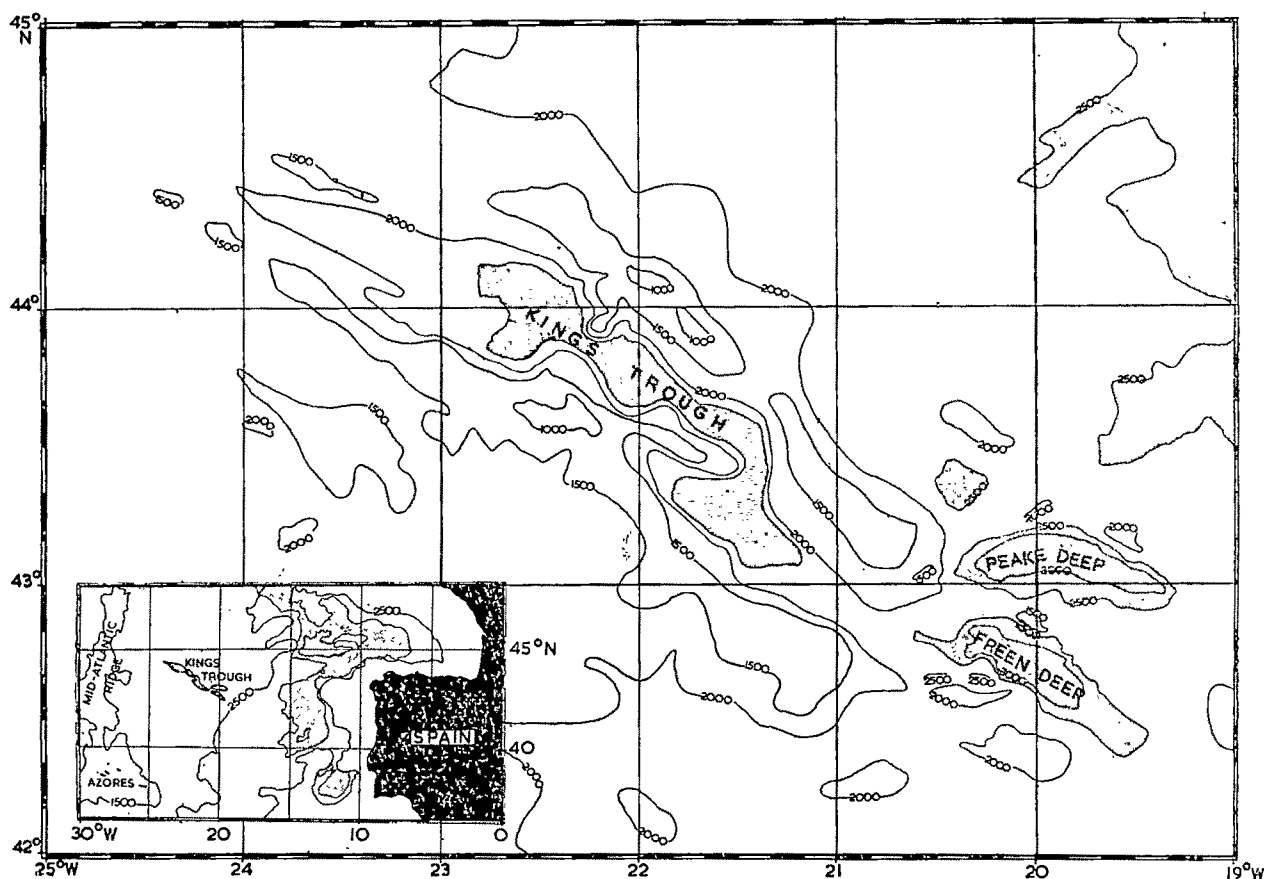


Fig. 1. Index map showing the regional bathymetry constructed from the charts of Dr. A. S. Laughton, National Institute of Oceanography.

and in height of their downslope scarp to 100 m. They can often be followed for more than 5 km.

Table 1 summarizes the results of the dredge hauls. Glacial erratics were distinguished from rocks in place by a rounded or faceted shape, by the presence of striations, by comparison with rocks dredged from presumed erratic-free parts of the oceans and by the principle of homogeneity. This last criterion is that if a haul contains several rocks very similar to one another, though preferably not identical, then these rocks may be assumed not to be erratics<sup>2</sup>. Further discussion of the nature of the erratics and the principles on which they are distinguished will be published elsewhere.

More than 120 pieces of limestone (indurated ooze) were recovered in eight hauls. The pieces are soft, slightly lithified, can often be broken by hand, and their colour ranges from white to grey or light brown. The Upper Tertiary samples (mid-Miocene to Pliocene), recovered in dredge hauls intersecting the terraces near the crest of the ridge, contain a relatively high proportion of planktonic foraminifera and appear to have been winnowed during deposition, much of the fine material having been swept away. The Lower and Middle Tertiary samples

(lower Eocene to Oligocene), on the other hand, dredged from the lower slopes of the ridge, are typical pelagic oozes with a high proportion of fine-grained carbonate material. A core station (5971) on the border of Peake Deep beyond the north end of the detailed survey area also brought up this material.

Serpentinite was the principal hard rock recovered in all four dredge hauls that cut across the crest of the ridge, which thus appears to be a linear outcrop of serpentinite; it was also found in two dredge hauls on the southern slopes. The rocks are completely serpentized, but where secondary alteration is slight they can be seen to be the common pseudomorphs after harzburgites, with large bastite pseudomorphs after orthopyroxenes and little evident clinopyroxene.

The amphibolites show evidence of two stages of metamorphism. The first, of lower amphibolite facies, produced green hornblende-plagioclase rocks from basalts and dolerites; the igneous texture and some pyroxene phenocrysts are well preserved. Widely spaced fractures or coarse brecciation are the only signs of stress during this metamorphism. Many of the amphibolites show later retrograde metamorphism in which plagioclase is

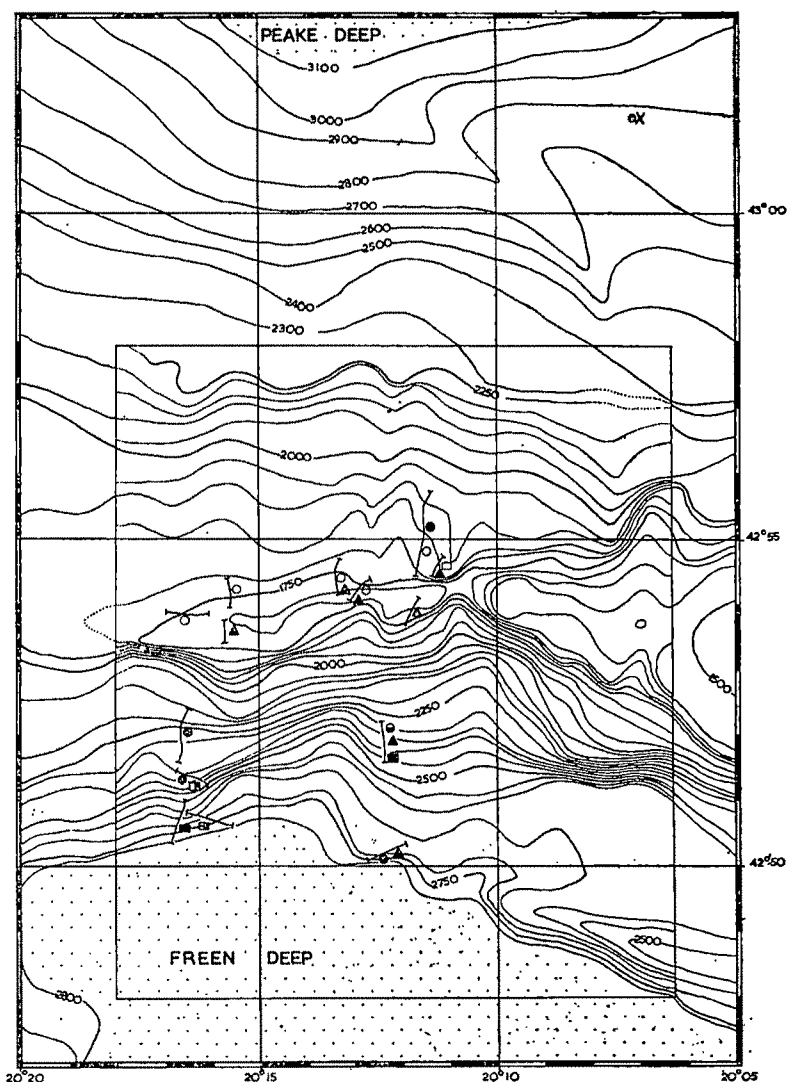


Fig. 2. Bathymetric map of the area surveyed in detail and the surrounding ocean floor. The rectangular box outlines the detailed survey area. Depths are in uncorrected fathoms assuming a velocity of sound in water of 800 fathoms/sec. Contours within the detailed survey area are at 50 fathom intervals, outside at 100 fathom intervals. The abyssal plains of Peake Deep and Freen Deep are stippled. Dredge stations are shown, with the following symbols for the in place rocks recovered: ○, Upper Tertiary limestones; ●, Middle Tertiary limestones; ●, Lower Tertiary limestones; ▲, serpentinites; ◆, amphibolites; ■, gabbros; ■, basalts and dolerites. Core station 5971 is shown by a cross and the appropriate symbol.



replaced by zeolites and the rock is rather sheared and has lost its igneous texture. Potassium-argon ages date the first metamorphism at  $60 \pm 6 \times 10^6$  yr (the freshest amphibolite), and measurements of three retrograded amphibolites ( $29 \pm 2$ ,  $21 \pm 5$ ,  $27 \pm 4 \times 10^6$  yr) suggest a best estimate for the age of the zeolitization and shearing of  $26 \pm 4 \times 10^6$  yr.

Two hauls made close together near the foot of the southern slope contained blocks of olivine-hypersthene gabbro in which most of the olivine had been replaced by iron oxide. In the neighbourhood of joint planes cutting the rock the ferro-hornblende minerals have been entirely replaced by green hornblende, though the plagioclase is unaffected. Evidently the gabbro has suffered, though to a lesser degree, the same metamorphism that has produced the amphibolites. Fresh or slightly weathered basalt and dolerite fragments were found in one haul near the crest of the ridge and a more profoundly weathered specimen in a haul from the borders of Freen Deep.

Table 1. LOCATIONS AND CONTENTS OF DREDGE HAULS

Station	Position	Depth (fathoms)	Probable age range of limestones	Hard rocks
5607	42° 53-9' N 20° 11-8' W	1,700	—	Serpentinites*
5608	42° 52-1' N 20° 16-8' W	2,150- 2,350	Lower to middle Eocene	—
5610	42° 50-8' N 20° 16-8' W	2,800- 2,600	—	1 weathered basalt 2 amphibolites*
5619	42° 53-8' N 20° 16-6' W	1,750	Middle-upper Miocene or Pliocene	—
5628	42° 50-3' N 20° 12-2' W	2,750- 2,650	Lower or middle Eocene	1 serpentinite
5669	42° 55-0' N 20° 11-5' W	1,800- 1,900	Middle or upper Eocene and Miocene or Pliocene	—
5675	42° 54-2' N 20° 12-8' W	1,700	Upper Tertiary (smear on serpentinite)	Serpentinites with carbonate veins
5676	42° 53-6' N 20° 15-7' W	1,700	—	One serpentinite breccia
5677	42° 54-2' N 20° 15-9' W	1,750	Upper Miocene or Pliocene	—
5678	42° 54-6' N 20° 11-2' W	1,850	—	Basalt, dolerite, serpentinite*
5679	42° 50-7' N 20° 16-2' W	2,800- 2,500	—	More or less unalitized gabbro*
5681	42° 51-5' N 20° 16-5' W	2,500	Lower or middle Eocene	More or less unalitized gabbro
5683	42° 54-4' N 20° 13-4' W	1,700	Middle to upper Miocene or Pliocene	Serpentinites*
5685	42° 52-0' N 20° 12-4' W	2,550- 2,250	Oligocene or lower Miocene	Amphibolites, serpentinites

\* Denotes hauls including glacial erratics.

Basement rocks (serpentinites, amphibolites and gabbros) and unmetamorphosed Lower to Middle Tertiary limestones were recovered together in four different hauls; this association suggests a depositional rather than an intrusive relationship. The formation of the basement and its exposure at the ocean floor must thus be earlier than the lower Eocene ( $50 \times 10^6$  yr) and this is confirmed by the age of  $60 \pm 6 \times 10^6$  yr of the freshest amphibolite.

Metamorphism at the lower amphibolite facies appears to require temperatures of about  $350^\circ\text{C}$ . Such temperatures are only reached well within the mantle beneath oceanic regions of normal heat flow ( $1.3 \times 10^{-6}$  cal  $\text{cm}^{-2}$   $\text{sec}^{-1}$ ), and the temperature at the base of normal oceanic crust can be calculated to be about  $200^\circ\text{C}$ . In regions of high heat flow (about  $6 \times 10^{-6}$  cal  $\text{cm}^{-2}$   $\text{sec}^{-1}$ ) the necessary temperatures can be reached at depths about 3 km or less beneath the ocean floor. Even at these shallow depths considerable tectonic activity is necessary (in the absence of erosion on the ocean floor) to expose amphibolites on the surface. The presence of basic igneous rocks indicates that the basement also formed in a region of igneous activity. These three requirements for the formation of the basement, high heat flow, tectonic activity and igneous activity, are met nowadays only at the crests of mid-ocean ridges, and it is suggested that when the basement was being formed this area was at the crest of the mid-Atlantic Ridge.

This complex basement, rather similar to that found at the crests of mid-ocean ridges today<sup>3,4</sup> (and probably

showing a similar topographic roughness), was then gradually covered with normal pelagic ooze from the lower Eocene (and probably from earlier times in local depressions that the dredge did not sample) to the Oligocene, and would probably have appeared a typical area of ocean bed.

The next event in the geological development of the region appears to have been the opening of the Kings Trough feature. This event can be dated by two concordant lines of evidence. First, the amphibolites affected by zeolitization and shearing, a phenomenon that seems to require a definite rise in temperature or increase in activity of circulating water, show K-Ar ages that converge at about  $26 \times 10^6$  yr, suggesting a geological event at this time. Second, the transition from normal pelagic to winnowed sediments which occurred in about lower Miocene times (dated at about the same age<sup>5</sup>), indicates that after that time sediments were deposited on a local topographic high, whereas before they were formed by normal accumulation on the ocean floor. Such a change in conditions could be brought about by the elevation of Palmer Ridge. The formation of the Kings Trough feature, or at least this part of it, can thus be dated with fair certainty at about  $26 \times 10^6$  yr.

After the formation of this feature pelagic ooze continued to accumulate through the Miocene and Pliocene on benches near the crest of the ridge, being somewhat winnowed as it was laid down, and formed the flat-topped terraces visible today. Seismic profiler records of a traverse across the ridge show such a wedge of sediment perched high up near the crest and containing horizontal layering (E. J. W. Jones, personal communication).

There remains the question of how the ridge was uplifted. The long narrow crest seems to be a continuous outcrop of serpentinite and may mark the axis of a long serpentinite intrusion which, at least at this place, has broken through the overlying rocks to form a level spine. Such intrusions of serpentinite are well documented from regions of recent folding<sup>6</sup>. It is suggested that this intrusion has tilted up the rocks on each side to form a broadly anticlinal structure, so that the pre-existent sediments now dip away from the crest down the flanks of the ridge. This would explain the frequent sampling of Lower Tertiary material from many different depths on the north and south slopes.

Table 2 summarizes the postulated geological evolution of this area.

Table 2

Date	Event
About $60 \times 10^6$ yr	Formation of complex igneous and metamorphic basement at the then mid-Atlantic ridge crest.
$60-26 \times 10^6$ yr	Normal pelagic sedimentation
About $26 \times 10^6$ yr	Uplift of Palmer Ridge accompanied by serpentinite intrusion
$26-0 \times 10^6$ yr	Accumulation of winnowed sediments near the crest of Palmer Ridge.

It was indicated here that the basement in this region must have been formed, on a uniformitarian hypothesis, at the crest of the mid-ocean ridge. Palmer Ridge is now 650 km from the ridge axis and has presumably been moved there by a process of ocean floor spreading. Other evidence exists to support the hypothesis of ocean floor spreading<sup>7-10</sup>, which now has a reputable standing. On the basis of the radiometric date of the freshest amphibolite and the age of the earliest sediments found, an average rate of spreading on one side of the ridge (half the rate of ocean opening) of from  $10-13$  km/ $10^6$  yr ( $1.0-1.3$  cm/yr) may be deduced for this part of the ocean over the past  $60 \times 10^6$  yr. These figures fall comfortably within the range of rates ( $0-1.75$  cm/yr) suggested by previous authors for comparable oceanic regions<sup>3,8,9,11,12</sup>.

We thank for assistance, advice and criticism the late Dr. M. N. Hill, Dr. A. S. Laughton, Dr. D. H. Matthews and all other scientists taking part in the two cruises of R.R.S. *Discovery*, and her officers and crew for their invaluable

assistance. We also thank Mr. J. Mitchell and Dr. R. Grasty for determining the radiometric ages.

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## Major Volcano-Tectonic Lineament in the Ethiopian Rift System

by

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Possible transform faults which offset the Wonji fault belt can be traced into lines of weakness dating back to the Jurassic in the surrounding Somalian and Ethiopian plateaux. This makes it unlikely that the Afar depression was produced by tensional separation during the formation of the Red Sea Rift.

THE preparation of a detailed 1:500,000 tectonic map and 1:1 million volcanological map of the Ethiopian Rift System, including Afar, for the UMC Subcommittee on African Rifts has revealed at once the complexity of this Rift System and yet its few dominating tectonic features.

The Main Ethiopian Rift, which extends from the Margherita-Chamo basin north-north-eastwards past Addis Ababa where it begins to funnel out into the Afar depression, is characterized by a peculiar, narrow belt of intense recent faulting. This has been termed the Wonji fault belt<sup>1</sup>, and all active and recent volcanicity along the Main Ethiopian Rift is situated on this belt. The fault belt does not follow the median axis of the Rift floor precisely (Fig. 1). It suffers several dextral transverse displacements along the Rift, particularly along the Chilalo-Guraghe line (X-X' on Fig. 1) and the Yerer-Gugu line (Y-Y' on Fig. 1). The Chilalo-Guraghe displacement coincides with the highest elevation of the Rift floor at the Awash-Maki watershed and is marked by severe gravity anomalies<sup>2</sup>, while the Yerer-Gugu line is responsible for the Rift embayment at Addis Ababa. Thus, although the Rift east of Addis Ababa seems to have a S.W.-N.E. orientation, the faulting of the Wonji fault belt remains S.S.W.-N.N.E. but with several dextral displacements, each of which is associated with a strong lineament crossing the Rift.

The Wonji fault belt was originally mapped northwards as far as the volcano Fant-ale<sup>1</sup>. Until the present work the continuation of the belt into Afar was a matter for speculation, though many years previously Dainelli<sup>3</sup> had made note of an alignment of volcanic centres north-eastwards from Fant-ale to Mussa-ali. The new mapping shows that the fault belt and its associated alkaline silicic volcanic centres continue north-north-eastwards from Fant-ale to the region immediately west of Lake Abbe, with a large dextral displacement along the Ajelu-Amoissa line (Z-Z' in Fig. 1). Between Fant-ale and Lake Abbe the Wonji fault belt widens in places to 40 km, but the volcanic centres lie on or close to the western margin of the belt.

Immediately north of Lake Abbe the fault belt turns abruptly almost northwards but is to a large extent obliterated by intense faulting which trends W.N.W.-E.S.E. and N.W.-S.E. and extends from the Gulf of Tadjura, curving round towards the Salt Plain. This faulting has produced local horsts and rift valleys, and some of the latter are associated with monoclinical warping.

It seems likely that the active Erta-ale basaltic volcanic chain, lying farther north-west within the Salt Plain, is associated with N.W.-S.E. monoclinical warping.

The N.-S. to N.N.E.-S.S.W. faulting of the Wonji belt reappears strongly north of latitude 12° N., although

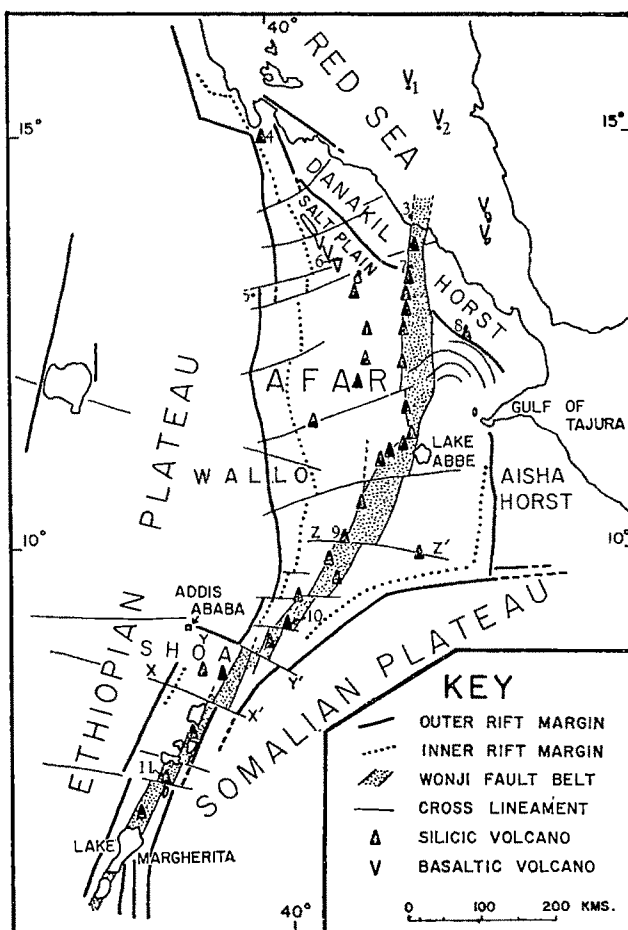


Fig. 1. Some important volcanic and tectonic lineaments in the Ethiopian Rift System. X-X' is the Chilalo-Guraghe line, Y-Y' is the Yerer-Gugu line, Z-Z' is the Ajelu-Amoissa line. 1, Jebel Teir; 2, Jebel Zebair; 3, Kod Ali; 4, Alid; 5, Amba Alaji; 6, Erta-ale; 7, Dubbi; 8, Mussa-ali; 9, Amoissa; 10, Fant-ale; 11, Chabbi.

it is complex in form. The direct alignment reappears at the site of a narrow belt of semicircular faulting of about 30–40 km radius. Preliminary evidence suggests that this pattern represents not subsidence or uplift but the results of clockwise rotation. Whatever the cause of this feature, the Wonji fault belt proceeds northwards from the west margin of the semicircle and forms an intense volcano-tectonic lineament in the Dubbi region of the Danakil Horst. This strong meridional lineament forms an abrupt south-eastern limit to the Salt Plain, and strikes directly across the Danakil Horst. The Danakil Horst is composed of Pre-Cambrian, Mesozoic and Lower Tertiary rocks like those of the Ethiopian and Somalian Plateaux but structurally more deformed. This block of ancient rocks is obliquely traversed by the continuation of the Wonji fault belt, and is covered with recent volcanic effluvia, both silicic and basaltic. The three Dubbi volcanoes with their huge calderas lie close to the western margin of the fault belt.

A northward prolongation of the fault belt into the Red Sea coincides initially and on its western margin with the peridotitic island of Kod Ali. To the N.N.E. the belt heads towards the volcanic islands of Jebel Teir and Jebel Zebair.

The Wonji fault belt is marked by dormant alkaline silicic volcanic centres. Many of the volcanoes have a simple or compound caldera, and have been sources of extensive ignimbritic flows in late Tertiary or Pleistocene times. In Holocene times flood basalts have emerged from fissures along this line.

The existence of this major volcano-tectonic line in Ethiopia is relevant to theories on the origin of the Afar depression and its relation to the Red Sea. The manner in which it cuts straight across structures of Red Sea trend is immediately evident, and if the Wonji fault belt is postulated to be a proto-development of the tensional central trough of the Red Sea<sup>3</sup> its discordance with this trough suggests that Afar structurally belongs first to the African Rift System and only secondarily to the Red Sea System. Some evidence also comes from the new 1:500,000 tectonic map. The gravity-geology surveys of Afar—published<sup>2,4</sup> and unpublished—suggest that the tectonic margin of the Red Sea trough south of latitude 15° N. is formed by the eastern margin of the Danakil Horst rather than the Afar-Ethiopian Plateau margin. Two other features which emerge from the new tectonic map suggest that the Afar area has persisted as a structural unit, contrary to some ideas of the proponents of continental drift. First, the Basement Mesozoic and Triassic rocks of the Ethiopian Plateau extend for a considerable distance eastwards into Afar, where they thicken and disappear below lavas and sediments (Desset Series) of the Afar floor. The distance between what has been termed<sup>3,6</sup> the inner and outer structural margins of western Afar reaches nearly 100 km in Wallo and north

Shoa (Fig. 1), a width wholly formed of downwarped old rocks. The extraordinary marginal graben of Afar<sup>4,6</sup> lie at the outer structural margin. The position of the inner structural margin of Afar is such that, if the Danakil Horst and the Aisha Horst are also taken into account there is insufficient space available for a reconstruction which superimposes south-western Arabia on Afar. Second, northern and western Afar are characterized, not only by the generally N.N.W.–S.S.E. oriented belts of intense, short curvilinear faulting, but also by long straight faults trending E.N.E.–W.S.W. No vertical displacements are present along these lines, some of which cut directly from the eastern Ethiopian Plateau, across Afar and over the Danakil Horst. From preliminary field-work I have tentatively identified these lineaments with approximately E.–W. tectonic lines on the Ethiopian Plateau, some of which (for example, Amba Alaji)<sup>4</sup> are known to have been active in the Jurassic. There is similar evidence from the deformed Mesozoic strata of the Danakil Horst. If the history of these lineaments extends back to the Mesozoic and their linearity is still extant, it would seem that the position of the Danakil Horst in relation to the Ethiopian Plateau has not altered during that time. The thickening and coarsening of the Mesozoic rocks towards the Salt Plain, both eastwards from the Ethiopian Plateau and westwards from the Danakil Horst, suggest that the Afar region was a subsiding basin of deposition from this early time. There is, therefore, no evidence of any tensional separation in Afar which has been produced by the formation of the Red Sea rift.

The importance of approximately E.–W. lineaments crossing the Ethiopian Rift System has only recently been recognized. Displacements both of the marginal rift faulting and of the Wonji fault belt occur along these lines. Although the faults are straight and extensive, no evidence of true transcurrent displacements along any of them has yet been recognized. Tentatively, therefore, it is considered that the E.–W. lineaments mark transform faults extending under the whole of the Ethiopian Swell, which are most strongly manifested superficially at the margins and within the Rift System.

The refinement of previous gravity data<sup>2,4</sup> has revealed that the regional Bouguer gradient over the northern Ethiopian Plateau has a strike of E.N.E.–W.S.W. This is precisely the direction of important lineaments in Afar, and is perpendicular to the Red Sea axis but also close to the Gulf of Aden trend.

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## Removing Viruses from Water by Polyelectrolytes

by  
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Polymers based on divinylbenzene-crosslinked styrene/maleic anhydride copolymer can adsorb 100 per cent tobacco mosaic virus and more than 99.99 per cent polio virus from aqueous suspensions. The technique may be useful for preparing potable water by removing viral infectivity which may survive conventional treatment. It may also be useful in detecting viral contamination.

THE contamination of effluent and drinking waters by viruses is a problem of increasing importance to public health<sup>1–4</sup>. Current procedures for treating water are considered to be inadequate for the elimination of danger in times of maximum contamination<sup>5,6</sup>. New approaches

to the effective removal of virus and/or bacteria have therefore been sought as a means of producing potable waters from contaminated effluent. Concentration of several types of virus from sera and contaminated sewage effluent using various ion-exchange resins has already been reported<sup>7,8</sup>.

\* Polymer No. 7, 325 mesh; TMV/polymer ratio, 1-84; shaken 1 h.

Table 3. TMV ADSORPTION IN HANKS SOLUTION

Polymer No.	Virus adsorption (per cent)*	
	Water	Hanks solution
(3)	9	70
(6)	75	95
(7)	97	98

\* 1 mg polymer (325 mesh) in 4 ml. of Hanks solution; shaken 1 h; TMV/polymer ratio, 1:84.

Because the polio experiments were planned in Hanks physiological saline solution, the adsorption of TMV in Hanks solution was determined (Table 3) and found to be better than that obtained with controls free of inorganic ions.

Adaptation of the technique to studies of animal viruses introduces contamination by foreign protein as a factor in adsorption. Quaternized 100 per cent imide was evaluated for the removal of bovine serum albumin (BSA) at various ratios of BSA to polymer (Table 4). One milligram of polymer removed 90 per cent of 5–10 mg quantities of BSA. The efficiency of removal dropped at both high and low concentrations of protein.

Table 4. REMOVAL OF BSA FROM AQUEOUS MEDIA

BSA (mg/5 ml.)	Percentage of removal*		
	1	2	Average
0.5	62	68	65
1.0	70	74	72
2.0	80	80	80
5.0	91	90	90.5
10.0	90	93	91.5
25.0	—	48	48

\* 1 mg of polymer No. 7 (325 mesh) shaken with BSA solution for 1 h, filtered and BSA content of filtrate determined with Folin reagent using a standard curve prepared for BSA.

The performance in mixtures of TMV and BSA was evaluated using the standard contacting procedure. The polymer-treated virus-BSA filtrate was compared in an analytical ultracentrifuge with a TMV control (no polymer or BSA). Removal of 80–90 per cent of the TMV was achieved. Analysis of the filtrate with Folin reagent showed that 90 per cent of the BSA was still present, which indicated a selective removal of TMV from a solution containing twenty times as much BSA.

Sterilization was not required in the TMV screen, but was customary in experiments on polio virus. Autoclaving polymer suspensions at about 120° C for 20 min could chemically and/or physically alter the polymers so as to affect virus adsorption. Samples of polymers (3), (6) and (7) were autoclaved and the infra-red spectra of the products were compared with those of the originals. The results indicated no significant hydrolysis of amides or imides and no changes in the content of quaternary salt.

Autoclaving aqueous suspensions of 66 per cent imide and 100 per cent imide destroyed their ability to adsorb TMV virus but still permitted good adsorption with 100 per cent imide-methiodide (Table 5). Autoclaving in Hanks solution, however, substantially reduced the latter adsorption.

Table 5. EFFECT OF AUTOCLAVING POLYMER SUSPENSIONS

Polymer No.	TMV adsorption (per cent)*		
	Control†	In water	In Hanks solution
(3)	86	1	0
(6)	90	3	16
(7)	95	97	43

\* 1 mg polymer (325 mesh) in 4 ml. of water of Hanks solution, autoclaved for 20 min at 120° C. Virus added to cooled solutions, shaken 1 h, filtered and virus content of filtrate assayed.

† Hanks solution (no autoclaving).

Visual observations and ultramicroscopic studies indicated that the polymers became more dense, with numbers (3) and (6) actually forming films. The 100 per cent imide-methiodide became denser and more particulate. Autoclaving thus destroyed polymeric surfaces available for

virus adsorption, probably by fusing individual polymer particles.

When investigations of the adsorption of polio virus were carried out, care was taken to avoid autoclaved adsorbent and salinity. Selected polymers were evaluated for their adsorption of polio virus by the procedure previously outlined (Table 6). Addition of polymers (3) and (6) reduced polio virus titre in final filtrates by more than 99.9–99.99 per cent. This contrasts with the lesser adsorption (99.2 per cent) of polio virus found for polyanionic maleic copolymer<sup>11</sup> using twenty-five times as much polymer relative to cationic systems. No significant reduction occurred in the presence of the 'Amberlite XE-119' control. Polymer (7) formed a viscous virus-polymer complex gel which, in this experiment, could not be separated to yield a clear aqueous phase. Meaningful values could not be obtained.

Table 6. REMOVAL OF POLIO VIRUS

Treatment	P.F.U./0.5 ml.*	Virus removal (per cent)
Control	$0.8 \times 10^8$	
Polymer No. (3)	$< 0.5 \times 10^8$	> 99.9
Polymer No. (6)	$< 0.5 \times 10^8$	> 99.9–99.99†
'Amberlite XE-119'	$0.8 \times 10^8$	0

\* Average of the two plate counts for the dilution giving discrete plaques.

† At the minimum dilution tested no plaque-forming units (P.F.U.) were observed.

This experiment does not distinguish between loss of virus activity due to adsorption by the polymer and possible inactivation in the presence of polymer. Published results indicate, however, that viable virus can be eluted from several ion-exchange resin-virus complexes. It is probable that this is so in these experiments on polio virus. Furthermore, it does not establish the minimal ratios of polymer to virus required for the complete removal of virus.

It is shown that these cationic polyelectrolyte derivatives are excellent adsorbents of plant and animal viruses from dilute aqueous suspensions. The ease and strength with which a virus or proteinaceous material is adsorbed to surfaces are related to several parameters. In the case of the adsorption of negatively charged viruses by anion-exchange resins or polybases, the results indicate that the availability of surface area for adsorption is an important factor. Polymers prepared by solvent-non-solvent techniques increase the area by a factor of about ten (that is, from 4 to 40 m<sup>2</sup>/g as measured by the Brunauer-Emmett-Teller method) when compared with the ion-exchange control. A potential hundred-fold increase in area has been shown to be possible in the case of uncollapsed films cast from solvents<sup>14</sup>. Such films cast from appropriate functional polymers represent a promising approach to commercialization with filter mat types of equipment. Differences in performance were also noted for different functional groups and for varying charge density of the adsorbent. Other parameters for future investigation include: (1) hydrophilic-hydrophobic ratio, (2) the degrees, type and extent of ionization, (3) charge distribution, and (4) influences imparted by interactions with neighbouring group. This technology should make possible the removal of viruses and bacteria which survive standard treatment of contaminated effluent waters.

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# Design of the Bronchial Tree

by

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The bronchial tree is so designed that the functions of the lung can be carried out with minimum entropy production.

A QUANTITATIVE description of some aspects of the architecture of the human lung has been provided by Weibel and Gomez<sup>1</sup>. One of the parameters which is described in their paper is the diameter of the tubes in the bronchial tree. They find that the average diameter  $d_z$  of the tubes after the  $z$ th bifurcation of the network is given by the expression,  $d_z = d_0 2^{-z/3}$ , where  $d_0$  is the diameter of the trachea. This is, of course, a simplified description of the system. Even if the model of a network of succeeding bifurcations in which the tubes of each generation can be assigned a common description is proper, there is, at least, a large variation of the properties of the  $z$ th generation about the average<sup>2</sup>. It does, however, seem to be a reasonable first step in the complete description of the passages of the lung and the form of this result is especially convenient for analysis.

The purpose of this article is to show that the value of tube diameter as a function of the location of the tube in the bronchial tree as reported by Weibel and Gomez is the same as the value for which gas of a given composition can be provided to the alveoli with minimum metabolism or entropy production in the respiratory musculature. The hypothesis that the characteristics of the design of biological systems take values for which a given function can be accomplished with minimum total entropy production has been tested previously in a description of ventilation as a function of total oxygen consumption and a description of capillary spacing in muscle tissue as a function of oxygen consumption in the muscle<sup>3,4</sup>.

In the application of the hypothesis to the description of the bronchial tree, the diameter of the tubes in the  $z$ th generation of the network is imagined to be variable while all other parameters of the respiratory system and the function of the respiratory system are held constant. It is assumed that the function of the tubes is to conduct gas between the atmosphere and the alveoli so that the alveolar oxygen and carbon dioxide tensions are held at given values. For a given oxygen consumption this requires that the alveolar ventilation  $\dot{V}_A$  must have a given value. The total ventilation  $\dot{V}$  is a function of the tube diameters since the dead space, which is the total volume of the bronchial tree, must be filled at each inspiration. The total ventilation  $\dot{V}$  is given by equation (1).

$$\dot{V} = \dot{V}_A + v \frac{\pi}{4} \sum_z d_z^2 l_z 2^z \quad (1)$$

In equation (1),  $v$  is the breathing frequency and  $l_z$  is the length of the tubes in the  $z$ th generation.

Another variable which depends on the geometry of the duct is the pressure drop between the atmosphere and the alveoli. For Poiseuille flow in a circular tube, the pressure drop across the  $z$ th generation of tubes  $(\Delta p)_z$  is given by equation (2).

$$(\Delta p)_z = \frac{128 \mu Q_z l_z}{\pi d_z^4} \quad (2)$$

In equation (2),  $Q_z$  is the rate of volume flow through a single tube in the  $z$ th generation and  $\mu$  is the coefficient of viscosity for air. The value of  $Q_z$  is approximately  $\dot{V}/2^z$ . The assumption that the flow is fully developed gives a lower limit for the estimate of  $(\Delta p)_z$ . The appro-

priate dimensionless number (Reynolds number)  $\times (d_z/l_z)$  has large enough values for the first few generations that the pressure drop cannot be taken to be independent of the entrance conditions<sup>5</sup>. If, however, we use an estimate for an upper limit for  $(\Delta p)_z$  corresponding to a flat velocity profile at the inlet, then the value of the diameter of the trachea would be increased by only 25 per cent over the value calculated below and the effect on the values of the diameters of the tubes of succeeding generations would quickly become negligible.

Since the alveolar ventilation is held fixed, independent of tube radii, the entropy production in the mixing of alveolar gas with air is fixed, independent of the radii of the tubes. The main entropy sources which depend on the tube radii are the viscous dissipation in the gas flow and the chemical and mechanical dissipation in the respiratory muscles. Because all the energy released in the chemical reactions in the muscles is eventually dissipated into heat, either within the muscle or in the gas flow and structural motion driven by the muscle, a good estimate of the total entropy production  $\dot{S}$  is given by the total energy released divided by the temperature.

$$\dot{S} = H \dot{V} \dot{O}_2 / T \quad (3)$$

In equation (3),  $H = 5$  cal/ml., the energy released per ml. of oxygen burned in the respiratory muscles,  $\dot{V} \dot{O}_2$  is the oxygen consumption of the respiratory musculature in ml./min and  $T = 300^\circ$  K. In the entropy balance for this process, the difference in entropy between the reactants and products of the chemical reaction is negligible compared with the heat flux.

We seek the value of  $d_z$  for which  $\dot{S}$  is a minimum. At this value,  $d\dot{S}/dd_z = 0$ . The main contributions to

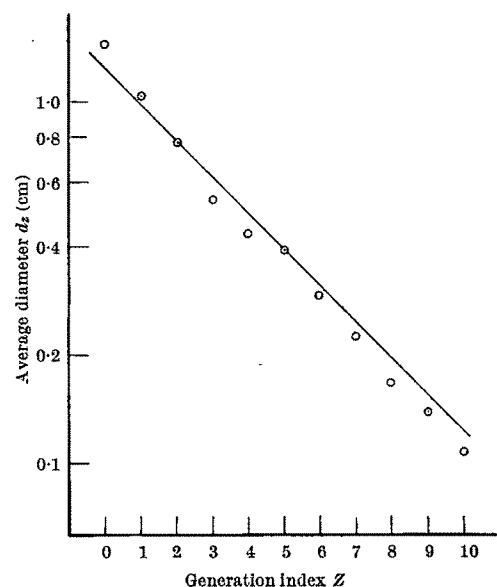


Fig. 1. The diameter of the tubes in the bronchial tree plotted on a logarithmic scale as a function of the location of the tube in the bronchial tree. —, Tube diameter for minimum entropy production;  $\circ$ , data of Weibel and Gomez.

the derivative are assumed to be due to the dependence of the total ventilation on  $d_z$  and the dependence of the pressure drop on  $d_z$ . The value of  $d_z$  which satisfies the design hypothesis is given by the value of  $d_z$  which satisfies equation (4).

$$\frac{dS}{dd_z} = \frac{H}{T} \left( \frac{\partial \dot{V}_{O_2}}{\partial \dot{V}} \frac{\partial \dot{V}}{\partial d_z} + \frac{\partial \dot{V}_{O_2}}{\partial (\Delta p)} \frac{\partial (\Delta p)}{\partial d_z} \right) = 0 \quad (4)$$

This equation is a quantitative expression of the idea that the tube diameter is limited at small values by the increasing flow resistance with decreasing tube diameter and is limited at large values by the increasing tube volume and dead space with increasing tube diameter.

It follows from equation (1) that  $\partial \dot{V} / \partial d_z = \frac{\pi}{2} v d_z l_z 2^z$  and from equation (2) that  $\partial (\Delta p) / \partial d_z = -512 \mu \dot{V} l_z / \pi d_z^5 2^z$ . Substituting these values in equation (4) and solving for  $d_z$  results in equation (5).

$$d_z = \left[ \left( \frac{1024 \mu V}{\pi^2 v} \right) \left( \frac{\partial \dot{V}_{O_2}}{\partial (\Delta p)} / \frac{\partial \dot{V}_{O_2}}{\partial \dot{V}} \right) \right]^{\frac{1}{3}} 2^{-z/3} \quad (5)$$

The coefficient in the relation can now be evaluated using the following values of the parameters:  $\mu = 1.8 \times 10^{-4}$  poise,  $v = 15$  breaths/min, and  $\dot{V} = 30$  l./min, a value in the middle of the range for this parameter. The values of the physiological parameters  $\partial \dot{V}_{O_2} / \partial \dot{V}$  and  $\partial \dot{V}_{O_2} / \partial (\Delta p)$  have been measured by Campbell, Westlake, and Cher-

niack<sup>6</sup>. They find that  $\partial \dot{V}_{O_2} / \partial \dot{V} = 0.25$  ml./l. over the range of ventilation up to 40 l./min. They also find that the differential efficiency of maintaining the same ventilation with increasing flow resistance is 10 per cent so that  $H(\partial \dot{V}_{O_2} / \partial (\Delta p)) = 10 \dot{V}$ . Then  $d_0 = 1.25$  cm and the value of  $d_z$  as calculated from the hypothesis is given by equation (6).

$$d_z = 1.25 \times 2^{-z/3} \text{ cm} \quad (6)$$

The curve represented by equation (6) is plotted in Fig. 1 along with the data of Weibel and Gomez.

The values of the diameters of the tubes making up the bronchial tree which have been calculated from the hypothesis agree with the values observed by Weibel and Gomez. It can be said, therefore, that the diameters of the conductive air passages of the lung are those for which a given alveolar ventilation can be maintained with minimum entropy production.

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## Origin of Genetic Variability: Combinations of Peroxidase Isozymes determine Multiple Allelism of the S Gene

by

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Electrophoresis suggests that peroxidase enzymes are involved in the expression of the S alleles which control incompatibility. This may be linked with the mechanism of action and the origin of S genes and thus with the origin of large scale variability.

FROM the evolutionary point of view a virtually unlimited variability at the level of the organism is advantageous, and this is made possible in nature through the evolution of sex. The key to this virtually inexhaustible potential lies in "recombination" at several levels of organization<sup>1-4</sup>. Looking at evolution as a whole, one principle seems to stand out as being consistent with evolution at all levels. Large-scale variation tends to be created by multiple combinations of a minimum number of suitably adaptive existing elements, rather than by the creation of new genetic elements *per se*.

Enzyme (protein) molecules, the many hundreds of forms of which catalyse all the reactions of living matter, are composed of one or more polypeptide chains, each the product of a single gene. All these molecules are composed of differing arrangements of twenty amino-acids, which are coded by the DNA molecule using non-overlapping triplet combinations of only four nucleotide bases<sup>5-7</sup>. All developmental and differentiations patterns appear to be produced by large-scale variation in the control, by combinations of regulator genes, of a comparatively small fixed number of elements—the structural genes. Finally, on the population level, a few thousand genes through recombination produce billions of individuals all different from each other. In this scheme of the origin of variation there is, however, one obvious gap: how does a large-scale neomorphic (equivalent) variation arise in the same gene?

*Origin of multiple S alleles.* S alleles controlling incompatibility behaviour in plants are an extreme example of

neomorphic variation. In species which have been tested approximately 80 per cent of the total number of alleles tested were found to be different. For example, Williams<sup>8</sup> in his work with red clover found seventy-eight different alleles out of a possible maximum of eighty-eight in forty-four plants tested. Thus, there appears to be no limit to the number of alleles. As far as the author is aware no neomorphic variability of a gene on such a scale is known in any other genetic system in any organism.

In the extensive genetic investigation of the S complex<sup>9-21</sup> no evidence is available to suggest that the large number of alleles has arisen through recombination between a relatively small number of genetic elements controlling the specificity of the S gene. Superficially it would seem that the extreme variability at the S locus defies the principle stated here. Here seemingly the same genetic element has assumed innumerable forms. The resolution of this dilemma depends on the understanding of the molecular basis of this variation.

Isozymes<sup>22</sup>, different molecular forms of the same enzyme, have been demonstrated by electrophoretic techniques in a large number of organisms. Different isozymes of the same protein have been shown in plants and animals to be implicated in normal growth and differentiation as well as in abnormality and disease<sup>23-25</sup>. The occurrence of isozymes has been shown to be controlled by a particular gene in only a very few cases. So far the most elaborate investigation of this type in higher plants has been made by Schwartz<sup>26</sup>, who investigated the origin of esterase isozymes in maize endosperm. No genetic

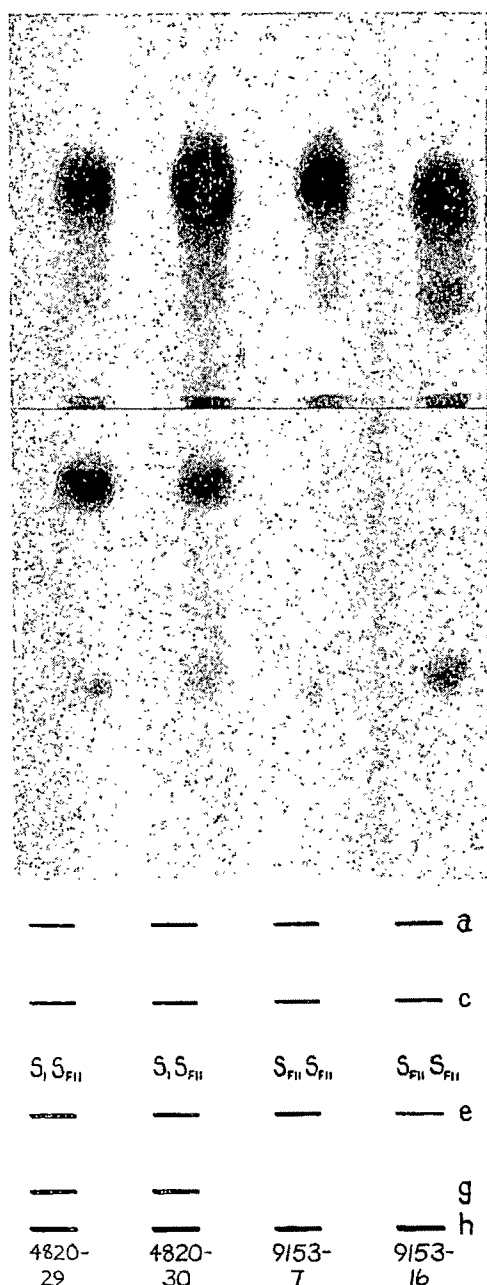


Fig. 1. Peroxidase isozymes in different *S* allelic styles. Different plants having the same *S* genotype show an identical electrophoretic pattern.

system in plants controlling multiple isozymes specified by the same gene has yet been investigated.

**Peroxidase isozymes in different *S* allelic styles.** Genetic<sup>9-21</sup>, immunogenetic<sup>27</sup> and biochemical<sup>28</sup> investigations of incompatibility suggest that the *S* gene acts by producing specific proteins in the pollen and style, each allele giving rise to different specificity. In the present investigation, control of stylar enzymatic activity by the *S* locus was examined in *Nicotiana*. The enzyme peroxidase was specifically chosen for investigation because of its suggested involvement in the control of protein activity<sup>29</sup>.

The following genotypes, four homozygous and three heterozygous for *S* alleles, were examined (usually several plants in each genotype):  $S_1S_1$  (four),  $S_3S_3$  (two),  $S_{F10}S_{F10}$  (two) and  $S_{F11}S_{F11}$  (four);  $S_1S_{F11}$  (two),  $S_3S_{F11}$  (one) and  $S_{F10}S_{F11}$  (two). Out of seventeen plants examined nine were pure *N. alata* (self-incompatible) and eight were extracted from hybrids between *N. alata* and *N. langsdorffii* (self-compatible)<sup>9-21</sup>. The latter plants

varied greatly among themselves, and from the pure *N. alata*, in flower size, shape and colour.

Styles from emasculated mature flowers were collected and stored at  $-20^\circ\text{C}$  for 1-3 months. Extracts were prepared by grinding approximately 1 g of styles with a few drops of 0.05 molar *tris* hydrochloric acid buffer at pH 7.5. The slurry was centrifuged at 15,000*g* for 20 min and the supernatant was dialysed overnight against the same buffer. All treatment was done at  $3^\circ\text{C}$  to prevent denaturation. Enzyme components were separated by electrophoresis on acrylamide gel blocks prepared according to Tombs<sup>30</sup>. As many as six samples were run together in the same block in strictly comparable conditions. Electrophoresis was carried out for 3 h at about 20 m.amp, after which the gel was stained for 30-45 min in a specific stain for peroxidase (for stock reagent: to 1 l. of 7 per cent acetic acid add 160 g of sodium acetate, saturate with 1 per cent EDTA, filter, saturate with benzidine dihydrochloride, and filter again; hydrogen peroxide (1 ml.) is added to 100 ml. of stock reagent just before use).

The results showed that each allele had its own specific isozymic bands and that they were identical in all plants with the same allele irrespective of whether the plants were pure species or were of hybrid origin. Plants with two alleles invariably produced bands of both alleles (Fig. 1). There was a difference in intensity of various isozymic bands. Fig. 2 gives a diagrammatic sketch of bands of the seven genotypes investigated.

Two plants, 4820/29 and 4820/30, both of the same genotype ( $S_1S_{F11}$ ) and extracted from the same hybrid family, had each an identical extra band overlapping one of the usual bands ("e" in Fig. 1). The extra band was, however, brilliant purple in colour, in contrast to the blue of usual bands, and lay exactly on the top of the latter. The significance of this band is not clear.

The results suggest that (1) peroxidase isozymes determine *S* gene specificity and (2) the basis of allelism of this gene lies in the particular combinations of the specific isozymes. Although at this stage of the investigation the unlikely probability of the involvement of other enzymes in the determination of *S* specificity cannot be entirely ruled out, the present work reveals for the first

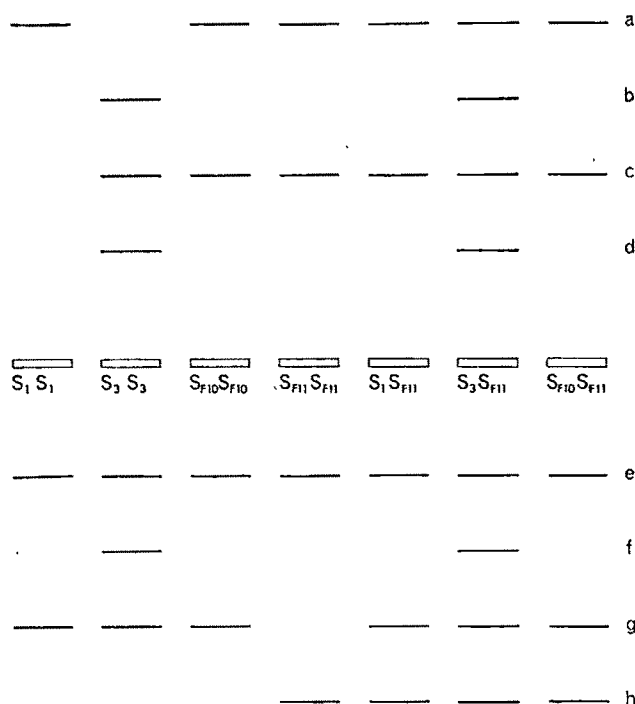


Fig. 2. Diagrammatic sketch of electrophoretic peroxidase isozymic patterns of seven *S* genotypes.

time a molecular basis of self-incompatibility, a widespread phenomenon in plants. In this context it is interesting to note that in the protozoan *Tetrahymena pyriformis* the multiple alleles of the mating type locus appear to control a number of esterase isozymes<sup>25</sup>, although in this case the picture is not so clear because the different isozymes controlled by the same allele do not appear at the same time. Further, the present work shows that at the gene level also, the basis of multiple variation is the same as at other levels, and therefore confirms the universality of the principle enunciated earlier, that large-scale variation in nature arises through recombinations of a comparatively few genetic elements rather than through creation of new genetic elements *per se*.

It is significant to find this principle so consistent throughout life, from the molecular level to the level of the highly complex multicellular organism. Apart from mathematical considerations, the necessity for such a principle seems obvious from another point of view: in the highly complex system of life processes the fewer the number of new unintegrated elements the fewer are the chances of error through interference at the molecular level<sup>21</sup>. So long as there is a built-in mechanism available to produce variation at all times, as there is in the DNA structure, a new variation can be incorporated when the system is ready and the circumstances favour it, and there is no need for the accumulation of entirely unintegrated new genetic elements. The very survival of the system depends on the elimination of redundancy.

**Mechanism of *S* gene action.** The discovery of the involvement of peroxidase in incompatibility enables us to take a fresh look at some of the still unresolved problems in this field. The two most important of these are concerned with (1) the physiological mechanism of *S* gene action, and (2) the nature of *S* gene mutations, natural and induced.

Peroxidase occurs very widely in plants<sup>22-24</sup>. It is known to be involved in the oxidative inactivation of certain biologically active proteins<sup>25</sup>, but the physiological significance of its activity in plants is not well understood. It is believed to participate directly, or through coupled oxidations, in various oxidative processes including respiration, lignification and degradation of auxin. In view of its very wide range of substrates<sup>22-24</sup> including certain proteins, amino-acids, hormones and enzymes, it probably plays a variety of parts in different physiological systems.

One of the physiological roles of peroxidase is its involvement in the destruction of the widely occurring growth promoting hormone indolyl-3-acetic acid (IAA). Evidence suggests that IAA, acting as inductor molecules, controls the activity of certain regulator genes involved in stem growth and flowering<sup>25,26</sup>. Thus the destruction of IAA would have the effect of repressing certain regulator genes which would otherwise be derepressed (activated) at that stage.

It is assumed here, with Lewis<sup>27</sup>, that the *S* gene complex produces an identical polypeptide molecule in pollen and style. This molecule is a dimer protein formed by polymerization of the identical primary molecules (monomers). In an incompatible pollination two identical dimers, one each from pollen and style, combine to form a tetramer with the aid of an allosteric molecule "which may be glucose, a protein or one of many small molecules". It is believed that only a tetramer is physiologically active in producing incompatibility; the dimers are inactive in this respect.

The present work suggests that the polypeptide molecule specified by the structural cistron of the *S* gene complex is peroxidase. It is proposed here that in an incompatible pollination the activated tetramer peroxidase destroys the growth promoting hormone IAA in the pollen tube. This destruction of the inductor molecule leads to repression of the regulator genes governing the production of growth substances for the pollen tube, thus

causing its inhibition. The activated peroxidase may further limit growth by blocking the pollen tube wall through the formation of a rigid lignin matrix in the membrane<sup>23,24</sup>, thus stopping the entrance of nutrients from the style to the pollen tube and probably also making it incapable of expansion. In a compatible pollination, where the dimers from the pollen and style are different, there is no such tetramerization. This allows the IAA to continue its derepression of the regulator genes, thus promoting pollen tube growth.

It is interesting to find that the peroxidase isozymes from plants with two different *S* alleles have only isozymes specified by each allele. No extra hybrid isozymes, having an intermediate electrophoretic mobility of parental isozymes, as reported by Schwartz<sup>28</sup>, are produced, thus providing a molecular basis for the lack of *S* allele interaction, so essential for the success of the gametophytic system of incompatibility.

**Mutation, and origin of new *S*<sub>I</sub> alleles.** The present work suggests that new self-incompatibility alleles generally arise by recombination of a small number of genetic elements specifying peroxidase isozymes. Each allelic specificity is determined by a unique combination of these isozymes. Changes in the constituent genetic elements of an *S* allele, giving rise to a new allelic specificity, may come about largely through loss, inactivation or suppression, or equal or unequal crossing over between the two homologous alleles, and rarely through a point mutation producing a new isozyme. Thus, only eight elements in the structural cistron could, through recombination, produce 255 alleles, assuming one element to be the minimum required for specifying an allele. The above hypothesis can explain the large number of self-incompatible alleles found in the natural population, but it does not explain by far the most puzzling feature of the results of work on the *S* gene, which is the inability in experimental conditions to obtain a single mutation from one self-incompatibility allele to another in intensive experiments<sup>5</sup> involving millions of pollen grains in *Oenothera*, *Prunus*, *Trifolium*, *Petunia* and *Nicotiana*. All induced mutations obtained so far were mutations in the regulator cistron producing self-compatibility; none was found in the structural cistron giving a new *S*<sub>I</sub> specificity. Nevertheless, the conclusion is that to account for such a large number of alleles in the population, the mutation rate must be high enough for the mutation to be normally detected in experimental conditions<sup>28,29</sup>. The fact that no such mutation has been identified leads me to the belief that there is a new aspect to the *S* gene mutation not yet illuminated by mutation studies.

Investigations of different forms of *S* alleles and their interactions in various breeding systems suggest that the activities of the *S* gene complex are an integral part of the physiology of reproduction including sporophytic as well as gametophytic phases of the life cycle. The role of *S* alleles in sexual mechanisms, including intra-specific and inter-specific, and homomorphic and heteromorphic, pollen-style incompatibility, is well known<sup>9-21</sup>. What is not so well known is that the action of certain elements of the *S* complex may be very early in the sporophytic phase, as is indicated by observations in *Theobroma* and *Trifolium* which show parallelism between interspecific hybridization and grafting<sup>40,41</sup>. The species or genotypes that were sexually compatible were also graft-compatible, and conversely, combinations that were sexually incompatible were also vegetatively incompatible.

These observations all point to one fact, that the genetic elements comprising the *S* gene complex have a very wide range of expression, certain elements being expressed very early in the sporophytic phase and others acting late in the gametophytic phase. There is evidence to suggest that IAA acts as an inductor (probably in conjunction with certain other hormones) to derepress certain elements of the *S* complex and some other genes governing

the reproductive phase<sup>35,36</sup>, long before the onset of flowering.

It is suggested here that the precursors of the substances determining the specificity of an *S* allele are already laid down in the cytoplasm early in the sporophytic phase, so that any mutation in the structural cistron occurring late in the life of the sporophyte or during the gametophytic phase is unable to express itself until the next sporophytic generation. This would mean that natural selection for *S* specificity mutation does not operate at the gametophytic level (basis of our mutation work), but only at the sporophytic level. This lag in the expression of *S* gene mutation might have required a comparatively high mutation rate, since it would be effective only through compatible pollinations, were it not that it is fully offset by the multiplication of each mutation many times over, through mitotic cell division, by the time it reaches the spore stage. This hypothesis, if correct, would explain our inability with present techniques to produce *S* specificity mutations, and at the same time account for the large number of *S* alleles occurring in nature.

A considerable gap between gene action and final expression of the phenotypic character appears to be a common feature of genes associated with reproduction<sup>42</sup> and phase variation<sup>43</sup>. The basis of monoecism, from algae to higher plants, is the mutually exclusive action of two sex determining genes which often act to fix the sex during early vegetative growth, although the observable sex differences are not manifested until later.

From these considerations it seems probable that the production of peroxidase isozymes is one of the last acts of the *S* gene complex, the earliest of which may be closer to seed germination than to microspore germination.

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## Influence of Temperature on Lizard Testes

by  
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SPECIES differences in thermal sensitivity are evident in numerous tissues and enzymes from lizards. These variations correlate well with differences in the preferred body temperatures of the species. (Preferred body temperatures are defined here as those temperatures maintained by lizards in thermal gradient chambers in which a wide range of temperatures is available. Temperatures maintained in such conditions are very similar to those maintained by animals active in nature<sup>1-4</sup>.) Thus, species appear to be physiologically adjusted to different temperatures commensurate with their thermal preferences. Evaluation of the extent of thermal divergence and the mechanisms involved therein is, however, limited, because the available data deal primarily with somatic functions. Reproductive tissues may not show similar relations to temperature. The male germinal epithelia of many plants and animals are generally considered to be less heat-

Incorporation of tritiated thymidine in organ culture shows that the temperature at which testicular cells begin to degenerate differs between two species of lizards. These differences parallel the differences in the preferred body temperature of the species.

resistant and less capable of adapting with respect to temperature than the somatic tissues<sup>5</sup>.

In lizards, exposures to body temperatures only slightly above preferred levels may cause testicular impairment and spermatogenic arrest<sup>6</sup>. Comparisons of the temperatures necessary to cause such impairment suggest some interspecific divergence in testicular heat-resistance. Interpretations of the effects of temperature *in vivo* are, however, hindered by concomitant systemic disorders, and it is not clear to what extent testicular damage results from the direct action of heat. To define better testicular heat-resistance and its relation to the preferred body temperatures of lizards, the thermal responses of testes from several species were compared *in vitro*.

Comparisons were based on two species of lizards with distinct thermal preferences: the American chameleon, *Anolis carolinensis*, from Louisiana, and the desert sand



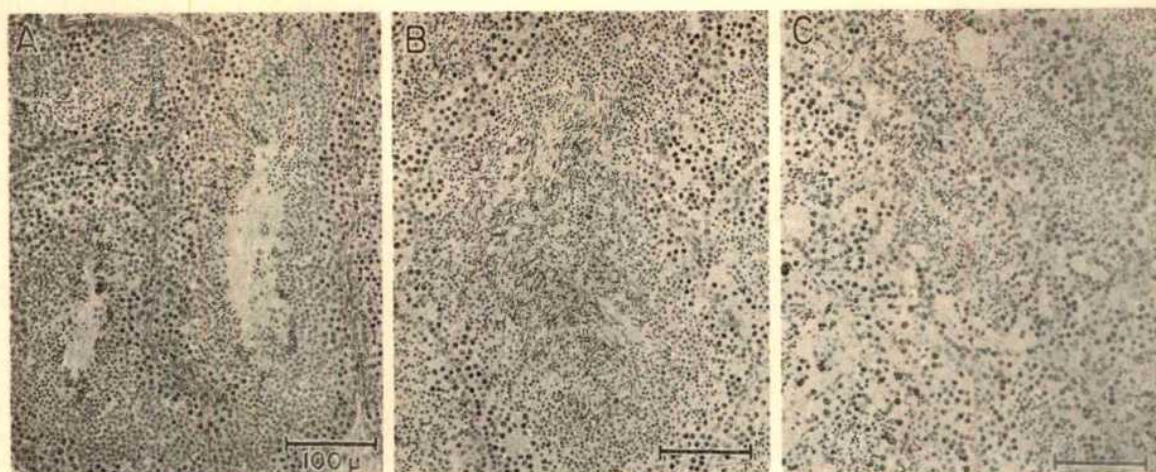


Fig. 1. The effects of temperature on testicular explants from a single *Uma scoparia*. (A) Tissue control stained with haematoxylin and eosin before cultivation; (B) autoradiograph, stained with haematoxylin, of tissue labelled with thymidine for 10 h and then cultured at 37° C for 5 days; (C) autoradiograph of explant labelled with thymidine and cultured at 40° C for 5 days. All tissues were fixed in Bouin, embedded in paraffin, and sectioned at 4µ.

lizard, *Uma scoparia*, from southern California. When provided with a wide choice of temperatures, the *Uma* maintain body temperatures between 34° C and 40° C, with an average of 37.5° C, whereas the *Anolis* regulate between 28° C and 36° C, with an average of 32.5° C (ref. 7).

Testes were divided into small explants, about 1–2 mm<sup>2</sup>. Each explant was cultured separately in 1 ml. of Waymouth's chemically defined medium (MB/752/1), using Chen's<sup>8</sup> modification of the watch glass method. Duplicate tests were made with Waymouth's medium supplemented with 5, 25, 50, or 100γ each of FSH and LH (follicle stimulating hormone, lyophilized porcine; and leuteinizing hormone, lyophilized equine), but these had little effect on the testicular cultures. Tissues were cultured for 3 and 5 days at various temperatures between 28° C and 44° C ( $\pm 0.2^\circ$  C) in a humidified atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. At the beginning of cultivation at each temperature, two explants from an individual were incubated for 10 h with 10–20 µc. of thymidine-methyl-<sup>3</sup>H (specific activity, 6.7 c./mmole). Another explant was incubated with the thymidine during the last 2 days of a 5 day cultivation. The viability and extent of differentiation of tissues were evaluated by the presence and localization of thymidine labelling and by comparing their general histological appearance with that of control tissues fixed before cultivation.

The effects of temperature on the testes of the two species are compared in Table 1. Maintenance of the germinal epithelium was best at the lowermost temperature used, 28° C, but there was little change or thymidine incorporation in these tissues. At intermediate temperatures, 32° C for *Anolis* and 37° C for *Uma*, thymidine incorporation was greatly enhanced, and there was evidence of several kinds of differentiation. A pronounced increase in the numbers of spermatozoa indicated that spermiogenesis had occurred in culture (Fig. 1B). Labelling was confined largely to spermatogonia and primary spermatocytes, with the latter labelled in all divisional

stages. Some labelled spermatids were present in all of the *Uma* cultures after 5 days at 37° C (Fig. 2), but these spermatids appeared to be newly formed and there was no labelling in spermatozoa. Thymidine incorporation, that is DNA synthesis, is completed before the onset of meiosis<sup>9,10</sup>, and so labelled spermatids must have differentiated from primary spermatocytes during cultivation. The spermiogenesis observed apparently involved differentiation of spermatids present before cultivation.

Spermatogenic arrest and progressive necrosis of all tissues occurred within 3–5 days at higher temperatures, starting at 35° C in *Anolis* and at 40° C in *Uma*. Extensive thymidine incorporation occurred even after 3 days, but there was no evidence of differentiation at these temperatures (Fig. 1C). Labelling was sparse or absent and all tissues were necrotic within the first 2–3 days in *Anolis* cultures at 40° C and in *Uma* cultures at 44° C. A marked reduction in spermatids at high temperatures suggests that these were the least heat-resistant cells in the germinal epithelium; spermatogonia appeared to be the most heat-resistant. These degenerative changes are similar to those described for the lizard testis *in vivo*<sup>6</sup>.

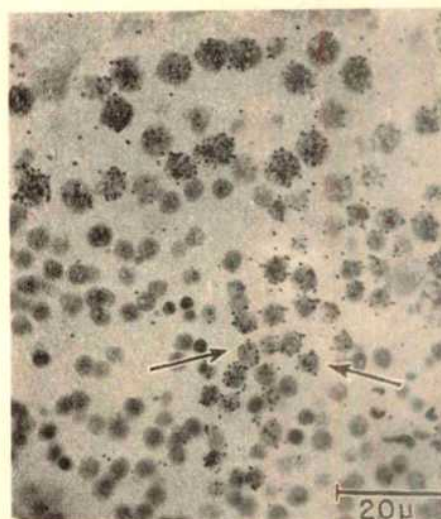


Fig. 2. Autoradiograph of Feulgen stained section (4µ) of a testicular explant from *Uma scoparia* labelled with 10 µc. of thymidine-<sup>3</sup>H for 10 h and cultured at 37° C for 5 days. Several spermatids labelled with thymidine are indicated by the arrows. The larger, more densely stained and labelled, primary spermatocytes are evident near the periphery of the seminiferous tubule, toward the top.

Table 1. INTERSPECIFIC EFFECTS OF TEMPERATURE ON THE CULTURED TESTIS

Temperature of Incubation ° C	<i>Anolis</i>	<i>Uma</i>
28	++	++
32	+++	++
35	+	—
37	0–+	+++
40	0	+
44		0

Three to five individuals were examined at each temperature. 0, Extensive degeneration within 3 days; +, labelling but with extensive necrosis within 5 days; ++, good maintenance but little activity; +++, maximal labelling and differentiation.



Although parallel responses to increasing temperature were observed in the testes of both species, the germinal epithelia of the more thermophilic *Uma* were clearly more heat-resistant (Table 1). The extent of this interspecific divergence in heat-resistance is about 5° C and thus corresponds closely to the difference between the average preferred body temperatures of the two lizards. This parallelism in the thermal responses of tissue and organism provides additional evidence of the physiological adjustments that underlie the selection of different body temperatures by the lizards. Furthermore, the divergence in testicular heat-resistance is very similar in magnitude to that observed in several somatic tissues from species with preferred temperatures similar to the two investigated here<sup>11</sup>.

The absolute heat-resistance of reproductive tissues and of somatic tissues in any one species may differ. If such a discordance exists, our data support the view<sup>6</sup> that it is less pronounced than in many other animals, especially the scrotal mammals. The performance of a tissue *in vitro* may not accurately reflect its performance *in vivo*, but the thermal responses of lizard testes in culture are consistent with observations on intact animals<sup>6</sup>. The testes of each species could be successfully cultured at temperatures corresponding to the range preferred by the species, but deterioration of the germinal epithelia occurred within a few days at temperatures only slightly above this level. In contrast, mammalian testes have not been successfully cultured at temperatures corresponding to their normal body temperatures, requiring instead the lower temperatures characteristic of the scrotum<sup>12</sup>.

Cowles<sup>6</sup> has postulated that the mammalian scrotum evolved during the development of homeothermy because "... the optimum body temperature eventually increased beyond the point at which reproduction was still pos-

sible. . . ." Lizards appear to have evolved the same high optimal body temperatures—their preferred levels—as have the mammals. Commensurate increases in the heat-resistance of the saurian germinal epithelium have, however, permitted the retention of the testis intra-abdominally. The longer exposure to high body temperatures in homeotherms could have been a factor in prohibiting the maintenance of the testis within the body cavity. In this regard, it would be interesting to have comparative data for the heat-resistance of germinal epithelia from homeotherms without scrota.

The saurian testes apparently represent excellent material for *in vitro* investigations of reproductive physiology. They lend themselves well to culture, and both the extent and rate of differentiation are far greater than that observed in cultured testes of other animals<sup>13</sup>.

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## On Concomitant Immunity in Tumour-bearing Hamsters

by

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Hamsters grafted with a lymphoblastic lymphoma are refractory to reinoculation with cells of the same tumour, although the initial graft grows progressively. The nature, strength, and development of this "concomitant immunity" have been investigated and its possible role in the control of metastases has been discussed.

EARLY in the study of tumour transplantation, Ehrlich<sup>1,2</sup> described the phenomenon whereby animals already bearing one tumour were sometimes resistant to a second graft; the new tumour was not accepted, even though the first lesion continued to grow progressively. Similar findings were reported by other investigators and the term "concomitant immunity" was introduced by Bashford *et al.* in 1908 (ref. 3). Many features of this process were, however, obscure: its incidence and specificity were disputed and the underlying mechanisms were completely unknown. Nutritional factors were implicated by Ehrlich in his theory of athrepsis, but this was quickly discarded. More specific immunological processes were later postulated although there was little direct evidence for such views. All attempts to transmit the immune state with serum were unsuccessful and, in 1929, Woglom<sup>4</sup> observed that "the athreptic theory . . . had been overthrown, but no explanation of the resistance had been substituted". In recent years, immunological factors

controlling the growth of tumours have been widely studied but almost all this work has been concerned with immunity induced by various procedures which *prevent* the immunizing graft from progressive growth<sup>5</sup>. By contrast, concomitant immunity—in which the immunizing tumour continues to grow—has attracted little attention and many of its paradoxical features are still obscure.

Two homotransplantable lymphomata from hamsters have been investigated in this laboratory, one of which regularly induces a classical state of concomitant immunity<sup>6</sup>. In the present paper, this experimental model has been used to investigate three aspects of the subject: the transfer of concomitant immunity, its development, and its strength. Functional implications are also briefly considered and the possible role of concomitant immunity in suppressing metastatic growth is emphasized.

In the first series of experiments, young Syrian golden hamsters from an inbred line (*MHA*)<sup>7</sup> were used, weighing 75–90 g. Initially, cell suspensions of the lymphoma were prepared according to a method previously described<sup>8</sup> and  $1 \times 10^7$  cells were injected subcutaneously into the right flank of twelve donor animals. Three weeks later, when the

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tumours were well developed and measured 2-4 cm in diameter, the hamsters were separated into two groups. Six animals were re-challenged with  $1 \times 10^8$  tumour cells grafted into the opposite flank and the remainder were killed. The tumours and spleens were removed and cell suspensions were prepared from each, mixed together in varying proportions, and inoculated into thirty-two normal isogenic recipients. Animals in the first group demonstrated the usual features of concomitant immunity; all the new tumour grafts were promptly rejected while the older lesions continued to grow. The findings in the second group are recorded in Table 1, which shows that spleen cells from tumour-bearing animals can suppress the growth of tumours in normal hamsters.

Table 1. EFFECT OF MIXING TUMOUR CELLS AND SPLEEN CELLS FROM TUMOUR-BEARING ANIMALS AND INOCULATING THE MIXTURE INTO ISOGENIC RECIPIENTS

No. of tumour cells/ml.	No. of spleen cells/ml.	No. of animals developing tumours
$2 \times 10^6$	$2 \times 10^6$	0/8
$2 \times 10^6$	$2 \times 10^7$	1/8
$2 \times 10^6$	$2 \times 10^8$	1/8
$2 \times 10^6$	$2 \times 10^9$	4/8
$2 \times 10^6$	—	8/8

Notes: (1) Tumour cells and spleen cells were mixed together and inoculated into the subcutaneous space at once, without a period of incubation; the final volume of cell suspension was 1 ml. (2) The presence of tumour in the recipient animals was assessed at autopsy, 3 weeks after grafting. These comments also apply to data presented in Tables 2 and 4.

Further experiments were carried out to test the specificity of this effect. These can be summarized as follows. (a) Cell suspensions of other organs from the original tumour-bearing hamsters—liver and kidney—failed to suppress the growth of tumours in normal recipients. (b) Spleen cells from normal *MHA* hamsters were ineffective; similarly, spleen cells from normal animals injected intravenously 2 days previously with 0.3 ml. of a solution containing 10 mg of 'Zymosan' (a non-specific stimulant of the reticuloendothelial system<sup>9</sup>) exerted no demonstrable effect. (c) Additional investigations were made, using a different homotransplantable lymphoma. The new tumour was readily accepted by animals which had recently rejected a second graft of the original tumour. Second, spleen cells from the original tumour-bearing hamsters did not inhibit the growth of the new lymphoma in normal recipients. Third, the reverse situation was also examined in which spleen cells from animals grafted with the new tumour were mixed with the original tumour and inoculated into normal hamsters; no suppression of the growth of the tumour followed. The main control experiments are shown in Table 2.

Table 2. EXPERIMENTS TO DEMONSTRATE THE SPECIFICITY OF THE SUPPRESSION OF TUMOUR GROWTH BY SPLEEN CELLS FROM TUMOUR-BEARING ANIMALS

Source and No. of tumour cells/ml.	Source and No. of spleen cells/ml.	No. of animals developing tumours
O $2 \times 10^6$	Normal $2 \times 10^7$	7/7
O $2 \times 10^6$	Normal $2 \times 10^8$	7/8
O $2 \times 10^6$	Treated with 'Zymosan' $2 \times 10^7$	5/5
O $2 \times 10^6$	Treated with 'Zymosan' $2 \times 10^8$	5/5
O $2 \times 10^6$	Animals bearing tumour D, $2 \times 10^7$	5/5
D $2 \times 10^6$	Animals bearing tumour O, $2 \times 10^7$	5/5
O $2 \times 10^6$	Animals bearing tumour O, $2 \times 10^7$	0/7

Note: In most of the tests the original tumour was used, but in two experiments a different homotransplantable lymphoma was used: the two tumours are listed as 'O' and 'D' respectively.

Repeated attempts to transfer concomitant immunity with serum from tumour-bearing animals were unsuccessful, despite the use of several different immunizing schedules; some animals received up to 5 ml. of serum, in divided doses. Similarly, all tests for cytotoxic antibodies in these sera were negative when the technique of Gorer and O'Gorman<sup>10</sup> was used. Three conclusions may be drawn. First, spleen cells from tumour-bearing hamsters can suppress tumour growth in normal isogenic recipients; second, such activity appears to be highly specific; third, serum from tumour-bearing animals is ineffective. These observations would seem to fulfil the usual criteria of an immune reaction mediated by cells.

Table 3. DEVELOPMENT AND STRENGTH OF CONCOMITANT IMMUNITY IN TUMOUR-BEARING HAMSTERS

Immunizing graft tumour cells/ml.	Second challenging graft tumour cells/ml.	Time of challenge days	No. of developing tumours
Test animals			
$1 \times 10^7$	$1 \times 10^6$	7	2/8
$1 \times 10^7$	$1 \times 10^6$	14	0/8
$1 \times 10^7$	$1 \times 10^6$	21	0/6
$1 \times 10^7$	$1 \times 10^7$	7	6/8
$1 \times 10^7$	$1 \times 10^7$	14	0/8
$1 \times 10^7$	$1 \times 10^7$	21	1/6
$1 \times 10^7$	$1 \times 10^8$	7	8/8
$1 \times 10^7$	$1 \times 10^8$	14	1/8
$1 \times 10^7$	$1 \times 10^8$	21	5/6

Control animals (hamsters from the same series which did not receive an immunizing tumour graft at day 1)

—	$1 \times 10^6$	7	4/5
—		14	5/5
—		21	5/5
—	$1 \times 10^8$	7	2/5
—		14	3/5
—		21	2/5

A second series of investigations was carried out to determine the strength of this immune state. Sixty-six normal hamsters received a subcutaneous graft of  $1 \times 10^7$  tumour cells and were subsequently re-challenged in the opposite flank with a second tumour graft. Different concentrations of cells and different times of challenge were tested and the results are summarized in Table 3. Predictably, the size of the challenging graft and the stage at which it was inoculated were found to be important factors, but it is clear that the state of concomitant immunity is quickly established and is remarkably potent. At its height, animals can reject 100,000 more cells than are necessary to produce tumours in normal control hamsters. Despite this, we would again emphasize that, in each case, the immunizing tumour continues to increase in size.

Additional experiments were performed to establish an approximate comparison between the strength of concomitant immunity and that obtained by other workers in specifically immunized animals. Old and his colleagues<sup>11-13</sup> investigated the transfer of immunity with peritoneal cells from such animals and their techniques were closely followed. Five donor *MHA* hamsters received a subcutaneous graft of  $1 \times 10^7$  cells. When the tumours were 2-4 cm in diameter, a peritoneal exudate was raised with a single injection of a 3 per cent starch solution, using the method of Bennett<sup>13</sup>. Two days later, the peritoneal cells were recovered by washing out the abdominal cavity with sterile culture medium ('M 199'); cell suspensions were prepared, mixed with tumour cells and inoculated into sixteen normal isogenic recipients. The results are summarized in Table 4 and indicate that peritoneal cells, combined with tumour cells in a ratio of 1:10, can suppress tumour growth in normal animals; peritoneal cells from normal animals are ineffective. The difficulties in comparing such findings with those previously reported, obtained from different experimental models, will be obvious; but it seems reasonable to conclude that the strength of the immune state is at least broadly comparable in the two circumstances.

The present findings show that concomitant immunity in tumour-bearing hamsters is a strong, highly specific process, mediated by immune cells which can suppress tumour growth in normal isogenic recipients. The co-existence of a progressively growing tumour and a large number of immune cells is particularly interesting in view of recent work by Mikulska *et al.*<sup>14</sup>, who demonstrated that autochthonous spleen cells from

Table 4. EFFECTS OF MIXING TUMOUR CELLS AND PERITONEAL CELLS FROM TUMOUR-BEARING ANIMALS AND INOCULATING THE MIXTURE INTO ISOGENIC RECIPIENTS

No. of tumour cells/ml.	No. of peritoneal cells/ml.	No. of animals developing tumours
$1 \times 10^6$	$1 \times 10^6$	0/8
$1 \times 10^6$	$1 \times 10^8$	0/8
$1 \times 10^6$	$1 \times 10^8$ normal cells	8/8

rats with fibrosarcomata induced chemically did not suppress tumour growth in isogenic recipients unless the primary tumour was resected 3 weeks before the spleen cells were obtained. It should, however, be emphasized that the experimental system used in this work differed in many respects from our own.

It is also clear that, judging by the quantitative results of the studies of cell transfer, concomitant immunity is extremely potent. Comparison with results from other experimental models is hazardous, but it appears that, at its height, the immune state is at least roughly comparable with that produced in animals immunized with tumours which do not grow progressively. In the face of this strong concomitant immunity, other mechanisms which have been postulated to explain the progressive growth of immunizing tumour grafts—self-enhancement<sup>15</sup>, inadequate antigenic stimulation<sup>16</sup>, impaired immune responses<sup>16</sup>—do not seem to be operative in the present circumstances.

It is likely that concomitant immunity has important functional implications, notably in suppressing metastatic growth. Evidence that immunological factors may participate in controlling the metastatic spread of tumours has been presented by Feldman and Yaffe<sup>17</sup>, and a direct association between concomitant immunity and the suppression of metastasis was postulated many years ago by Gay<sup>18</sup>; but, as in so many other aspects of this subject, the older data are conflicting. Four features suggest that such a relationship may exist, at least with the hamster lymphoma under investigation. (i) Although this tumour is poorly differentiated and grows very rapidly, it does not metastasize: at least 2,000 autopsies have been carried out on these animals since 1964, but deposits of secondary growth have never been found. (ii) Despite this, tumour cells soon appear in the circulation and persist until the death of the animal. They are clearly viable because subcutaneous inoculation of whole blood into normal recipients produces a high incidence of tumours<sup>6,19</sup>. (iii) Tumour cells are also demonstrable in the regional lymph nodes<sup>8,20,21</sup>. A vigorous histological reaction is mounted but, again, viable cells must be present in considerable numbers; if cell suspensions are prepared from these nodes and inoculated into normal animals, many of the recipients develop tumours, an effect which is demonstrable throughout the life span of the tumour-bearing animal<sup>19</sup>. (iv) Some preliminary observations indicate that if the tumour is grafted into animals which are treated with an immunosuppressive agent—a rabbit anti-hamster thymocyte serum, raised according to the method

of Levey and Medawar<sup>22</sup>—important changes are produced: first, the tumour quickly metastasizes to regional and mediastinal nodes; second, concomitant immunity appears to be abrogated. These observations are now being extended and, when complete, will be reported separately, but they are clearly relevant to the present argument.

Certain limitations of our experimental model will be obvious. The tumour used to induce concomitant immunity has been maintained by serial transplantation for several years: its antigenic structure is, as yet, unknown and it is not isologous with respect to the test animals. The findings reported here emphasize, rather than resolve, the paradox of concomitant immunity and no mention has been made of possible mechanisms whereby the immunizing tumour is able to resist destruction and grow progressively; this aspect has, however, been under investigation and will be the subject of a separate paper. The most important aspect of the present work is the demonstration that progressive growth of a tumour is not incompatible with a strong, specific immune response.

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## Inhibition of Tumour Cell Viability by DNA

by

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Specific preparations of DNA inhibit the viability of specific tumour cell lines. The effect may be due to some special characteristic of the DNA molecule, such as a specific region of nucleotide sequence, as well as molecular size, shape or charge.

It has been demonstrated that mouse thymus DNA specifically inhibits the growth of L1210 tumours, when L1210 cells are incubated with DNA and then injected subcutaneously into mice<sup>1-3</sup>. DNA from both L1210 and *Escherichia coli* is ineffective. Degradation by deoxyribonuclease (DNase), denaturation by heating to 100° C, or ultra-violet irradiation of the mouse thymus DNA abolish its ability to inhibit growth. Treatment with ribonuclease (RNase), or heating to 60° C, however,

permits the inhibition by DNA of tumour growth. Additional work has shown that mouse thymus DNA but not L1210 DNA, *E. coli* DNA, or DNA from mouse thymus treated with DNase, inhibits the viability of L1210 cells *in vitro*<sup>2-5</sup>. The mouse thymus DNA, but not L1210 DNA or *E. coli* DNA, also stimulates synthesis of DNA and RNA before inhibiting the viability of L1210 cells<sup>4,6</sup>. Results obtained with the use of specific metabolic inhibitors at particular intervals of time suggest that at least some of

the RNA enhanced with DNA is directly transcribed by the mouse thymus DNA rather than only by the DNA of the L1210 cells (Glick, J. L., and Sahler, C., to be published). These experiments all indicate that the action of mouse thymus DNA on L1210 cells may result from some special characteristic of the DNA, such as a specific region of nucleotide sequence, besides more general properties such as molecular size, shape, and charge. The present report supports this hypothesis, because only specific preparations of DNA were found to inhibit the viability of specific cell lines.

DNA was prepared from mouse thymus, mouse liver, mouse spleen, calf thymus, human spleen, human leukaemia, L1210 mouse leukaemia, and SB1 hamster small bowel carcinoma. Except for the DNA from calf thymus, all the other preparations of DNA were isolated by the method of Szybalska and Szybalski<sup>6</sup> in this laboratory. The calf thymus DNA was obtained commercially but was purified<sup>6</sup> by us. Human spleen DNA was obtained separately from two female patients (aged 46 and 48 years, respectively), whose spleens were diagnosed free of disease. Human leukaemia DNA was obtained from cell line RPMI No. 5630 (originating from a chronic myelogenous leukaemia) cultured in medium RPMI No. 1640 plus 10 per cent foetal calf serum. The mouse DNA preparations were all obtained from young male DBA2 mice. L1210 DNA and SB1 DNA were obtained from L1210 cells cultured in medium RPMI No. 1634 plus 5 per cent foetal calf serum, and from SB1 cells cultured in medium RPMI No. 1640 plus 5 per cent foetal calf serum, respectively. All DNA preparations were treated with RNase and repurified<sup>6</sup> before use. In some cases mouse thymus DNA and human spleen DNA were degraded with DNase<sup>6</sup> in order to make them biologically inactive.

The following cell lines were treated with DNA: L1210 mouse leukaemia (RPMI No. 3116), S-37 mouse sarcoma, S-180 mouse sarcoma, Ehrlich mouse carcinoma, human leukaemia RPMI No. 1245 (originating from a chronic myelogenous leukaemia), human lymphoma P-3J (a Burkitt lymphoma), and human mesothelioma (RPMI No. 212). L1210 cells were grown *in vitro*, as already noted. S-180 and Ehrlich cells were maintained in the ascitic form in male Swiss mice. S-37, human leukaemia RPMI No. 1245, human lymphoma, and human mesothelioma cells were cultured in media RPMI No. 1640 plus 20 per cent foetal calf serum, RPMI No. 1634 plus 10 or 20 per cent foetal calf serum, RPMI No. 1640 plus 10 per cent foetal calf serum, and McCoy medium plus 10 per cent foetal calf serum, respectively. In all experiments cells were washed several times at 2°C and then very slowly shaken at 37°C in 4 ml. of a phosphate-buffered saline<sup>6</sup>, pH 7.5, containing 5.5 mmolar glucose and 0.2 mmolar spermine tetrahydrochloride. The cells (10<sup>6</sup>/ml.) were treated for 30 min with DNA (60 µg/ml.), then washed at 2°C and re-incubated at 37°C in 4 ml. of phosphate-buffered saline containing glucose and free of DNA but not spermine. Control cells were incubated for 30 min without DNA and then washed and re-incubated in a similar fashion to the cells treated with DNA.

Cell viability was determined by adding 0.2 ml. of 0.1 per cent trypan blue (dissolved in Hanks balanced salt solution) to 0.2 ml. of cell suspension; only unstained cells were considered viable<sup>7,8</sup>. Furthermore, cell lysis proceeded most readily when the percentage of stained cells was high, thus indicating that uptake of dye reflected the death of cells rather than mere changes in permeability<sup>4</sup>. In the experiments described here the number of viable cells in each group treated with DNA was compared with the number of viable cells in the corresponding control group. This viability index of the cells treated with DNA was then expressed as a percentage change from the control cells.

The results listed in Table 1 summarize the effects of various DNA preparations on the viability of L1210 cells. Those DNA preparations which depressed cell viability

were about equally effective. In Fig. 1a, therefore, the results of the inhibitory DNA solutions (group I) were pooled, as were the results of the non-inhibitory DNA solutions (group II). At least three points are worth considering. (i) Inhibition of cell viability was not apparent during the initial 30-min exposure of L1210 cells to "group I DNA". Such inhibition, however, did develop on re-incubation of the cells in the absence of DNA. (ii) Cell death was induced with mouse DNA isolated from two other normal tissues besides thymus. In contrast, L1210 DNA, which was obtained from malignant mouse cells grown either *in vivo*<sup>2,3</sup> or *in vitro*, had no effect on cell viability. (iii) Inhibition by DNA of the viability of L1210 cells was dependent on the species from which the DNA was derived. Thus, DNA prepared from calf or human normal tissues or from hamster malignant tissue was not inhibitory.

Table 1. RESPONSE OF L1210 CELLS TO VARIOUS DNA PREPARATIONS

DNA preparation	Inhibition of cell viability
Group I	
Mouse thymus DNA	+
Mouse liver DNA	+
Mouse spleen DNA	+
Group II	
Calf thymus DNA	0
Human spleen DNA	0
Mouse leukaemia L1210 DNA	0
Hamster carcinoma SB1 DNA	0
DNase-treated mouse thymus DNA	0

+, Inhibition (cell viability at least 20 per cent less than that of the control cells, at 90 and 120 min after removal of the DNA); 0, no effect (cell viability within  $\pm 5$  per cent of that of the control cells, at 90 and 120 min after removal of the DNA).

Table 2. RESPONSE OF VARIOUS CELL LINES TO MOUSE THYMUS DNA

Cell line	Viability inhibited
Group A	
Mouse leukaemia L1210	+
Mouse sarcoma S-37	+
Mouse sarcoma S-180	+
Group B	
Mouse carcinoma (Ehrlich)	0
Human leukaemia RPMI No. 1245	0
Human mesothelioma RPMI No. 212	0

+, Inhibition; 0, no effect. See Table 1 for definitions of inhibition and no effect.

Table 2 lists several cell lines which were susceptible to mouse thymus DNA, as well as those cell lines which were not affected. Although three different mouse tumour lines were inhibited to the same degree by mouse thymus DNA, a fourth line—Ehrlich ascites cells—was not affected. Two different human tumour lines also were not inhibited by mouse thymus DNA. Fig. 1b combines the results of mouse thymus DNA on the susceptible cell lines (group A) and on the resistant lines (group B). The extent of inhibition caused by mouse thymus DNA on any of the three susceptible cell lines was similar to that caused by any of the group I preparations of DNA on L1210 cells (Fig. 1).

Figs. 2 and 3 demonstrate the inhibitory properties of the different human DNA preparations. Normal human spleen DNA inhibited the viability of two different human tumour cell lines—leukaemia RPMI No. 1245 (Fig. 2) and lymphoma P-3J (results not shown)—although it had no effect on L1210 cells (Table 1). Degradation of human DNA by DNase removed its inhibitory property (Fig. 2). Intact DNA prepared from human leukaemia RPMI No. 5630 did not inhibit cell viability of leukaemia RPMI No. 1245 but did depress cell viability of lymphoma P-3J (Fig. 3).

Smith and Cress<sup>9,10</sup> observed DNA inhibition of cell viability, but their results did not indicate the type of specificity reported here. They found that DNA preparations from both tumours and normal tissues were toxic to L cells in tissue culture. High concentrations of DNA (250–5,000 µg/ml.) were required, however, to cause the cells to die, and the DNA was added permanently to the culture medium. Toxic effects were not observed until



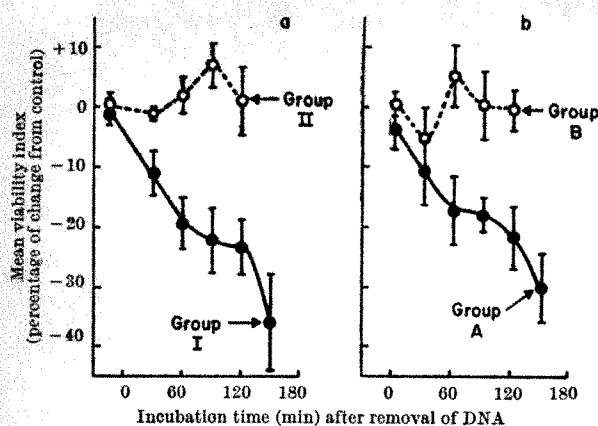


Fig. 1. *a*, Effects of various DNA preparations on L1210 cells; *b*, effects of mouse thymus DNA on various cell lines. The results obtained with the various DNA preparations listed under groups I and II in Table 1 were pooled respectively in *a*. The results obtained with the various cell lines classified under groups A and B in Table 2 were pooled respectively in *b*. Each of the three types of DNA belonging to group I was used in three or four experiments, a total of ten experiments being performed. Likewise, duplicate experiments were performed with each of the five types of DNA belonging to group II. Triplicate experiments with mouse thymus DNA were performed on each of the three cell lines belonging to group A, and duplicate experiments with mouse thymus DNA were performed on each of the three cell lines belonging to group B. The solid line designated by group I and the broken line designated by group II each represent the mean of ten experiments,  $\pm$  S.E.M. The solid line designated by group A represents the mean of nine experiments,  $\pm$  S.E.M., and the broken line designated by group B represents the mean of six experiments,  $\pm$  S.E.M. Control experiments, in which cells were incubated without DNA, were performed with each DNA experiment.

12 h after DNA had been added. Thus, the disparity between our results and those of Smith and Cress may perhaps be attributed to the different conditions of incubation. Our results demonstrate that under appropriate conditions the origins of both the exogenous DNA and the host cell line determine whether or not the DNA inhibits the viability of the cells. This finding is consistent with the suggestion that the base sequence of the DNA is somehow involved in causing the cells to die. There are several possibilities which may determine whether a given cell line will remain unaffected after exposure to a specific exogenous DNA. (i) Various cell lines may be able to

degrade the entering nucleic acid almost immediately<sup>11,12</sup>. (ii) Some cell lines may either repress transcription of information initiated by exogenous DNA or inactivate end-products or proteins resulting from the translation of this information. (iii) Other cell lines may lessen the availability of their enzymes for synthetic processes involving a foreign DNA. (iv) Exogenous DNA may bring about the death of cells by inducing the formation of gene products which may interact only with specific antigenic constituents of particular cell lines (Glick, J. L., and Sahler, C., to be published). The absence of these antigenic constituents in other cell lines would preclude the recognition and destruction of the host cells by the newly formed gene products. (v) The viability of the cells can be enhanced by DNA when incubation conditions are altered<sup>13</sup>; this phenomenon is independent of the type of DNA used and does not appear to be caused by extensive degradation of the DNA. Thus, in some cell lines specific inhibition of cell viability might be counterbalanced by non-specific enhancement, so that cell viability would not be affected by the DNA.

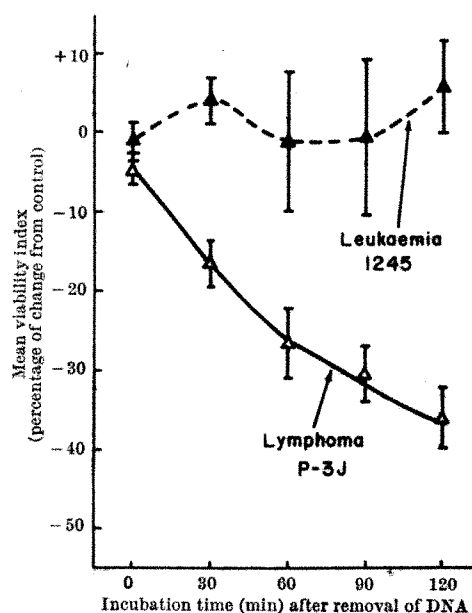


Fig. 3. Effects of intact DNA which had been obtained from human leukaemia RPMI No. 5630 on human leukaemia RPMI No. 1245 and human lymphoma P-3J. The solid line designated by lymphoma P-3J represents the mean of four experiments,  $\pm$  S.E.M. The broken line designated by leukaemia 1245 represents the mean of seven experiments,  $\pm$  S.E.M. Control experiments, in which cells were incubated without DNA, were performed with each DNA experiment.

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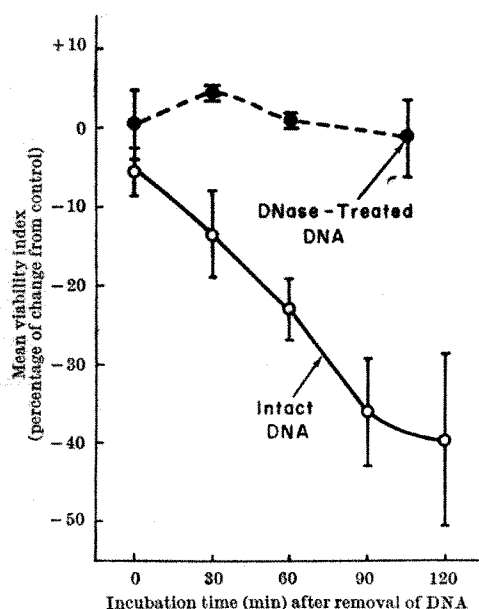


Fig. 2. Effects of intact and human spleen DNA preparations treated with DNase on human leukaemia RPMI No. 1245. The solid line designated by intact DNA represents the mean of seven experiments,  $\pm$  S.E.M. The broken line designated by DNA treated with DNase represents the mean of two experiments,  $\pm$  S.E.M. Control experiments, in which cells were incubated without DNA, were performed with each DNA experiment.

## Two ATPases in the Sarcoplasmic Reticulum of Skeletal Muscle Fibres

by

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Two distinct ATPase activities have been localized on the sarcoplasmic reticulum of muscle cells. They may play a part in contraction and relaxation.

THERE are two systems of membranes between the myofibrils in the sarcoplasmic spaces of skeletal muscle fibres: the sarcoplasmic reticulum (SR) and the transverse tubular system (T)<sup>1</sup>. They are in intimate contact in correspondence of the triads<sup>2</sup> but they do not communicate directly with each other.

Recent investigations<sup>3-7</sup> make it clear that the T system is an invagination of the sarcolemmal membrane, thus fulfilling the main requirement for a pathway to spread activation to the fibrils. On the other hand, the sarcoplasmic reticulum is a prominent part of the contraction-relaxation mechanism<sup>8-11</sup>. The probable physiological activator of contraction, that is, calcium ions<sup>12</sup>, is captured within the lateral sacs of the triad when the muscle is in the resting state and is released from them during contraction<sup>13,14</sup>. ATP localized in restricted areas of the reticulum<sup>15</sup>, which might correspond to the triadic sites, could furnish the high energy phosphates needed for this phenomenon to take place<sup>9,16</sup>.

Histochemistry under the optical microscope indicates the existence of an ATPase at the level of sarcoplasmic reticulum<sup>17</sup> and triads<sup>18</sup> of skeletal muscles. Moreover, biochemical investigations have shown that the sarcotubular fraction has a magnesium-stimulated ATPase which is inhibited by calcium in the presence of magnesium<sup>19-21</sup>. Moreover, a sodium-activated ATPase has been found in the microsomal fraction of frog<sup>22</sup> and rabbit striated muscle<sup>23</sup>. Biochemical techniques do not, however, allow a precise localization of these enzyme activities in relation to the differentiations of structure in the sarcoplasmic reticulum. Histochemical techniques applied to electron microscopy may well overcome these limitations. In fact, recent electron microscope observations showed that a magnesium-activated ATPase occurs in the lateral sacs of the diads and triads of dog<sup>24</sup> and rat<sup>25,26</sup> myocardium, in the sarcoplasmic reticulum and T system of frog skeletal muscle<sup>27</sup> and in the correspondence of the triad components of *Opsanus tau* gas bladder muscle<sup>28</sup>. In the present article the characterization and localization of ATPase in the sarcoplasmic reticulum of frog and rat muscles have been investigated using this technique.

Frog ileofibularis and rat extensor digitorum longus muscles were tied at their resting lengths on strips of wood and fixed in cold 6.25 per cent glutaraldehyde buffered with 0.2 molar sodium cacodylate, pH 7.2, for 30-45 min. The fixed muscles were cut transversely in 3-4 mm pieces, washed in cold 0.1 molar sodium cacodylate, pH 7.2, and stored for 36 h in the same buffer at 0°-4° C. Thick (50-60 $\mu$ ) sections were cut on a freezing microtome and immersed in buffer for 15-20 min. Freely floating sections, briefly washed in distilled water, were incubated for 5-60 min at 37° C in a Wachstein and Meisel<sup>29</sup> medium

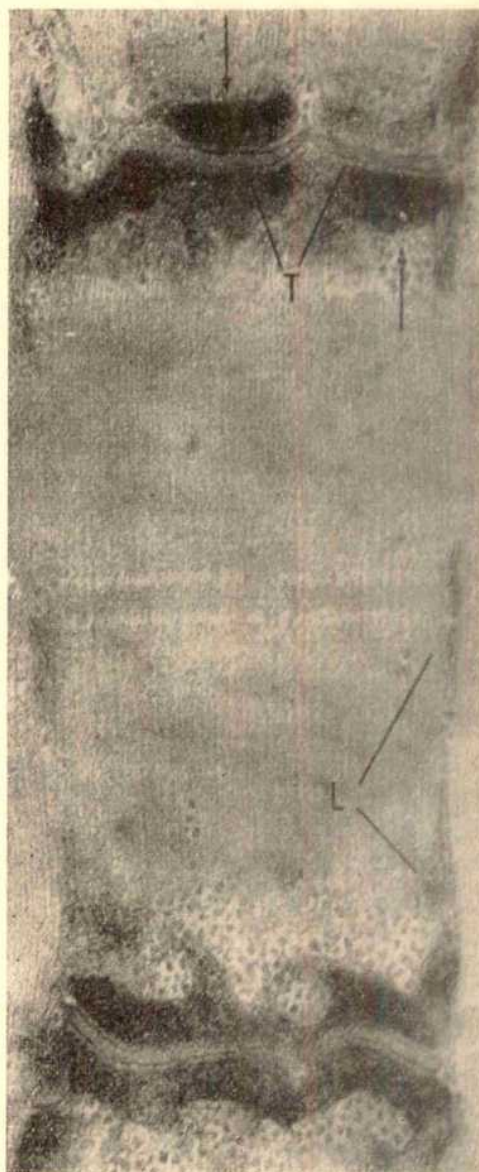


Fig. 1. Frozen section from frog muscle incubated for 60 min with magnesium and ATP as substrate. Reaction product is concentrated within the lateral sacs of the triad (arrows). Longitudinal portion of the SR (L) shows very low amounts of reaction product; none is present in the central tubule of the triad (T). ( $\times 45,600$ .)



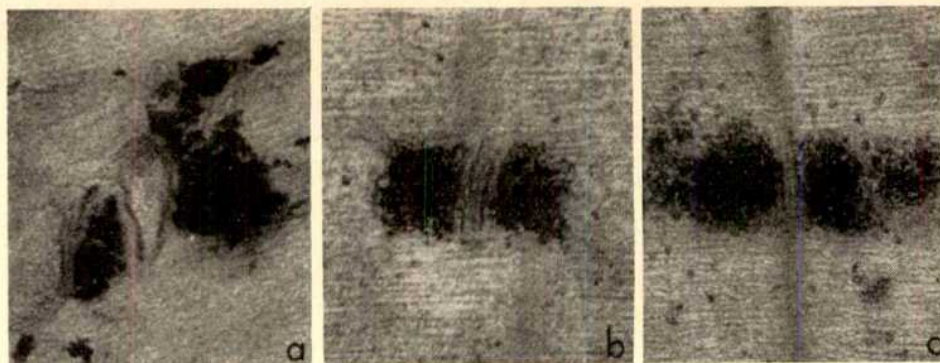


Fig. 2. Frozen sections from frog muscle incubated for 15 min in the presence of (a) magnesium alone, (b) calcium alone, (c) magnesium and calcium, with ATP as substrate. The reaction product is restricted to the lateral sacs of the triad. Smaller deposits of final product can be seen when magnesium is replaced by calcium (b); no significant decrease of reaction product when both magnesium and calcium are present (c). ( $\times 52,800$ .)

containing substrates, lead nitrate as the capture reagent and equimolar concentrations of ATP and magnesium<sup>30,31</sup>.

Similar media at the same pH, containing AMP, ADP, IDP, ITP, TPP or  $\beta$ -glycerophosphate as substrates, were also tested. In each case control preparations were incubated in media from which the substrates were omitted.

After incubation the sections were rinsed briefly in distilled water, post-fixed in cold 2 per cent osmium tetroxide buffered with veronal-acetate to pH 7.4, with added sucrose, for 30 min, dehydrated in graded ethanol and embedded in 'Epon 812'<sup>32</sup>. Thin sections were cut on a Porter-Blum microtome, and examined under an electron microscope.

When the effects of activators or inhibitors of the enzyme activity were studied, thick sections prepared from the same piece of muscle were incubated and treated in parallel. The results were assessed by examining thin sections prepared from at least three blocks derived from different thick frozen sections. Only the results obtained in the well fixed peripheral region of the muscle were retained.

In longitudinal sections, incubated with magnesium and using ATP as substrate, the final product of the enzyme activity is mainly concentrated within the lateral sacs of the triad, while the longitudinal portion of the sarcoplasmic reticulum shows much smaller amounts of lead phosphate (Fig. 1).

With ADP, IDP, ITP or TPP as substrates the location of the precipitate is similar to that with ATP. The sections incubated with AMP show no reaction product within the reticulum.

Incubations which were run simultaneously in the presence of magnesium alone, calcium alone or magnesium and calcium (all 2 mmolar), under ATP as substrate, show smaller deposits of the final product when magnesium is replaced by calcium and no significant decrease of the final product when both magnesium and calcium are present (Fig. 2). Possibly, the failure to find inhibition by calcium is due to the retention of sufficient amounts of calcium in the SR of the fibre in the basal condition of incubation.

If magnesium and ATP are used in equimolar concentrations the simultaneous presence of sodium (125 mmolar) and potassium (12.5 mmolar) leads to a marked additional deposition of lead phosphate within the lateral sacs of the triad (Figs. 3 and 4).

Ouabain (1 mmolar), a specific inhibitor of transport ATPase, completely inhibits this additional activation of the magnesium dependent ATPase activity (Figs. 3 and 4). These effects are more evident when shorter incubation times (5–15 min) are used. At the level of the sarcolemma, particularly on the membrane of the pinocytotic vesicles, activation of ATPase by monovalent cations and inhibition by ouabain were also evident.

In sections incubated with  $\beta$ -glycerophosphate as substrate no reaction product is encountered in the terminal segments of the sarcoplasmic reticulum, whereas lead phosphate is deposited at the level of the sarcolemmal membrane.

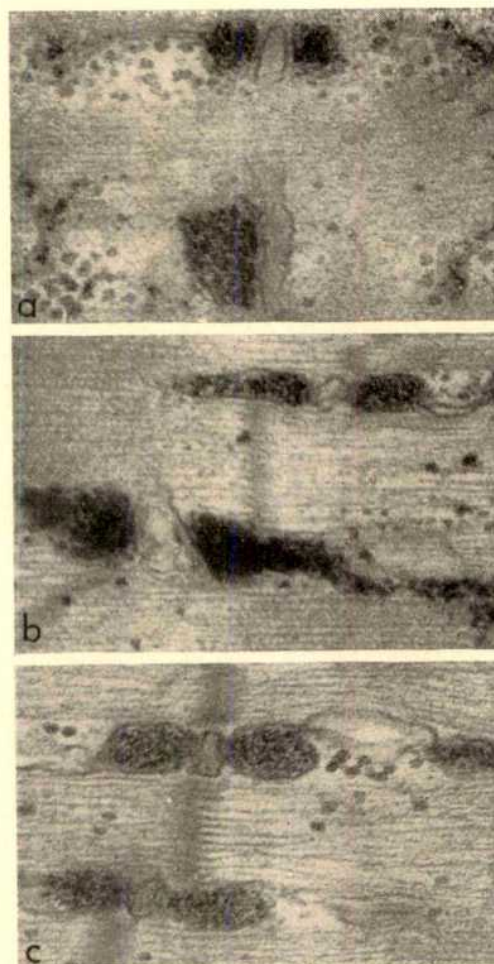


Fig. 3. Frog muscle incubated in parallel for 15 min with magnesium-ATP equivalent medium. (a) Fine granular lead phosphate deposit in the lateral sacs of the triad in the presence of magnesium alone. (b) Heavy lead phosphate precipitate can be seen when sodium and potassium are simultaneously added. (c) Ouabain (1 mmolar) clearly decreases the additional amount of precipitate of the final product seen in b. ( $\times 53,200$ .)



In order to test the specificity of the localization of the enzyme activity, floating sections were immersed in 1 per cent buffered lead nitrate, pH 7.2, for 15 min and then incubated for the same length of time in a solution of inorganic phosphate in order to produce lead phosphate (Page and Gillis, personal communication).

This control showed, both in the frog and in the rat muscles, that lead phosphate deposits were absent from the reticulum but present in coarse aggregates on the myofibrils, mainly in conjunction with the corresponding peripheral part of the A band (Fig. 5).

Our observations show that both ATPase activities, one stimulated by magnesium and the other activated by sodium and potassium, are located chiefly at the level of the outer sacs of the triad, and are far less evident, although they are not absent, at the correspondence of the longitudinal portion of the sarcoplasmic reticulum.

This topographical difference may reflect the real situation, or may be due to the effect of different susceptibilities of the enzymes present in the two parts of the SR to the inactivating action of the fixative. In both cases, however, the inactivating action of the fixative shows a clear heterogeneity.

In the intermediate component of the triad, the T tubule, no ATPase or other phosphatase activity was ever detected. This contrasts with the findings of Zebe<sup>27</sup> obtained after very short fixation with osmium tetroxide, with the possibility of artefacts due to the diffusion of soluble enzymes from unfixed tissue.

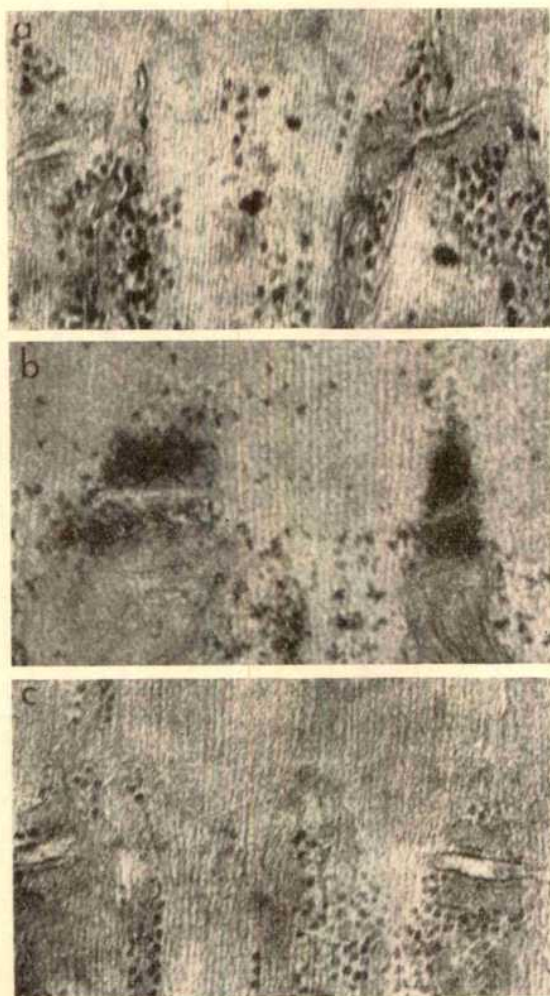


Fig. 4. Rat muscle incubated in parallel for 5 min with magnesium-ATP equivalent medium. (b) Reaction product is present when sodium and potassium are added. No reaction product can be seen if magnesium is used alone (a) or ouabain is added (c) to the medium used in (b). ( $\times 59,200$ )



Fig. 5. Frog muscle immersed in 1 per cent lead nitrate for 15 min and then incubated in an inorganic phosphate solution for 15 min. Coarse aggregates of reaction product can be seen at the peripheral part of the A band. ( $\times 9,200$ )

Deposition of lead precipitate has regularly been observed within the terminal sacs of the triad. This finding is similar to that of Essner *et al.*<sup>25</sup> in the sarcoplasmic reticulum of rat heart. The question then arises whether this localization is apparent, that is, is due to enzyme activity at the level of the membrane which limits the cisternae, with subsequent diffusion and fixation of the reaction product in the interior of the cisternae, or whether it is real, implying that both ATPase activities are present inside the terminal sacs of the SR. Although it does not at present seem possible to settle this question, it must be emphasized that such localization has also been observed after very short periods of incubation (down to 5 min), a situation which should minimize possible diffusion artefacts. The contents of the cisternae may have a strong affinity for the lead precipitate, but the negative results of the controls with phosphate and lead do not support this possibility. Finally, it is known that, particularly after fixation with glutaraldehyde, the outer sacs of the triad show a clear opaque content<sup>6,23-25</sup>, which may be the structural framework for the enzymes the activity of which has been detected here. The discrepancies between our demonstration of the presence of an ATPase activated by sodium and potassium and inhibited by ouabain in the lateral sacs of the triad, and the failure of Fratantoni and Askari<sup>23</sup> to find such activity in a sarcotubular cell fraction, may be due to the composition of the microsomal fraction tested, which had a very low calcium uptake, and from which the lateral sacs of the triad might have been almost absent.

So far as the physiological significance of these findings is concerned, the striking fact emerges that the sites which, according to Hasselbach<sup>13</sup> and Costantin, Franzini-Armstrong and Podolsky<sup>14</sup>, function as calcium sinks during relaxation of the muscle fibre are also the seats of at least two distinct ATPase activities. On the basis of biochemical experiments on sarcotubular fractions there seems to be a relationship between ATPase and calcium sequestration<sup>8-10,19,21,26</sup>. The present results, which show

an exact structural coincidence between these two processes, give further weight to such an association. Besides, the experiments of Hill in toad muscle<sup>15</sup> showed that ATP is located between the fibrils in the I band, almost certainly in a component of the reticulum, although there is insufficient evidence for its identification with the triadic elements. This finding at least indicates a very close proximity between the two ATPases and their specific substrate.

As regards the ATPase stimulated by sodium and potassium, it should be noted that its presence in the SR parallels the finding that this enzyme is present in the microsomal fraction isolated from various other types of cells. In these, however, fragments of plasma membrane, which is known to possess ATPase activity, are likely to be present, and this could prevent a strict intracellular localization of the enzyme, whereas in our experiments it has been possible to localize it.

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## Toxicity of Serum from Irradiated Donors

by

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The "radiation sickness" which many mammals suffer may be due to a circulating by-product of energy absorption with toxin-like effects. When rats drink water flavoured with saccharin and are injected with serum from irradiated donors, they develop an aversion to this fluid. This effect had previously been observed in directly irradiated and poisoned animals.

MANY mammalian species, including man, display the syndrome of "radiation sickness" within a few hours after an exposure to sublethal doses of ionizing radiation. This may include nausea, emesis, and gastro-intestinal malaise. One of the most sensitive measures of this effect in experimental animals is the conditioned aversion test<sup>1</sup>. In essence, this test consists of giving an animal a distinctive solution (for example, water flavoured with saccharin) to drink in conjunction with radiation exposure. A single association of drinking the distinctive solution followed by radiation sickness will cause animals to learn to avoid the test fluid. Thus, a whole-body exposure to 10-30 r. will produce a significant reduction in the preference of a rat for saccharin<sup>2</sup>.

Various investigations suggest that this effect results from a circulating substance produced by the absorption of energy in the tissues of the exposed animal. First, the magnitude of the conditioned aversion response is related to total dose and is relatively independent of dose rate. Equivalent total doses produce similar effects though

exposure durations vary from minutes to hours. Second, the conditioned aversion can be produced by irradiation of a portion of the body, but the effect is not so great as if the whole body were exposed<sup>3</sup>. If two rats are joined by skin-vascular anastomosis, then the conditioned aversion response can be observed in a shielded animal when its partner is irradiated<sup>3</sup>.

If absorption of ionizing radiation by the organism does result in a stable circulating by-product with toxin-like effects on the behaving organism, then the serum from irradiated donor animals should produce the conditioned aversion response in non-irradiated recipient rats. The present experiment was conducted to test this inference.

White adult laboratory rats (Sprague-Dawley) were used as donors and recipients. The experimental donors received approximately 30 krads in 45 sec in a cobalt-60 irradiation unit recently described<sup>4</sup>. Two to four hours after irradiation, they were bled by cardiac puncture. The blood was allowed to stand for about 30 min-2 h before it was centrifuged at 2,800 r.p.m. for 20 min and



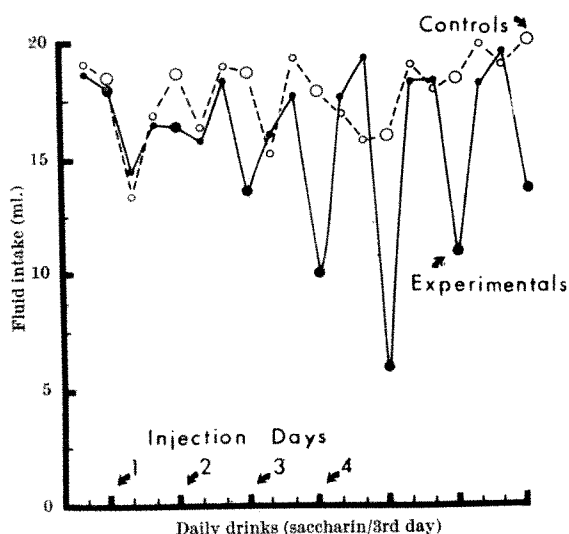


Fig. 1. The effect on the consumption of saccharin-water (large dots) by recipient rats, induced by associated injections of serum from irradiated (30 krad) donor rats. The small dots indicate the intake of tap-water on days between treatments. Control recipients, injected with serum from non-irradiated donors, did not learn to reject differentially the saccharin-water associated with injections.

then the serum was pipetted off and pooled. Thus, the recipients of serum received injections approximately 7 h after irradiation and 3–5 h after bleeding. The control donors were treated in an identical fashion except that they were not irradiated. Because 30 krad produced an obvious stress in the irradiated donors, the control donors were also stressed with a series of shocks (ten 0.5 sec pulses at 1.0 m.amp) during the period of confinement. These animals were lightly anaesthetized with ether for the cardiac puncture.

The recipient animals were maintained in individual cages and dry 'Purina' laboratory chow was freely available to them. Drinking was restricted to a 10 min period each day and the amount of the fluid intake was recorded. After a week of habituation to this schedule, the experimental treatment began.

Experimental treatment consisted of flavouring the drinking water by adding 1 g of saccharin/l. of water and allowing the animals to drink for 10 min as usual. Five minutes after the drinking period, the animals received an intraperitoneal injection of 4 ml. serum from irradiated donors. Two more injections were given at 15 min intervals so that each rat received 12 ml. on each treatment day. The recipient controls were treated identically except that they received serum from the non-irradiated control donors.

All recipients were offered water flavoured with saccharin every third day, and they received the treatment of multiple injections after drinking saccharin on the first four occasions. The last three occasions served as a measure of recovery of the effect of the injection series.

A second experiment was conducted with identical radiation and treatment conditions except that all the serum was collected from non-irradiated donors and frozen for storage. Several weeks later the serum was thawed to room temperature and then equal portions were exposed to 30 krad and injected into experimental recipients. Control animals received equal portions which had not been exposed.

After a single association of drinking water flavoured with saccharin followed by injections of serum from irradiated donors, the experimental recipients drank less water flavoured with saccharin than did the control recipients which were injected with serum from non-irradiated donors ( $P=0.05$ , ranks test). It should be recalled that the treatment of multiple injections follows the period of drinking saccharin so that the effect of the

first treatment is seen on the next period of drinking saccharin 3 days later (Fig. 1). The difference between experimental and control recipients increased with each treatment, so that after two injections this difference was highly significant ( $P<0.01$ ), and after four treatments there was no overlap in the distribution of saccharin intake scores of the two groups. In the absence of further treatment, experimental recipients began to increase their intake on the final two saccharin days, but the difference between the two groups remained significant ( $P<0.01$ ).

In contrast, no differential effect was observed in the second experiment, where the serum was exposed to 30 krad *in vitro*, that is after it was extracted from the donors. A statistical summary of the results of both experiments following four treatments is presented in Table 1. These results indicate that the aversive response does not arise from a direct effect of radiation on serum. Furthermore, because the control donors were stressed with electric shock and confinement, the effect is probably not due to a general stress factor such as an elevated concentration of adrenocortical steroids.

Table 1. CONSUMPTION OF SACCHARIN-WATER BY RECIPIENT RATS AFTER FOUR ASSOCIATIONS OF DRINKING SACCHARIN-WATER FOLLOWED BY INJECTIONS OF 12 ML. SERUM

Serum	N	Mean (ml.)	Range (ml.)
From irradiated donors	9	6	1–11
From non-irradiated donors	9	16	13–19
Irradiated <i>in vitro</i>	4	17	15–19
Non-irradiated <i>in vitro</i>	3	19	13–24

The aversive response displayed by the animals is equivalent to that observed when animals are directly treated with a series of 10–30 rads. Although it is not possible to be certain that the mechanism underlying the response produced by direct exposure of the animal is identical with that produced by injection of serum from irradiated donors, it is interesting to note that direct collimated exposure of the hip region of the rat is as effective as head exposure which indicates that the former effect probably does not result from irradiation of a specific class of tissue. On the other hand, exposure of a comparable area of the abdomen is about five times as effective as any other region. This may mean that the interaction of ionizing radiation with intestinal flora, waste, or food metabolic products is especially effective in the production of toxin-like substance or substances<sup>1</sup>.

In a series of investigations which in many ways parallel the saccharin aversion studies but differ in method, Steadman<sup>2,3</sup> has reported the isolation of a toxic substance from the urine of irradiated rabbits which, when injected intraperitoneally into non-irradiated test rabbits, produces many of the symptoms of acute radiation sickness. Only a small amount of this substance was found in the urine from normal controls. Furthermore, the time course of symptoms, particularly tachycardia, after injection of the toxic material from the urine of irradiated animals is similar to the time course of the development of the saccharin aversion after irradiation. Thus, it may be that this substance of yet unknown chemical composition may be the by-product of radiation exposure which produces radiation sickness and the conditioned aversion response.

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# Proteins in the Cell Walls of Some Green Algae

by

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Analysis of algal cell wall fractions suggests the presence of protein bound to the wall much as in higher plants.

THE frequently expressed belief that protein is present in plant cell walls, otherwise exclusively carbohydrate, seems finally to have been confirmed<sup>1</sup>. This confirmation stems from the demonstration that when a cell is fractionated a protein containing hydroxyproline also described elsewhere<sup>2</sup> separates in the main with the cell wall fraction and is firmly bound to the cell wall. This is one of the few proteins known other than collagen to contain this amino-acid. Unlike collagen, however, the protein—if a single protein only is involved—also contains cystine and, while hydroxyproline could serve only as a marker, it is to cystine that Lamporte looks for the function of the protein, based obliquely on the known effects of S-S bond splitting agents on yeast cell walls<sup>3</sup>. He gives good reason for the view that the protein is involved in the extensibility of cell walls during cell growth, and he has provisionally given it the name "extensin"<sup>1</sup>. All the evidence for the occurrence of this protein comes from higher plants; but it is evident that if it is of any fundamental significance it must also occur in lower plants. We have therefore examined a number of algae in an attempt to determine (a) whether a protein containing hydroxyproline occurs also in these plants; (b) whether the protein is associated with the cell wall; and (c) whether the protein is of a kind making it a likely candidate for the proposed role in extension. This is a preliminary account of our findings which are positive on all counts; the full results will be given elsewhere. During the writing of this article a carbohydrate-polypeptide complex has been reported in *Ascophyllum*<sup>4</sup>, but the amino-acid composition of the polypeptide moiety has not been mentioned.

The plants we have examined are the freshwater green alga *Nitella opaca*, the green seaweeds *Cladophora* and *Chaetomorpha*, both with cellulosic walls, and *Codium*, a green seaweed with mannan walls<sup>5</sup>. Cell wall fractions were prepared by each of four methods. (1) The whole plant was thoroughly washed in distilled water and ground finely with a pestle and mortar. Mild centrifugation then yielded a crude cell wall fraction which was shaken with glass ballotini in water for up to 3 h in a Mickel shaker. The resulting homogenate was centrifuged at 1,000g for 1 min and the centrifugate was washed ten times in water and dried from acetone. This formed the standard method. On occasion the process was repeated several times until approximately constant weight and nitrogen content were achieved. (2) Cell wall fractions obtained by method (1) were shaken with glass beads in the protein solvents; phenol-acetic acid-water (1:1:1—w/v/v); phenol-ethanol (85 g:15 ml.); 50 per cent urea. Again, the process was repeated several times and with a combination of all three solvents. (3) With *Codium* the three solvents used in method (2) were

applied to material decolorized in *n*-butanol and acetone. (4) Methods (1) and (2) were applied to internodal cells of *Nitella* from which the bulk of the cytoplasm had been removed by mechanical pressure, and to specimens of *Chaetomorpha* from which the protoplasm had been ejected by sporulation. These, and particularly the latter, gave the cleanest wall fractions. Contamination of the material with glass powder as a result of repeated extractions is allowed for in the results presented.

The total protein content was determined in each case by Kjeldahl's method. Amino-acid analyses were made on cell wall fractions hydrolysed in 6 normal hydrochloric acid for 24 h and with the acid then removed in a vacuum desiccator; the amino-acids were dissolved in 0.1 normal hydrochloric acid containing 0.1  $\mu$ moles of norleucine, and 1 ml. aliquots placed on the column of a 'Technicon' automatic amino-acid analyser.

All the cell wall fractions contained protein (Table 1).

Table 1. KJELDAHL PROTEIN DETERMINATIONS

Sample	Method of preparation	Percentage protein (Kjeldahl)	Percentage protein (corrected for ash content)
Whole <i>Nitella</i>	(1)	8.3	10.3
(A, Table 2)	(2) (phenol-ethanol)	6.4	6.6
Whole <i>Nitella</i>	(2) (phenol-acetic acid-water)	6.8	—
Internodal cells, <i>Nitella</i>	(4)	5.1	7.8
<i>Chaetomorpha</i> (B, Table 2)	(1)	9.3	—
<i>Chaetomorpha</i>	(2) (phenol-ethanol)	7.3	—
<i>Codium</i>	(1) (one extraction)	8.9	—
"	(1) (two extractions)	6.4	—
" (C, Table 2)	(1) (nine extractions)	2.6	5.4
"	(2) (phenol-ethanol)	5.2	6.0
"	(2) (phenol-acetic acid-water)	5.7	6.1
"	(3) (50 per cent urea, one extraction)	8.0	—
"	(3) (50 per cent urea, three extractions)	2.9	5.2

With *Nitella*, extraction with water, phenol-ethanol, or phenol-acetic acid-water all left uniformly a wall fraction with a protein content lying between 6.5 per cent and 10 per cent. With *Codium* little change occurred either in wall yield or in protein content between the sixth and ninth water extract, and three extractions with 50 per cent urea gave a yield and a protein content similar to that after nine extractions with water; the yield of 5 per cent to 6 per cent protein is almost certainly the true wall protein value for this alga. With the exception of *Nitella*, 4-hydroxyproline was found in all wall hydrolysates (Table 2). A protein with characteristics resembling that

found in higher plants certainly occurs, therefore, in some algae. Of the other plants examined, *Cladophora* and *Chaetomorpha* contain less, and *Codium* very much more, hydroxyproline than do many of the higher plants so far reported<sup>1</sup>. With *Codium*, hydroxyproline formed the most abundant amino-acid in the protein. More significantly, in view of current suggestions as to the role of this protein, all cell wall fractions showed a peak in the position of cysteic acid, most prominent (Table 2) with *Nitella* wall fractions from which hydroxyproline is absent. The derivation of this peak from cystine was demonstrated in the walls of sporulated *Chaetomorpha* by hydrolysis under nitrogen, when the cysteic acid peak was reduced from 65 residues/1,000 to 1 residue/1,000 and cystine came off the column at a later stage.

Table 2. AMINO-ACID COMPOSITIONS OF VARIOUS ALGAL FRACTIONS

	Residues/1,000						
	<i>Nitella</i> A (Table 1)	<i>Chaetomorpha</i> B (Table 1)	Phenol- acetic acid- water	Sporu- lated	<i>Clado- phora</i> (Method 1)	<i>Codium</i> C (Table 1)	Cyto- plasmic fraction
Cysteic acid	85	64	65	1	64	23	22
4-Hydroxy- proline	—	34	37	64	46	108	26
Aspartic acid	140	119	109	145	130	72	107
Threonine	35	41	45	49	39	60	68
Serine	53	49	57	55	34	68	75
Glutamic acid	48	109	108	106	117	88	114
Proline	106	75	69	80	52	53	54
Glycine	123	119	107	117	146	87	99
Alanine	95	73	70	74	65	97	102
Valine	39	57	34	41	55	91	70
Cystine (half value)	7	—	—	36	—	—	—
Methionine	Trace	2	6	10	—	7	3
Isoleucine	30	26	30	22	26	31	40
Leucine	38	75	59	65	46	61	73
Tyrosine	15	27	28	27	35	20	22
Phenyl- alanine	23	21	20	23	20	25	40
Lysine	108	70	75	63	71	61	52
Histidine	12	7	7	4	13	10	6
Arginine	36	30	25	25	nd	20	25
Peak 1	—	14	21	12	23	16	2

nd, Not determined.

In addition to the amino-acids already recognized for the wall protein of higher plants, several peaks were observed with these algae which seem not so far to have been recorded. In all cell wall fractions of *Cladophora*, *Chaetomorpha* and *Codium*, a double peak (peak 1, Table 1) came off the column in the basic region immediately before ornithine and lysine, in the same position, and with the same characteristics, as hydroxylysine—another amino-acid found in collagen but not in higher plant walls under tissue culture<sup>1</sup>, or in Douglas fir xylem<sup>6</sup>. The double peak seems to be due to the presence, in varying proportions, of two isomers of hydroxylysine as evidenced by the observation that, when cell wall hydrolysates are run on the column with added hydroxylysine, the double peak occurs in identically the same position but enlarged. Two other peaks, as yet not identified with certainty, came off the column after cysteic acid. These are distinguished by the feature that, after reacting with ninhydrin, the material of these peaks absorbs light more strongly at 440 $\mu$  than at 570 $\mu$ , as with proline and hydroxyproline. There is, therefore, the faint possibility that these peaks represent two isomers of 4-hydroxyproline. The peaks also occur in the water-soluble cytoplasmic fraction of *Codium*.

The presence of protein in sporulated plants of *Chaetomorpha*—yielding certainly the cleanest wall fraction of any investigation made of this kind—and the very similar amino-acid composition of the proteins from these plants and from all the other wall fractions dealt with here, seem to us to yield exceptionally good evidence that the protein involved is a wall-bound protein as claimed for higher plants<sup>1</sup>. This is further supported by the observa-

tion that, with *Chaetomorpha*, the wall protein content derived by a summation of all the amino-acids present is  $8.2 \pm 0.6$  per cent, closely similar to the figure yielded by the Kjeldahl estimate (Table 1). As a further check on this point, the cytoplasmic proteins of *Codium* (supplied by Dr. W. Mackie of this Department) were analysed and the analysis is recorded in Table 2. In these proteins the hydroxyproline content is very much less than in the wall protein (26 residues/1,000 as against 108 residues/1,000), the double peak tentatively credited to hydroxylysine is virtually absent (2–3 residues/1,000 at most), and the two unidentified fractions absorbing strongly at 440 $\mu$  are very much reduced. This seems to rule out any remaining possibility that the wall protein represents contamination of the wall by cytoplasmic proteins.

Table 3. PERCENTAGES OF TOTAL AMINO-ACIDS IN THE TWO UNIDENTIFIED PEAKS

	<i>Nitella</i>	<i>Chaeto- morpha</i>	<i>Clado- phora</i>	<i>Codium</i> (wall)	<i>Codium</i> (cytoplasm)	Carrot culture
Peak 2	3.7	1.0	0.9	3.3	0.3	0.05
Peak 3	—	0.2	1.5	6.2	0.9	0.05

We regard it, therefore, as established that proteins of the type considered to occur in the walls of higher plant cells are not confined to higher plants, with the corollary that any part played by wall proteins in cell growth may well be a general phenomenon. The absence of 4-hydroxyproline from *Nitella* wall protein is not at the moment to be regarded as of any great significance, because this amino-acid seems to be more remarkable as a convenient distinguishable label for wall proteins than for any particular function ascribed to it. Greater significance attaches to the presence in all wall hydrolysates of cysteic acid almost certainly derived, during extraction (and proved so with *Chaetomorpha*), from cystine. If it is true, as suggested, that S-S bonds are involved in wall extensibility, then these algae provide much more favourable material on which an estimate of their importance may be based than do almost any cells of higher plants. This has given impetus in this laboratory to an investigation of the tensile properties of walls of growing cells in the algae discussed in this article, which is now proceeding. The known effects of ions, of auxins, and of other exogenous materials or environmental conditions on both the growth rates of cells and the tensile properties of their cell walls lead us to suspect that S-S bonds are not the only bonds to be effective. The indications are, however, that the factors controlling cell wall expansion are more complex than envisaged by considerations confined to inter-carbohydrate bonding only. In particular, it is satisfactory to find a protein associated with the walls of *Codium* in which not only is the structural polysaccharide a mannan but in which virtually no polysaccharide other than mannan and a little glucan can be detected, because this plant and the other seaweeds known to have similar walls<sup>5</sup> demonstrate that no concept of a wall growth process based entirely on the carbohydrate architecture of cellulosic walls can possibly of itself be universal.

We thank Dr. G. N. Graham of this Department for instructing one of us (E. W. T.) in the use of the amino-acid analyser, and for bringing to our notice the possible significance of the double peak.

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# LETTERS TO THE EDITOR

## PLANETARY SCIENCE

### Forecasting of Earthquakes—Correlation between Deep Foci and Shallow Events in Melanesia

In the Solomons Region of the S.W. Pacific, the deep focus earthquakes which have been detected since 1912 have been concentrated at the north-western end of the Bougainville-New Ireland, and in a region to the south-east of the Santa Cruz Group. Recent research has established a *prima facie* case for a systematic time-space correlation between deep foci and sequences of shallower seismic events similar to that found in the New Hebrides by Dr. Claude Blot in the course of several years of detailed investigation of thousands of seismic and volcanic events.

Beyond the Western Solomons deep focus region, and a little to the east, five separate deep shocks have been located beneath the New Georgia Group. If these are ignored, a distance of 1,600 km separates the two main regions of known deep focus shocks (Fig. 1, heavy stipple on map).

The structural picture which is revealed from the seismic zone in plan and longitudinal section along the island chain (to the same scale) is of considerable interest. In the map and section shown, the vertical interval equals the horizontal interval; deep foci take the form of two broad abutments at different depths which support a narrowing arch of seismicity along which phenomena travel upwards towards the centre in the San Cristobal area. If we consider the seismicity since 1963, the year when epicentres could be plotted more accurately because of the U.S.C.G.S. World Wide Standard Stations, then on the western side of the arch, the deepest intermediate foci occur at a shallower depth (about 250 km) than those on the eastern side of the arch (about 300 km). It will be

noted that generally the deep foci themselves also occur at a shallower depth in the west than in the east—about 450 km compared to 625 km.

The zone of apparent seismicity for 1963–66 has been lightly stippled on the map (Fig. 1). The seismicity for any one year shows (a) only a few deep foci in the west and in the east; (b) a greater number of intermediate shocks; (c) very many shallow shocks; density of earthquakes increases from the depths to the surface.

The almost complete absence of shocks between the deep foci and the deepest intermediate depths (250 km in the west, 300 km in the east) is notable, yet Blot's research in the New Hebrides had led him to postulate that there is a time-space correlation between deep foci and the shallower events, both for earthquakes and volcanic eruptions<sup>1</sup>. This has now been established for the Solomons as well. Blot and Grover used the hypothesis to predict volcanic eruptions some months before the event in the New Hebrides, Santa Cruz, and Solomon Islands<sup>2</sup>. Because of the difficulty of applying the method without the use of computers, as yet it has not been widely used. A complete list of these forecasts of eruptions is now being prepared in readiness for the I.U.G.G. meetings in Trieste and Zurich in 1967. The list includes the warning of the impending eruption of Tinakula in the Santa Cruz Group, quiescent for a decade, and that of Gaua in the New Hebrides, quiescent probably for centuries. The main difficulty in this region is the obtaining of reliable reports from these remote volcanic islands which do not possess radio communication.

It appears that deep focus shocks initiate thermo-energetic phenomena which radiate upwards along tectonic zones and trigger shallower earthquakes of the same order of magnitude as the deep shock, except where phenomena converge. The delays between a deep-shock and its related earthquakes are a function of the distance from, and the magnitude and depth of the deep shock. Convergence of thermo-energetic phenomena produces greater earthquakes. Their approximate epicentres may be predicted graphically by the intersection of the depth curves of time-epicentral distance graphs while their approximate dates of occurrence depend on the triggering depth (that is, the depth of the point of maximum concentration of energy) and may also be predicted graphi-

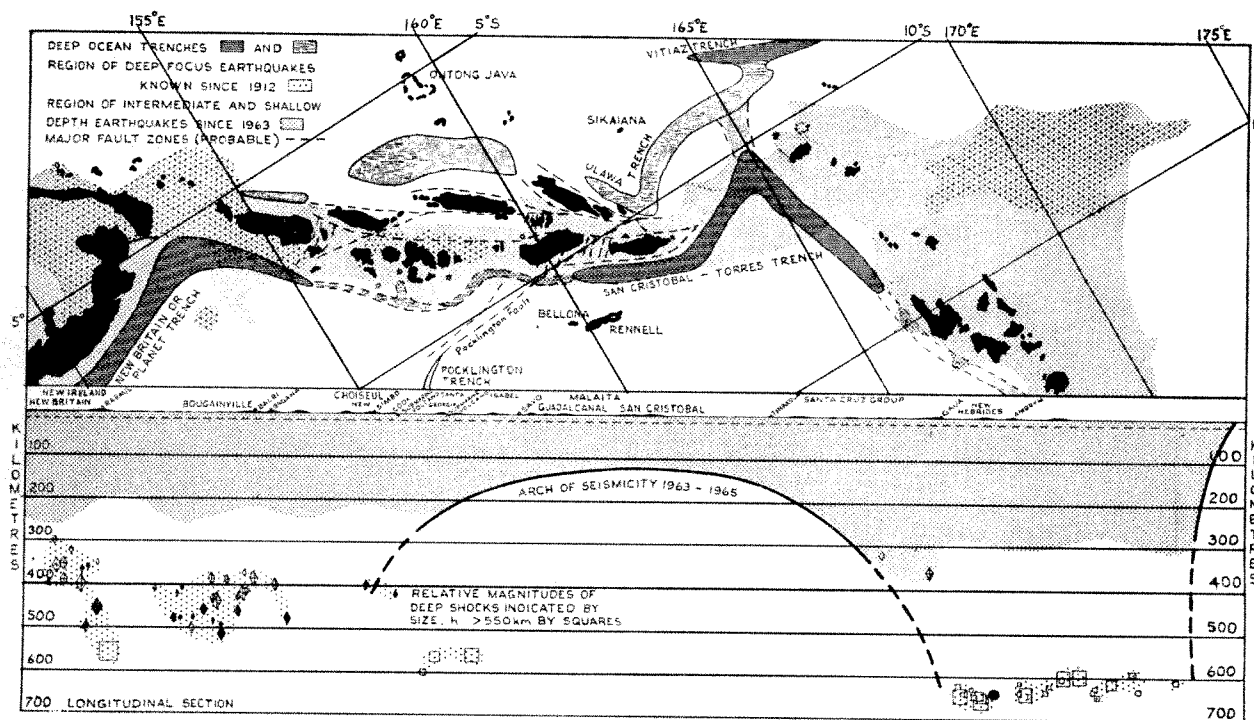


Fig. 1.

cally. The greatest magnitude earthquakes are caused by the convergence of thermo-energetic phenomena from deep shocks of great magnitude.

This would appear valid for all earthquakes in the Solomons region since 1963, and the data of the shocks triggering the important seismic crisis of June 15, 1966 (including two shocks of mag. 7+PAS) and that of December 31, 1966, in the Santa Cruz Group (also two shocks of mag. 7+) fit the theoretical hypocentres.

It should be noted that in this hypothesis deep events are considered which occur two and three years before the movement of surface faults. In the Solomon Islands gravity, magnetic, geological and bathymetric data, and recent movement, point to the existence of megashears; unfortunately, these occur mostly under the sea, and are only now being confirmed by the expedition by the Hawaii Institute of Geophysics. One fault with a vertical displacement of 2,000 m in the sea east of Malaita was reported by Professor A. Malahoff and L. Kroenke on December 6, 1966. It has proved unnecessary to consider faulting in prediction of great earthquakes because faults appear to be the ultimate effect of deep-seated phenomena; nevertheless some of these shears appear to continue for hundreds of kilometres, and therefore must be very deep-seated. As the mantle is almost certainly very close to the surface in the Solomons (according to the 1963-65 gravity data) the principal features described must extend below the shallow crust into the mantle itself, in which nearly all the earthquakes appear to occur.

I thank Dr. C. Blot and Professor S. W. Carey for helpful discussion.

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### Third Brine Pool in the Red Sea

DURING the autumn of 1966 the R.V. *Chain* (cruise number 61) discovered a third area of hot, highly saline water in the rift valley of the Red Sea where two areas of similar water had previously been reported<sup>1-3</sup>. The two known areas, now called the Discovery Deep (21° 17' N., 38° 02' E.) and Atlantis II Deep (21° 21' N., 38° 04' E.), have salinities of about 255 parts per thousand and temperatures of 44° and 56° C respectively (refs. 2-4, and footnote (†) to Table 1). This communication describes the dimensions and characteristics of a third hole (Chain Deep, 21° 17' N., 38° 03-5' E.) and its relationship to the other two areas.

The third brine pool was discovered during a survey of the rift valley between the Atlantis II and Discovery Deeps. About fifteen traverses were made of this area to determine if the Atlantis II and Discovery Deeps were in direct communication. The survey shows a saddle crossing the rift valley with a narrow elongated trough on the eastern side of the saddle. The deepest part of this trough is at 2,066 m. Because the higher temperatures and salinities in the Discovery and the Atlantis II holes start around 2,000 m, it was important to determine if hot water was also present in this new deep. A temperature telemetering pinger, which was used to test all deep holes on R.V. *Chain*, was lowered at the position shown in Fig. 1. A maximum temperature of 34° C was recorded at a depth of 2,042 m (Fig. 2). Water samples, taken on the same lowering, had a maximum salinity of 74.2 parts per thousand at a depth of 2,024 m (Table 1). The increase in temperature and salinity accompanied by a marked decrease in oxygen is characteristic of the water in the other two deeps and defines the Chain Deep as a third brine pool. Unfortunately the deepest part of the hole was not

tested. Attempts to core the Chain Deep with both free fall and a 'Kasten' corer were unsuccessful.

Data from a depth recorder (Fig. 3) show the Chain Deep to be a narrow rift with slopes as high as 20°. Its length as defined by the 2,000 m contour interval is about 2.6 km and its maximum width is 0.7 km. The floor of the Chain Deep is V-shaped and more irregular when compared with the flat floored areas of the other two deeps.

The Chain Deep is separated from the Discovery Deep by a sill at 1,980 m, which is sufficiently high to prevent exchange of hot water between these two holes. The shallowest area observed between the Atlantis II and Chain Deeps is 2,009 m. If this is the shallowest area, exchange of hot water between the Atlantis II and Chain Deeps is possible. Temperature profiles from the three hot holes (Fig. 4), however, show distinct differences which suggest that the hot waters of the three holes are not in

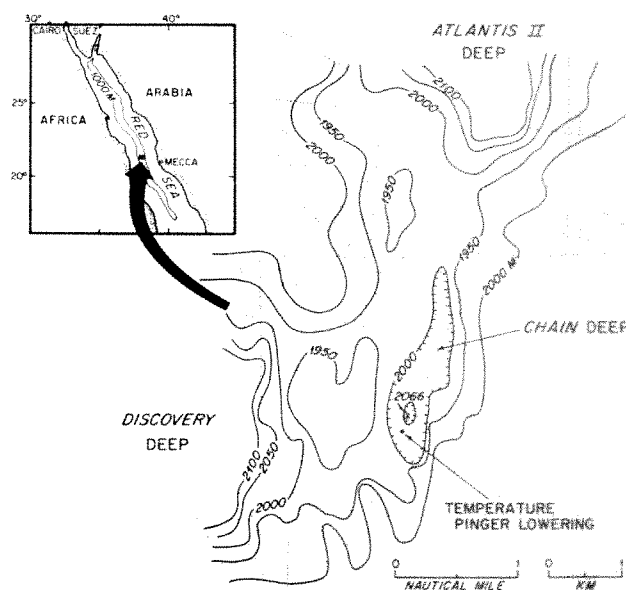


Fig. 1. Bathymetry of the Chain Deep. Depth contours in metres corrected for sound velocity. Relative positions determined by radar fixes on an anchored buoy. Dotted lines indicate control.

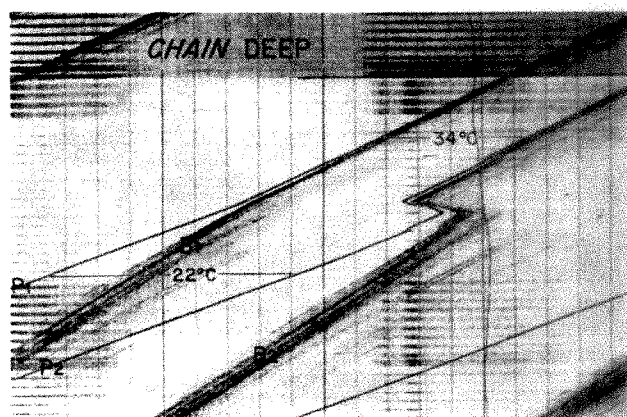


Fig. 2. Precision Graphic Recorder record of the temperature telemetering pinger lowering into the Chain Deep. The pinger emits two pulses within a 1-sec interval. The first pulse ( $P_1$ ) is emitted every second, a following pulse ( $P_2$ ) is emitted after the first by a period of time that varies with temperature.  $B_1$  and  $B_2$  are the bottom returns from the two pulses. The distance between a pulse and its bottom return indicates the depth of the instrument above the bottom. Going from left to right in the figure,  $P_2$  shows several jumps which do not indicate a step-like pattern in the temperature distribution but are caused by stopping and starting of the winch. The accuracy of the instrument is  $\pm 1.25^\circ \text{C}$ . In the upper right-hand corner the pinger has reached the bottom and a temperature of  $34^\circ \text{C}$  is indicated.



Table 1. GENERAL HYDROGRAPHIC DATA FROM THE CHAIN DEEP

Corrected depth (m)	Temperature (°C)	Salinity (parts per thousands)	Oxygen (ml./l.)
1,730	21.94	40.63	2.15
1,830	21.97	40.63	2.19
1,930	22.06	40.88	2.19
1,999	23.80	42.01	1.52
2,013	22.0*	—	—
2,018	22.2	—	—
2,019	25.98	55.6†	0.15
2,021	23.0	—	—
2,024	29.07	74.2	0.10
2,025	25.0	—	—
2,029	28.4	—	—
2,031	32.2	—	—
2,037	33.0	—	—
2,042	34.0	—	—

\* Temperature values without salinity or oxygen measurements are selected measurements from the temperature telemetering pinger lowering (Fig. 2). Differences in depth for a given temperature as determined from the depth recorder and the reversing thermometers may be the result of many causes, one of which is the increase in the speed of sound caused by the increased temperature and salinity. This would make the true depths greater than the recorded depths. Normal Mathews corrections for the Red Sea were applied.

† The ionic ratio of these highly saline waters is different from that expected from evaporation of normal sea water. Thus the salinity values should be accepted only as an approximation. Chlorinity values for the Discovery Deep have been calculated by Brewer<sup>2</sup> to be about 155.5 parts per thousand and for the water at 44° C and for the Atlantis II Deep by Miller<sup>3</sup> to be about 155.5 parts per thousand for the 55.9° C water.

direct communication at present. This implies that a sill shallower than 2,009 m exists between the Atlantis II and Chain Deeps.

Temperature profiles obtained in the three holes with the telemetering pinger are shown in Fig. 4. The curves for the Atlantis II and Discovery Deeps are similar in general shape to those reported by Krause and Ziegenbein<sup>5</sup> using a continuous temperature recorder. The curve for the new Chain Deep shows a rapid increase in temperature from

about 21° to 32° C in the same depth range (2,020–2,030 m) where the Discovery and Atlantis II waters reach their highest temperature.

We do not believe that the three holes are now in direct communication because of the distinct differences in temperature profiles and the presence of the sills. The similarities in high temperature, high salinity, and low oxygen suggest, however, that the hot water in the three holes has a similar origin—either from the same source or by the mixing of hot water between the holes at some time in the recent past.

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### Thermal Conductivity of Lunite as Dependent on Temperature

It has already been pointed out<sup>1,2</sup> that the value of the midnight temperature of the lunar surface  $T_n = 100^\circ \text{K}$  (ref. 3) is in conflict with the precision measurements of the constant radiotemperature component averaged over the disk. In fact, for  $T_n = 100^\circ \text{K}$  the constant component of the surface temperature at the disk centre<sup>1</sup> is equal to

$$T_0(0) = 219^\circ \text{K} \quad (1)$$

On the other hand, the constant component of the effective temperature measured over the disk for  $\lambda = 3 \text{ cm}$  is equal to  $T_{e0} = 212^\circ \pm 2^\circ \text{K}$  (ref. 1) and corresponds to the temperature at a depth of about 50 cm, where temperature increases by  $2^\circ$  at the most as a result of heat flow from the Moon's interior. Thus the constant component caused by solar heating alone at this depth, averaged over the disk, must be equal to

$$T_{e0} = 210^\circ \pm 2^\circ \text{K} \quad (2)$$

The effective temperature averaged over the disk is related to the constant component of the true temperature for the disk centre by the expression<sup>4</sup>

$$T_{e0} = (1 - \bar{R}) 0.964 T_0(0) \quad (3)$$

where  $\bar{R}$  is the mean spherical coefficient of wave reflexion from the Moon's surface, and depends on the dielectric constant  $\epsilon$ .

For  $\epsilon = 1.5$ ,  $1 - \bar{R} = 0.95$ ; for  $\epsilon = 2$ ,  $1 - \bar{R} = 0.92$  (ref. 5). Thus the constant component of the surface temperature at the centre of the disk, as obtained from radio measurements, is

$$T_0(0) = \begin{cases} 229 & \epsilon = 1.5 \\ 237 & \epsilon = 2 \end{cases} \quad (4)$$

Comparison with (1) reveals that the foregoing constant component is superior by

$$\Delta T = 10^\circ \div 18^\circ \quad (5)$$

to that obtained at the surface by infra-red measurements. I think that the discrepancy observed is caused by the existence of a real difference in the constant com-

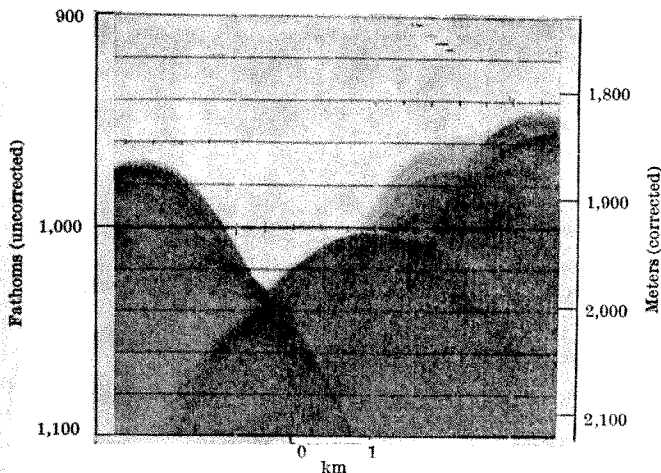


Fig. 3. Precision Graphic Recorder record of a crossing of the Chain Deep.

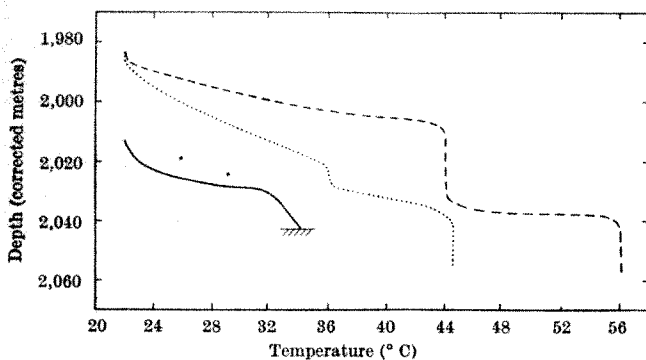


Fig. 4. General temperature distribution in the Atlantis II (---), Discovery (· · · ·) and Chain (—) Deeps. Data obtained by temperature telemetering pinger lowerings made during Chain cruise 61.

ponent at the surface and at the 50 cm depth, which is not related to a flow of heat from the interior. This difference is possible<sup>7</sup> if the thermal conductivity and thermal capacity of lunite are dependent on temperature.

As is known, the temperature dependence of thermal conductivity is mainly related to a transfer of radiation energy. In a porous body the latter may take place even in a substance which strongly absorbs radiation by transfer through the pores. In that case the radiation component of thermal conductivity will be

$$K_s(T) = 4\sigma \frac{A}{2-A} T^3 S l_p \quad (6)$$

where  $\sigma$  is the Stefan-Boltzman constant,  $A$  is the emissivity of the material,  $l_p$  is the size of a pore,  $S$  is the proportional area occupied by pores. In the case of a radiation which penetrates through the substance the radiation thermal conductivity is

$$K_s(T) = \frac{16}{3} \sigma \epsilon_i T^3 \bar{l}_\lambda$$

where  $\epsilon_i$  is the dielectric constant of the substance for infra-red waves,  $\bar{l}_\lambda$  is the average mean-free quantum path for all frequencies (Rossel and mean).

$$\bar{l}_\lambda = \frac{\int_0^\infty l_\lambda \frac{dB}{dT} d\lambda}{\int_0^\infty \frac{dB}{dT} d\lambda} \quad (7)$$

where  $B_\lambda$  is Planck's function.

The theory admits that for dielectrics in the infra-red range  $l_\lambda = a\lambda$  as in the radio range. Thus if this is substituted in (7) we obtain

$$\bar{l}_\lambda = a \frac{0.36}{T} \quad (8)$$

This gives a square dependence of the radiation thermal conductivity on temperature

$$K_s(T) = 2 \sigma \epsilon_i a T^2 \quad (9)$$

The total thermal conductivity will be equal to the sum of the molecular  $K_0$  and radiation  $K_n$  ( $n = 2, 3$ ) thermal conductivities

$$K(T) = K_0 + K_n \quad n = 2, 3 \quad (10)$$

where  $K_n$  is found from formulae (6) and (9).

Taking (10) and the specific heat dependence in the form of  $C(T) = C_1 T$ , thermal conditions were computed for a homogeneous model. An increase of the constant component for a given  $\gamma(T) = (K_0 C)^{-1}$  was found to be dependent on the ratio of the radiation to the molecular component of the thermal conductivity  $K_n/K_0$ . This ratio can best be characterized by its value at temperature  $T_k = 300^\circ \text{K}$ . As revealed by computations on an electronic computer, for  $\gamma(300) = 600$  an increase of the constant component of the temperature  $\Delta T = 15^\circ \text{K}$  is possible, if

$$K_s(300) = 0.4 K_0 \quad (11)$$

or

$$K_s(300) = 0.25 K_0 \quad (12)$$

For  $\gamma(300) = 600$ ,  $C(300) = 0.2$  and lunite density  $\rho = 1$  the value of the molecular thermal conductivity  $K_0 = 10^{-5} \text{ cal cm}^{-1} \text{ sec}^{-1} \text{ deg}^{-1}$ . Comparing (12) and (6) for  $T = 300^\circ \text{K}$  we obtain the effective size of pores  $S l_p = 170 \mu$  ( $A = 1$ ). For the 50 per cent porosity  $S \approx 0.5$  the actual size of pores will be

$$l_p = 350 \mu$$

A similar value for pore size was derived in ref. 8 and in ref. 9, where the radiation component of thermal con-

ductivity was determined from the character of temperature variation at the Moon's surface at sunset.

Comparing (11) and (9) we obtain for  $\epsilon_i = 1.4$  that  $a = 13$ . This gives the depth of penetration of infra-red waves into lunite

$$l_\lambda = 13 \lambda \quad (13)$$

It is interesting to note that for radiowaves within the  $0.1 \text{ cm} \leq \lambda \leq 10 \text{ cm}$  range the relationship  $l_\lambda = 26 \lambda$  (ref. 1) holds for the depth of penetration into lunite. According to (8) an average depth of penetration for  $T = 300^\circ \text{K}$  is about  $170 \mu$ . It is worth noting that a quantity of a similar order ( $150$ – $250 \mu$ ) is obtainable from the measurements in the case of certain terrestrial rocks with grains of the order of  $70 \mu$  and less<sup>10</sup>. This testifies to a pretty high validity of expression (13). As pointed out earlier, the low thermal conductivity of lunite is evidence of the fact that the pores are not closed and are formed of gaps in solidly interconnected particles. The area of contact of a particle, as revealed by computations<sup>11</sup> should be much less than the grain area. The newly obtained data favour a conclusion that the lunite pores may not exceed  $350 \mu$ . For a porosity of 50–70 per cent lunite the pores and the particles must be approximately equal in size. Then in the case of a non-transparent substance the particles may not exceed  $350 \mu$ . A smaller size of pores is possible if the base substance is transparent to infra-red waves. In that case, for the aforementioned porosity of lunite half the infra-red quantum path will coincide with the total length of pores and the other half with the total thickness of transparent particles. The number of penetrated particles may not at any frequency be less than one, consequently its size will not exceed  $0.5 \bar{l}_\lambda = 80$ – $100 \mu$ . The particle size in that case is most likely to be of the order of the infra-red wavelength, that is  $10$ – $20 \mu$ .

I thank Boris Konstantinov, who suggested that I should study the effect of radiation heat transfer in lunar matter.

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## THE SOLID STATE

### Hydrogen Isotope Diffusion

HYDROGEN isotope diffusion in metals and polymers has generally been treated in the literature as a single body problem with the motion of the hydrogen regarded as the important parameter. On this basis the equation for interstitial diffusion of hydrogen atoms in metals or molecular diffusion in polymers follows an equation of the form

$$D = \frac{v}{6} \frac{Ed^2}{RT} \exp(-E/RT) \quad (1)$$

where  $D$  is the diffusion coefficient,  $v$  a frequency which corresponds to the vibration of the atoms or molecule in

the solvent lattice,  $d$  the distance traversed in an elementary displacement and  $E$  the diffusional activation energy<sup>1</sup>. For the single body approximation,  $v$  is inversely proportional to the square root of the isotopic mass since hydrogen is much lighter than the molecules or atoms with which it interacts, so that

$$D_H/D_D = 2 \frac{E_H}{E_D} \exp(E_D - E_H)/RT \quad (2)$$

The temperature effect on the diffusion coefficient ratio for the hydrogen isotopes is negligible both for polymer systems<sup>2</sup> and metals<sup>3-6</sup> as would be expected from equation (2), because the H<sub>2</sub>-polymer and individual metal-H bonds are weak and will have small zero point energies, so that  $E_H \approx E_D$ . Thus the single body approximation leads to the conclusion that the ratio  $D_H/D_D$  should have a value close to 1.4 as has been found in some strongly interacting metal hydrogen systems, namely, palladium-hydrogen<sup>7</sup> and zirconium-hydrogen<sup>8</sup>. For polymer systems and a number of metals studied recently by myself and my colleagues, this has not proved to be the case, however, as shown in Table 1. We can only conclude that for these systems the motion of solute molecules or atoms in the solvent lattice is not rate controlling and that the diffusion rate is dependent on the solvent lattice itself.

Table 1. ISOTOPE EFFECTS ON DIFFUSION AND SOLUBILITY OF HYDROGEN

Material	Temperature range (°C)	$D_H/D_D$	$D_H/D_T$	$S_H/S_D$	$S_H/S_T$
Epoxy resin <sup>2</sup>	25-55	0.96 ± 0.06		1.16 ± 0.1	
Araldite <sup>2</sup>	20-40		1.01 ± 0.04		1.18 ± 0.07
Neoprene <sup>10</sup>		1.04 ± 0.10	1.07 ± 0.07	1.03 ± 0.14	1.02 ± 0.12
Maraged steel <sup>3</sup>	150-250	0.87 ± 0.07		1.44 ± 0.12	
En 58E steel <sup>4</sup>	250-400	0.99 ± 0.07		1.38	
Nilo-K <sup>5</sup>	200-350	0.96 ± 0.06		1.39 ± 0.08	
SAE 4130 <sup>9</sup>	—	1.14			
S110 steel <sup>6</sup>	250-400	1.17 ± 0.06			
Palladium <sup>7</sup>	200-300	1.31			
Zirconium <sup>8</sup>	—	1.50			

The motion required of the lattice atoms will be much less than that for self diffusion of solvent material and will clearly depend on solute molecule size, which is, of course, the same for hydrogen and deuterium within close limits. Furthermore, whether lattice motion or hydrogen molecule or atom motion are rate controlling will depend on a number of factors such as temperature, solute-solvent interaction and solvent material. The exothermic occluders—palladium<sup>7</sup> and zirconium<sup>8</sup>—with strong hydrogen atom-metal interaction, give large isotope effects on the diffusion coefficients. This shows that interstitial diffusion with hydrogen atom transport as the rate controlling step applies for these systems, while some steels<sup>6,9</sup> appear to be intermediate in behaviour (Table 1).

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## Carbon Fibres of High Strength and High Breaking Strain

SOME details have recently been given about carbon fibres with high strength and high modulus produced at the Royal Aircraft Establishment (R.A.E.), Farnborough<sup>1</sup>, and by Rolls Royce, Ltd.<sup>2</sup>. Carbon fibres with average tensile strengths ( $\sigma_B$ ) of 250–300 × 10<sup>8</sup> lb./in.<sup>2</sup> and Young's moduli ( $E$ ) of about 60 × 10<sup>6</sup> lb./in.<sup>2</sup> have been reported, and this makes them extremely promising as a means of reinforcing plastics and metals. The purpose of this communication is to report that fibres with much higher strengths but with lower  $E$  and consequently higher breaking strains ( $\epsilon_B$ ) can be produced by means of a suitable heat treatment. The way in which  $E$  and  $\sigma_B$  of the R.A.E. carbon fibres vary with the heat-treatment temperature used is shown in Fig. 1. Each point on the graphs represents an average value obtained from twenty fibres tested with a tensile test machine at a strain rate of 0.05 cm/min.

For applications where fibres of high  $E$  are required it can be seen that heat treatment at a temperature of 2,500° C or more is necessary. On the other hand, when fibres with maximum  $\sigma_B$  and  $\epsilon_B$  are needed, the optimum heat-treatment temperature is only 1,500°–1,600° C. The highest  $\epsilon_B$  is 1.3 per cent, occurring as a result of heating to 1,500° C. This is more than twice the  $\epsilon_B$  of 0.6 per cent found for fibres of high  $E$  heated to 2,500° C. The strength distribution for fibres heated to 1,500° C is shown in Fig. 2, the  $\sigma_B$  for this sample being 444 × 10<sup>8</sup>

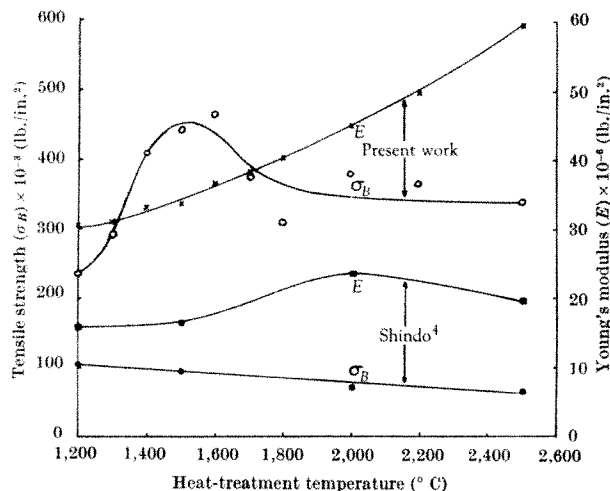


Fig. 1. Effect of heat-treatment temperature on the mechanical properties of carbon fibres at room temperature.

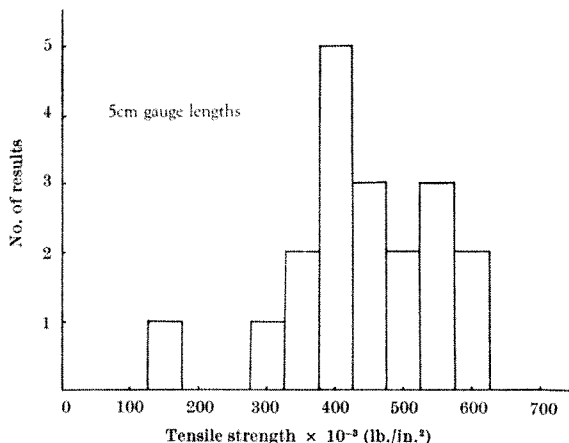


Fig. 2. Histogram of tensile strengths of carbon fibres after heat-treatment to 1,500° C.

lb./in.<sup>2</sup>. Thus carbon fibres have been made with  $\sigma_B$  higher than the value of  $250 \times 10^3$  lb./in.<sup>2</sup> quoted for *E*-glass fibres in use and approaching the figure of  $500 \times 10^3$  lb./in.<sup>2</sup> given for virgin fibres<sup>3</sup>. They also have higher *E*, that is,  $34 \times 10^6$  lb./in.<sup>2</sup> as compared with  $12.4 \times 10^6$  lb./in.<sup>2</sup>.

In a similar study, Shindo<sup>4</sup> found no trace of an increase in  $\sigma_B$  of carbon fibres as the treatment temperature was increased over the range 1,000°–3,000° C, and his results for *E* and  $\sigma_B$  are much lower than those reported here, as can be seen from Fig. 1. He did find a slight maximum for *E* after a heat treatment at 2,000° C. No trace of such a maximum was found with R.A.E. carbon fibres; a decrease in *E* suggests a change in structure, for example, in preferred orientation, which would be unlikely to be produced by a higher treatment temperature. The reason for the reported decrease in *E* by Shindo is therefore obscure.

A further point is that the strengths of R.A.E. carbon fibres given in Fig. 1 were obtained using 5 cm gauge lengths. When tests were done using 1 cm lengths the  $\sigma_B$  for fibres treated at 1,500° C was found to be  $496 \times 10^3$  lb./in.<sup>2</sup>, that is, 12 per cent higher than obtained with 5 cm lengths. This is due to the scatter of fibre strengths, the chance of including a weak spot in the test-piece being less when using shorter lengths. The highest  $\sigma_B$  found for a fibre treated to 1,600° C was  $745 \times 10^3$  lb./in.<sup>2</sup> for a 1 cm length, one-quarter of the maximum  $\sigma_B$  measured by Bacon<sup>5</sup> for graphite whiskers.

At present there is no evidence to suggest that tensile failures are due to surface damage on the fibres and it is possible that flaws are present at grain boundaries within the fibres because they are polycrystalline<sup>1</sup>. With this in mind the fibre structures are now being studied using an ultra-microtome to obtain thin sections for electron microscopy.

The results shown here indicate that a wide range of carbon fibres can now be produced. It is thought that there will be many applications for both the high *E* and the high  $\sigma_B$  high  $\epsilon_B$  fibres. We thank Mr. C. Addison and Mr. D. Wright for their help with fibre testing.

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## CHEMISTRY

### 1,2-Benzanthracene Derivatives in a Kuwait Mineral Oil

THE isolation of 1,2-benzanthracene and 4'-methyl-1,2-benzanthracene from a crystalline mixture of benzanthracene homologues, obtained from fractions of a Kuwait mineral oil by reaction with maleic anhydride, was described in an earlier communication<sup>1</sup>. We have now isolated another isomer from the mixture and identified it as 7-methyl-1,2-benzanthracene, and obtained some insight into the composition of the monomethylbenzanthracene fraction by gas-liquid chromatographic analysis.

Gas-liquid chromatography of the crude benzanthracene fraction showed that it was a mixture of benzanthra-

cene itself with mono- and di-methyl derivatives<sup>1</sup>, and this was confirmed by mass spectrometry which revealed that small amounts of tri- and tetra-methyl compounds were present as well. We were unable to separate the mono- or di-methyl fractions from the mixture by chromatography on alumina or silica gel, but a useful partial separation was achieved by countercurrent distribution between hexane and nitromethane. After eighty transfers a fraction (35 mg) consisting essentially of monomethylbenzanthracenes was obtained. After further purification by chromatography on alumina and crystallization of the picrate, it was fractionally sublimed in a stream of nitrogen, affording a number of fractions, one of which, on crystallization from ethanol-benzene, gave 7-methyl-1,2-benzanthracene as colourless plates, melting point 177°–180° C undepressed when mixed with an authentic specimen, melting point 182° C. The 8-trinitrobenzene complex formed orange-red needles, melting point and mixed melting point 169°–171° C (authentic melting point 173°–174° C; we found that C = 65.8; H = 3.8; N = 8.1 per cent. C<sub>22</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub> requires C = 65.9; H = 3.8; N = 9.2 per cent).

Because of the very small amounts of material available, and the complexity of the mixture, we were unable to achieve any further separation of pure constituents, and we were led to consider analysis of the mixture by gas-liquid chromatography. This technique has been used to analyse mixtures of polycyclic aromatic hydrocarbons of different types<sup>2</sup>, but there have been very few reports of its application to mixtures of methyl homologues<sup>3</sup> of the same hydrocarbon. We studied a number of systems employing both packed and capillary columns; the best results (Table 1) were obtained with a stainless steel capillary column coated with silicone gum, used in conjunction with a 'Perkin Elmer F11' instrument fitted with a flame ionization detector. Even under the most favourable conditions, however, only partial separation of a synthetic mixture of equal amounts of all the monomethylbenzanthracenes was achieved, the twelve isomers being resolved into five groups as shown in Table 2.

Table 1. GAS-LIQUID CHROMATOGRAPHY OF METHYL-1,2-BENZANTHRACENES

Compound	Retention time (min)	Relative retention time	Compound	Retention time (min)	Relative retention time
1,2-Benzanthracene	46.0	1			
1'-Methyl-	63.0	1.37	5-Methyl-	68.0	1.48
2'-Methyl-	63.0	1.37	6-Methyl-	65.3	1.42
3'-Methyl-	66.2	1.44	7-Methyl-	66.2	1.44
4'-Methyl-	70.2	1.53	8-Methyl-	63.1	1.37
3-Methyl-	69.8	1.52	9-Methyl-	68.4	1.49
4-Methyl-	67.6	1.47	10-Methyl-	76.4	1.66

Column, 200 ft. stainless steel capillary, 0.020 in. internal diameter, coated with silicone gum 'MS 2211', at 215° C, nitrogen flow rate 6 ml./min.

Table 2

Peak	Methylbenzanthracenes corresponding
1	1', 2' and 8-methyl-
2	3', 6-, and 7-methyl-
3	4-, 5-, and 9-methyl-
4	4', and 3-methyl-
5	10-methyl-

Analysis of the mixture of benzanthracenes from the oil under the same conditions gave two major and two minor peaks in the region corresponding to monomethylbenzanthracenes (Table 3). Tentative assignments were made for each peak on the basis of retention times, and confirmed by addition of authentic material and rechroma-

Table 3. GAS-LIQUID CHROMATOGRAPHY OF MIXTURE OF 1,2-BENZANTHRACENE DERIVATIVES FROM KUWAIT OIL

Peak	Retention time (min)	Relative retention time	Area per cent	Coincident with
1	45.8	1.00	14.6	Parent
2	57.6	1.26	2.0	—
3	61.7	1.36	2.0	1', 2', and 8-methyl-
4	65.6	1.43	20.1	3', 6-, and 7-methyl-
5	68.0	1.47	5.0	4- and 5-methyl-
6	71.3	1.56	23.0	4' and 3-methyl-
7	83.3	1.86	2.0	—
8	93.6	2.08	7.5	—
9	97.3	2.17	6.0	—
10	102.0	2.28	12.6	—
11	106.0	2.37	5.6	6,7-dimethyl-

tography; increase in peak height was taken as evidence of identical retention time. The results confirm the complexity of the mixture of methylbenzanthracenes from the oil. It is evident that the 1', 2', 4-, 5- and 8-isomers together represent less than 10 per cent of the crystalline material examined; not all of these may be present, though the mixture undoubtedly contains some of them in small amount. At least two monomethyl derivatives are present in substantial amounts, but as many as five could be present to make up the two major peaks. It is of interest that the two isomers which have been isolated, the 4'- and 7-methyl compounds, fall one in each of the two major peaks. The parent hydrocarbon, which has also been isolated from the mixture, forms a third large peak. No peaks which correspond to the strongly carcinogenic 9- and 10-methyl isomers are found in the chromatograms, and this was confirmed by addition of authentic specimens of these hydrocarbons and re-chromatography, when peaks appeared at the expected positions. Later peaks are thought to be caused by dimethylbenzanthracenes, but could not be assigned because of lack of reference data.

Biological tests (private communication from Dr. D. L. Woodhouse, University of Birmingham) have shown that the extracts obtained from high-boiling fractions of the Kuwait oil by reaction with maleic anhydride, and subsequent regeneration from the acidic adducts, are carcinogenic. It is reasonable to suppose that the activity of these materials may be at least in part a result of their content of benzantracene homologues, some of which are potent carcinogens<sup>4</sup>, although none of the compounds so far isolated has more than marginal activity. Benzantracene derivatives, however, are evidently not the only type of carcinogen in the oil, for the bulk of the fractions which did not react with maleic anhydride were also strongly active (Woodhouse), and it was shown in model experiments using radioactive 1,2-benzanthracene that the carcinogenic activity of these materials could not be caused by residual amounts of benzantracene homologues which had not been removed by the maleic anhydride treatment.

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### Membranes for measuring Low Molecular Weights by Osmotic Pressure

THE determination of molecular weight from measurements of equilibrium osmotic pressure using aqueous solutions has usually been limited to values greater than 15,000, although it is possible to measure molecular weights as low as 4,000 with ancillary measurements<sup>1</sup> using solute permeable membranes.

Membranes have recently been developed for the ultra-filtration of micro-solutes with nominal lower limits of molecular weights of 350, 1,000 and 10,000. These membranes are hydrous polymers and are described as the interaction product of polyanions and polycations. They have an ionically cross-linked structure and are sold under

the name of 'Diaplex' by the Amicon Corp., Cambridge, Massachusetts. It will be shown that these membranes are also very suitable for measuring molecular weight.

The osmotic pressures of some polyethylene glycol (PEG) solutions were measured using a Pinner-Stabin<sup>2</sup> osmometer fitted with 350 and 1,000 'Diaplex' membranes. The solvent was 0.3 normal potassium chloride containing 0.04 per cent octan-2-ol. The latter was used to suppress surface tension effects. All measurements were made at  $25^\circ \pm 0.01^\circ \text{C}$  and osmotic equilibrium was attained in 24 h if there was no diffusion of solute across the membrane.

The results for two samples of PEG are presented in Table 1. The manufacturer gives a molecular weight range of 950–1,050 for the PEG 1,000 (Shell Chemical Co.) and 3,000–3,700 for the PEG 4,000 (Union Carbide).

Table 1. MOLECULAR WEIGHT OF POLYETHYLENE GLYCOLS

Sample	Membrane	$M_n$	S.E.	Number of observations
PEG 4,000	350	3,070 $\pm$ 60		7
" 4,000	1,000	*3,880 $\pm$ 60		3
" 1,000	350	*1,360 $\pm$ 70		4

\* Obtained by extrapolation to zero time.

Equilibrium measurements were obtained with the 350 membrane and PEG 4,000. There was no systematic trend in the ratio of osmotic pressure ( $\pi$ ) to concentration ( $c$ ) when the concentration ranged from 0.02 to 0.1 g/100 g solution. The values of  $\pi/c$  could therefore be averaged for the calculation of  $M_n$  from  $RT\pi/c$ .

Results for the PEG 4,000 and the 1,000 membrane gave a higher average molecular weight because the membrane was slightly permeable to this solute, and the osmotic pressure decreased slowly with time. It was necessary to extrapolate the linear relationship of  $\log \pi$  to time to zero time to obtain the initial osmotic pressure. The further corrections described by Gardon and Mason<sup>3</sup> are clearly needed for this situation.

Solute diffusion was also observed when PEG 1,000 was measured in a 350 membrane and again corrections should be applied.

The values of  $\pi$  for a sample of polyvinyl alcohol of nominal molecular weight 80,000 depended on the concentration. A plot of  $(\pi/c)^{1/2}$  against  $c$  was extrapolated to zero concentration in accordance with the graphical method of Berghlund<sup>4</sup> (Fig. 1). A linear correlation coefficient of 0.977 was obtained and the intercept of the regression line showed a molecular weight of 82,200 with a standard error of 500.

'Diaplex' membranes are well suited to osmometry in aqueous solution because of their high permeability to water (10–100 times that of 'Cellophane') and low permeability to solutes even of quite low molecular weights,

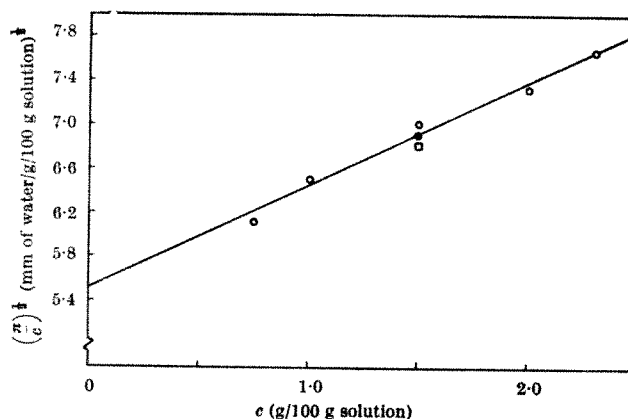


Fig. 1. The regression line  $(\pi/c)^{1/2} = 0.919c + 5.55$  and the experimental points obtained for polyvinyl alcohol.  $\circ$ , 1,000 membrane;  $\bullet$ , 1,000 membrane and osmometer with the volume reduced by half with a stainless steel block;  $\square$ , 350 membrane and osmometer with reduced volume.



which allows a rapid attainment of equilibrium. Even where some permeability to solute is evident, if it is not too great, correction may be made by a simple extrapolation of the curve of the log  $\pi$  against time. The low molecular weight cut-offs of the membranes allow molecular weights as low as 3,000 to be measured by equilibrium methods. Values as low as 1,000 can be determined using extrapolation techniques provided the required corrections<sup>1</sup> are made.

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### Electronic Spectra of the Allyl and $\beta$ -Methallyl Free Radicals in the Far Ultra-violet

THE electronic states of the free allyl radical



have been the subject of several theoretical investigations<sup>1</sup>. Longuet-Higgins and Pople<sup>2</sup> predicted that the lowest doublet excited states (<sup>2</sup>B<sub>1</sub>) should have energies of 2.74 eV (4520 Å) and 5.29 eV (2340 Å) above the <sup>2</sup>A<sub>2</sub> ground state. Although both electric dipole transitions are allowed, from the spatial electron distributions, the long wavelength system was predicted to be weak and the short wavelength system strong. Currie and Ramsay<sup>3</sup> have recently observed a weak system with a 0-0 band at 4083 Å in a variety of allyl compounds after flash photolysis. On substantial chemical evidence, this was assigned to the predicted long wavelength transition of the allyl radical.

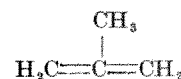
The object of this communication is to report some experiments on the flash photolysis of olefines and some allylic compounds in the gas phase, which have revealed a new band system in the far ultra-violet with an intensity maximum at 2250 Å; combining chemical evidence with established photochemical mechanisms, it has been possible to conclude that the transition corresponds with the predicted short wavelength system of the free allyl radical. A number of investigators have detected absorption bands in the far ultra-violet in irradiated polyethylene which they have tentatively assigned to the allyl radical<sup>4-5</sup>. We also record here a new band system in the far ultra-violet region, observed in flashed compounds containing the  $\beta$ -methallyl group.

The flash photolysis apparatus was of standard design. With the photolysis lamp and reaction vessel constructed of 'Spectrosil' grade quartz, and with the volume contained by the reflector flushed out with nitrogen, photolysis occurred down to about 1600 Å. Hilger small, medium and Littrow quartz-prism spectrographs were used in various aspects of the work with flash energies from 300 to 3,000 J. Spectra were recorded on 'Ilford HP3' plates sensitized with sodium salicylate.

Fig. 1a shows a series of absorption spectra taken during and after flash photolysis of butene-1; in addition to the Herzberg methyl radical band<sup>6</sup> at 2160 Å, a strong system occurs in the 2200-2300 Å region. Because of the high intensity of the new bands they can conveniently be recorded with low flash energies of about 300 J, with a single optical pass. The same system has been observed in the flash photolysis of eight compounds containing the allyl group, including propylene. The spectrum was not observed in a variety of compounds not containing the allyl group. Although the spectrum is banded, there is an

underlying continuum from 2270 Å to shorter wavelengths. The long wavelength limit of the strong bands is 2310 Å; other faint bands were detected at wavelengths up to above 2400 Å. Lossing, Marsden and Farmer<sup>7</sup> used mass-spectrometry to investigate the mercury photosensitized decomposition of a number of hydrocarbons. The principal transients detected in butene-1 were the methyl and allyl radicals, while substantial yields of the allyl radical were also observed in propylene. Notwithstanding the established hazards of attempting to assign spectra without a rotational analysis, we consider that detection of the same bands from eight compounds containing the allyl group, taken with the theoretical energy and intensity prediction and the photochemical results of Lossing *et al.*, establishes rather conclusively that the carrier of the spectrum of Fig. 1a is the free allyl radical.

Fig. 1b is a series of absorption spectra taken during the flash photolysis of isobutene. In addition to the methyl band, a transient absorption appears in the 2300 Å to 2420 Å region, consisting of a progression of about eight broad and apparently diffuse bands, together with a pair of comparatively narrow bands at 2230 and 2250 Å. The bands appear to be due to a single carrier, because they show no significant variation in relative intensity either during their decay or from one parent molecule to another. In their investigation of the mercury photosensitized decomposition of isobutene, Lossing *et al.*<sup>7</sup> detected a high yield of the  $\beta$ -methallyl radical



We have detected the spectrum of Fig. 1b in the flash photolysis of four compounds containing the  $\beta$ -methallyl group, and consider that the  $\beta$ -methallyl radical is the carrier of the bands.

A curious feature of this work has been the detection of the free methyl radical in varying yields, not only from every compound containing the methyl group, but also

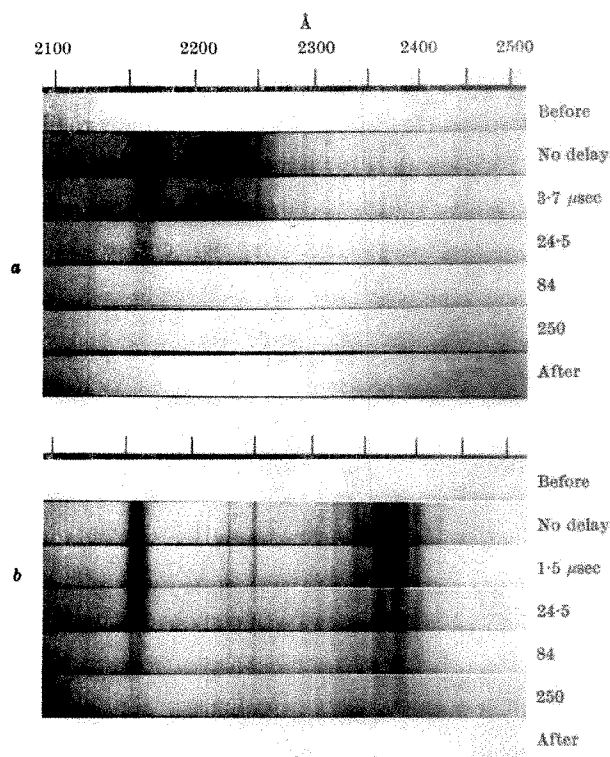


Fig. 1. a, Decay of allyl radical with time. 4 mm butene-1 + 100 mm argon. Flash energy = 320 J. b, Decay of methallyl radical with time. 0.5 mm isobutene + 100 mm argon. Flash energy = 1,608 J.

from allyl alcohol, diallyl ether, allyl amine, allyl bromide, allyl cyanide, diallyl, butadiene and ethylene. Hydrogen atom migration with subsequent scission of the C-C bond occurs with all the molecules in the latter group, though the overall quantum yield is generally small. Production of the methyl radical in flashed ethylene may be biphotonic. The observations do, however, provide direct evidence for the postulate that ethylidene participates in the photochemistry of ethylene<sup>8</sup>. We have not yet detected the CH fragment, and it is known to react very rapidly with ethylene (ref. 9 and J. R. McNesby, personal communication).

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## PHYSICS

### Correction of Some Erroneous Calculations of the Einstein A Coefficient for the 18 cm Transition of OH

In a recent note<sup>1</sup> Turner pointed out the confusion over the value of the Einstein A coefficient for the  $^2\Pi_{3/2}$ ,  $J=3/2$ ,  $\Lambda$ -doublet transition of OH. This coefficient is required to derive the interstellar abundance of OH from the intensity of the OH absorption lines observed in radio astronomy. Turner points out an error in previous calculations which may be traced to an incorrect matrix element in equation (2-16) of Townes and Schawlow<sup>2</sup>. His own calculation, however, is also in error in some respects. The calculation of the transition probability can most conveniently be done in two steps. First the probability can be calculated for the pure  $\Lambda$ -doublet transition, without regard to hyperfine structure. Second, the effect of the hyperfine structure can be treated by well-known methods. The Einstein A coefficient for the  $\Lambda$ -doublet transition is related to the dipole matrix element  $\mu_{ij}$  by

$$A_{\Lambda} = \frac{64\pi^4\nu^3}{3hc^3} |\mu_{ij}|^2$$

where  $|\mu_{ij}|^2$  is given by<sup>3</sup>

$$|\mu_{ij}|^2 = \sum_{M'} |\mu_x(JMJ'M')|^2 + |\mu_y(JMJ'M')|^2 + |\mu_z(JMJ'M')|^2$$

The summation is carried out only over  $M'$ , since  $A_{\Lambda}$  represents the probability that a molecule in one particular state  $M$  of the upper level will make a transition to any state  $M'$  of the lower level. The dipole matrix element may be related to the line strength  $S_{ij}$  in the usual manner

$$|\mu_{ij}|^2 = S_{ij} \mu^2 (2J+1)^{-1}$$

where  $\mu$  is the permanent electric moment.  $S_{ij}$  is identical with the familiar symmetric rotor line strength<sup>4</sup> if  $K$  (the component of angular momentum along the molecular axis) is replaced by  $\langle \Omega \rangle$ ; we thus have

$$S_{ij} = \frac{(2J+1) \langle \Omega \rangle^2}{J(J+1)}$$

so that

$$|\mu_{ij}|^2 = \frac{\langle \Omega \rangle^2 \mu^2}{J(J+1)}$$

With the use of the most recent molecular constants of Radford<sup>5</sup>, which lead to  $\langle \Omega \rangle = 1.470$  and the accurate dipole moment ( $1.660 \pm 0.010 D$ ) of Powell and Lide<sup>6</sup>, we finally obtain for the  $^2\Pi_{3/2}$ ,  $J=3/2$  transition

$$A_{\Lambda} = 8.53 \times 10^{-11} \text{ sec}^{-1}$$

This result is just one-half the value obtained by Turner. The reason lies in the incorrect factor of  $2I+1$  which appears in his equation (4); the  $\Lambda$  doublet transition probability clearly cannot depend on the nuclear spin  $I$ . Turner's error comes from the fact that his definition of  $|\mu_{\Lambda}|^2$  in equation (2) is inconsistent with his equation for  $A_{\Lambda}$ . If he chooses to sum over all magnetic quantum numbers  $M_J$ ,  $M'_J$ ,  $M_I$ ,  $M'_I$  in equation (2), then the total statistical weight factor  $(2J+1)^{-1} (2I+1)^{-1}$  must appear in the definition of  $A_{\Lambda}$ .

We must now consider the effect of hyperfine structure. The apportionment of intensity among the hyperfine components can easily be found from the familiar formulas<sup>7,8</sup> or tables<sup>9</sup> for the weak coupling of two angular momenta. The result is that the hyperfine components should have intensities in the ratio 1:5:9:1 (assuming that thermal equilibrium applies), as previously given by several authors<sup>10,11</sup>. The relative intensities involve both a statistical weight and a transition probability factor, however, and it must be recalled that the principle of spectroscopic stability applies to the intensities, not to the Einstein coefficients. We can calculate the Einstein coefficient  $A_{FF'}$  for an individual hyperfine component ( $F \rightarrow F'$ ) in the following way. We first write the intensity  $I_{FF'}$  for emission from an upper level  $F$  to a lower level  $F'$  as

$$I_{FF'} = \alpha_{FF'} I_{\Lambda},$$

$$\sum_{FF'} \alpha_{FF'} = 1$$

where  $I_{\Lambda}$  is the total intensity of the  $\Lambda$  doublet transition and  $\alpha_{FF'}$  is the normalized relative intensity mentioned above (and listed in Table 1). Then in order to maintain the interpretation of the Einstein coefficient as a transition probability, we must define  $A_{FF'}$  as

$$A_{FF'} = A_{\Lambda} \alpha_{FF'} (\sum_F g_F) / g_{F'}$$

where  $g_F = 2F+1$  is the statistical weight of an upper hyperfine level. This leads to the values of  $A_{FF'}$  for the OH transitions shown in Table 1.

It may be noted that

$$A_{12} + A_{11} = A_{22} + A_{21} = 8.53 \times 10^{-11} \text{ sec}^{-1} = A_{\Lambda}$$

In other words, the probability that a molecule in one particular hyperfine sub-level of the upper  $\Lambda$  doublet level will radiate into one or the other sub-level of the lower  $\Lambda$  doublet level is identical with the basic transition probability  $A_{\Lambda}$ . This is expected, as the nuclear spin interactions cannot affect the net transition probability in this order of approximation.

Turner's calculation for the individual hyperfine components is basically correct because he has included the proper degeneracy factor,  $(2F+1)^{-1}$ , in his definition of  $A_{FF'}$ . Unfortunately, his numerical values are not consistent with his equations, although the difference is trivial for the two strong components.

A value of  $A_{22} = 2.66 \times 10^{-11} \text{ sec}^{-1}$  for the 1,667 Mc/s transition has been given by Barrett<sup>11-13</sup>. It seems likely that his calculation made use of the erroneous matrix element from Townes and Schawlow<sup>2</sup> (repeated by Dousmanis, Sanders and Townes<sup>14</sup>); his value should thus be

Table 1

Transition (in emission)	Frequency (Mc/s)	$\alpha_{FF'}$	$A_{FF'}$ (sec <sup>-1</sup> )
$F=1 \rightarrow 2$	1,612	0.0625	$1.42 \times 10^{-11}$
$F=1 \rightarrow 1$	1,665	0.3125	$7.11 \times 10^{-11}$
$F=2 \rightarrow 2$	1,667	0.5625	$7.68 \times 10^{-11}$
$F=2 \rightarrow 1$	1,720	0.0625	$0.85 \times 10^{-11}$



multiplied by  $(2J+1)/J=8/3$ . Also, he used<sup>13</sup> a slightly smaller value of the dipole moment of OH (1.60 D). When these corrections are made, Barrett's value of  $A_{22}$  becomes  $7.64 \times 10^{-11} \text{ sec}^{-1}$ , in good agreement with the value obtained here.

A still different value of  $A_{22}=0.964 \times 10^{-11} \text{ sec}^{-1}$  has been quoted by Goss and Spinrad<sup>13</sup>, without details of the calculation. It is noted that this is just 1/8 of the value obtained here, which suggests that their calculation of  $A_{FF'}$  omitted the factor  $\sum_F g_F=8$ .

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## BIOPHYSICS

### Orientation of Collagen Fibres in Rat Dentine

It is well known that the dominant part of the organic matrix of dentine is collagen, the fibres of which are preferentially orientated. The accepted view is that the collagen bundles, apart from a narrow layer of "mantle dentine" formed first, lie predominantly in the plane of the mineralizing front, that is approximately parallel to the enamel-dentine junction, the pulpal surface and the incremental lines of growth<sup>1</sup>. The dentinal tubules tend to run at right angles to the incremental lines, so that sections transverse to the tubules show collagen bundles of the intertubular matrix lying largely in the plane of the section and sweeping at tangents to the tubules. In human dentine there is a "peritubular collagen-free zone"<sup>2</sup> which can be shown to become

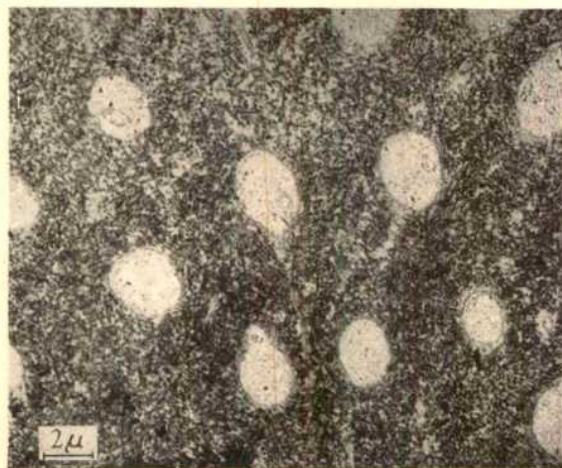


Fig. 1. Ultra-thin section of incisor predentine stained with uranyl acetate. Both the odontoblast processes and most of the collagen bundles are cut transversely. Some collagen fibres, however, lie in the plane of the section. ( $\times c. 3,350$ .)

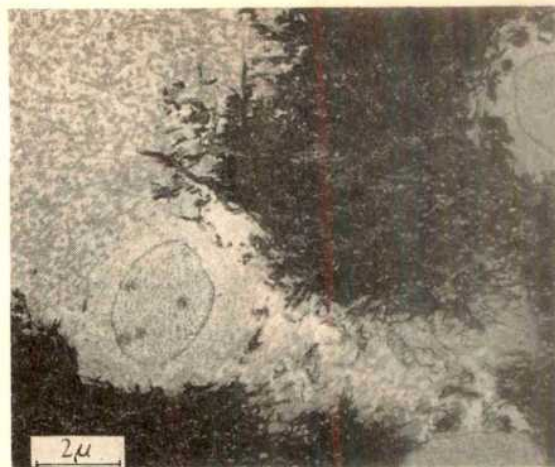


Fig. 2. The mineralizing front in developing molar dentine again showing the odontoblast processes and most of the collagen bundles cut transversely. Note the "peritubular collagen-free zones". Unstained. ( $\times 5,700$ .)

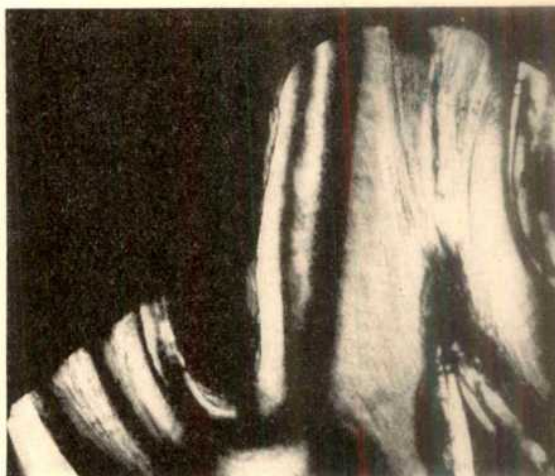


Fig. 3. Longitudinal ground section through rat lower molar cusps seen between crossed nicols. The dentinal fibres leave the enamel-dentine junction approximately at right angles but then bend abruptly to descend almost parallel to the long axis of the cusp. The dentine matrix in this area is birefringent, and appears green when the tubules are parallel with the slow (positive) axis of a quartz-sensitive tint. The collagen is therefore largely parallel to this tubule direction. ( $\times 60$ .)

hypermineralized with respect to the remainder of the matrix<sup>3</sup>. There is, however, some evidence that collagen exists in this peritubular zone and that the fibres<sup>4</sup> and the hydroxyapatite crystals<sup>5,6</sup> are arranged parallel to the long axes of the tubules.

During a study of the ultrastructure of developing coronal dentine in the molars and incisors of the rat it was observed that most of the collagen fibres were parallel to, rather than perpendicular to, the tubules (Figs. 1 and 2). Some tubules were observed in predentine with a peripheral zone  $1\mu$  wide which was relatively free from organized collagen, and some tubules in the calcified dentine were surrounded by a zone of greater electron density. This peritubular zone frequently accepts heavy metal stains more readily than the surrounding dentine, indicating a greater concentration of reactive ground substance.

Further information was sought by surveying a number of ground sections with the polarizing microscope. In most of the molar dentine the orientation of the collagen fibres is in accord with the accepted view. The steepness of the molar cusps, however, is such that the tubules in this area ascend along the long axis of the cusp and are almost parallel to the enamel-dentine junction for most of their course (Fig. 3). The dentine in this region is positively birefringent with respect to the long axis of

the cusp, and, since the birefringence of collagen predominates<sup>1</sup>, most of the fibres must also lie in this direction. This explains the appearance under the electron microscope of fibres lying along the tubules. In the incisor dentine it was deduced that collagen fibres, for the most part, lie perpendicular to the tubules and parallel to the incremental lines. A narrow band was seen in all sections immediately underneath the enamel where the birefringence differed from that of the rest of the dentine, indicating a collagen orientation more parallel to the tubules; this constitutes the layer of von Korff or "mantle dentine". Some teeth, however, had broad areas in the dentine nearer the centre of the tooth, in which the mean direction of the collagen fibres appeared to be at 40°–45° to that of the tubules and at 20°–30° to the incremental lines. Sections from such areas could give the electron microscope appearance referred to already.

Evidence from the molars thus supports the view that, as each increment of dentine is formed, the collagen is laid down in sheets of randomly interwoven fibres in the plane of the formative front and that the tubules are not involved in their orientation.

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### Effects of Ultra-violet Microbeam Irradiation of Various Sites in HeLa Cells on the Synthesis of RNA, DNA and Protein

THERE have been a number of studies on the effects of ultra-violet microbeam irradiation of delimited areas within cells in culture. In experiments with mitotic cells<sup>1–10</sup>, it has been found that irradiation of a chromosome produces a loss of DNA and histone at the site of irradiation<sup>3,4,9</sup>, and also that the spindle diminishes in size or disappears temporarily following the irradiation of any part of the cell<sup>1,2,5–8,10</sup>. In experiments with interphase cells<sup>11,12</sup>, some authors have reported that about two-thirds of the labelling of cytoplasmic RNA is suppressed by nucleolar irradiation<sup>11</sup>, while extranuclear nuclear irradiation results in a progressive inhibition of DNA synthesis for several hours after the irradiation<sup>12</sup>. We have carried out an autoradiographic study on the effects of ultra-violet microbeam irradiation of different cellular sites on synthesis of RNA, DNA and protein in order to compare the pattern of inhibition in a single cell type.

For our experiments we used a standard HeLa cell line, which had been propagated as a cell-sheet on a glass surface. The cells were cultivated on a quartz coverslip in the culture medium and by a method of culture described previously<sup>9</sup>. The irradiation apparatus was of the Uretz type<sup>5</sup>, and the spot of the heterochromatic microbeam was about 2  $\mu$  in diameter in the focal plane. Each cell was irradiated at only one site for 25 sec unless otherwise indicated, using a high pressure quartz mercury arc lamp (Toshiba 80 W) as the irradiation source. At various times over a period of 12 h, or at 24 h after the irradiation, a group of the coverslip cultures was labelled for 30 min in a culture medium supplemented either

with 2  $\mu$ c./ml. <sup>3</sup>H-cytidine (specific activity: 2.02 c./mmole) or with 1  $\mu$ c./ml. <sup>3</sup>H-thymidine (specific activity: 8.6 c./mmole), or with 16  $\mu$ c./ml. <sup>3</sup>H-phenylalanine (specific activity: 441 mc./mmole). Autoradiographs were prepared by the method of Ficq<sup>13</sup>, using 'Kodak AR10' stripping film. The slides were exposed for 2 weeks and stained with Giemsa solution.

The irradiated cells were relocated on the autoradiographic slides using diagrams made at the time of irradiation, and were then photographed. The number of silver grains over the nucleolar, extranuclear nuclear and cytoplasmic areas was counted on the photographs and tabulated after making a correction for background. An estimate of the synthetic activity for each area in the irradiated cells was computed as a percentage of the average grain count for the corresponding area of unirradiated cells in the same fields.

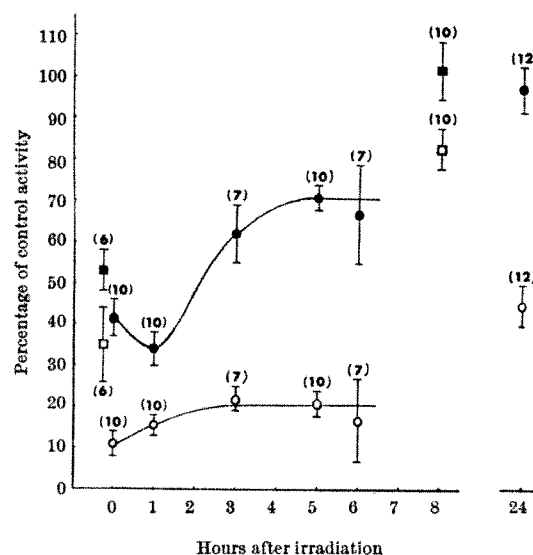


Fig. 1. Effect of irradiation of one nuclear site on nuclear <sup>3</sup>H-cytidine labelling. ○, Nucleolar irradiation, nucleolar activity; ●, nucleolar irradiation, extranuclear nuclear activity; □, extranuclear nuclear irradiation, extranuclear nuclear activity. Figures in parentheses show number of experimental cells.

Effects of nucleolar irradiation on nucleolar and nuclear cytidine uptake are summarized in Fig. 1. In this experiment, cells bearing only a nucleolus were selected and the centre of the nucleolus was irradiated. Four to five hours after the irradiation, the mean activity of the irradiated nucleolus had increased from about 10 per cent to about 20 per cent of the activity in control cells, while that of the extranuclear area was restored to about 70 per cent after first showing a decrease to about 35 per cent 1 h after irradiation. These activities were further restored 24 h after irradiation. The form of the curve of the extranuclear nuclear side effect suggests that about 1 h is required for substance(s) produced at the irradiated site to diffuse throughout the nucleus and affect the enzyme systems in the rest of the nucleus. Irradiations at doses other than 25 sec showed similar patterns in the processes of inhibition and recovery. At lower doses the nuclear side effect was smaller, but the inhibition of the nucleolar activity was also reduced. At higher doses nucleolar inhibition was higher but the side effect was also greater and the recovery was slower. The maximum difference between the two effects was about 50 per cent and was obtained with an exposure time of 25 sec. Morphologically, a circular area bearing fewer grains was found in the nucleus around the irradiated nucleolus for several hours after the irradiation.

Effects of the nucleolar irradiation on the cytoplasmic



RNA were examined by a procedure of labelling with 0.4  $\mu\text{C}/\text{ml}$ .  $^3\text{H}$ -cytidine for 4 h starting 3 h after irradiation. These intervals were selected to measure the dependence of cytoplasmic labelling on nuclear and nucleolar inhibition during the stable stage following the first recovery period. In 15 cells with irradiated nucleoli, the mean of the percentage control activities of the extranucleolar areas of the nuclei was  $79.9 \pm 4.4$  per cent, while that of the cytoplasm was  $57 \pm 3.5$  per cent. There was no significant inhibition in the cytidine uptake, however, either in the cytoplasm of cells, the nuclei of which had been irradiated, or in the nuclei of the cytoplasm irradiated cells a few hours after the irradiation.

Effects of the irradiation on the labelling of the nucleus with thymidine were examined in the cells where either an extranucleolar nuclear area, or a cytoplasmic area, or all nucleoli in a cell, were irradiated with a total dose of 25 sec in different cells in the same fields. The results are summarized in Fig. 2. The irradiated cells without grains were excluded from the data. For all nuclear sites the irradiation caused an inhibition in the rate of DNA synthesis, which developed progressively for several hours after the irradiation. There appears to be no significant difference between the effects of the nucleolar and extranucleolar nuclear irradiations. These results are in good agreement with those obtained by Dendy and Smith<sup>12</sup> in their comparative study on the effects of ultra-violet and  $\alpha$ -particle microbeams on the inhibition of DNA synthesis in mouse fibroblasts. Our results for cytoplasmic irradiation differ from theirs, however, because their rate of the reduction of DNA synthesis was smaller. This might be caused by differences in the experimental conditions or the cell species used.

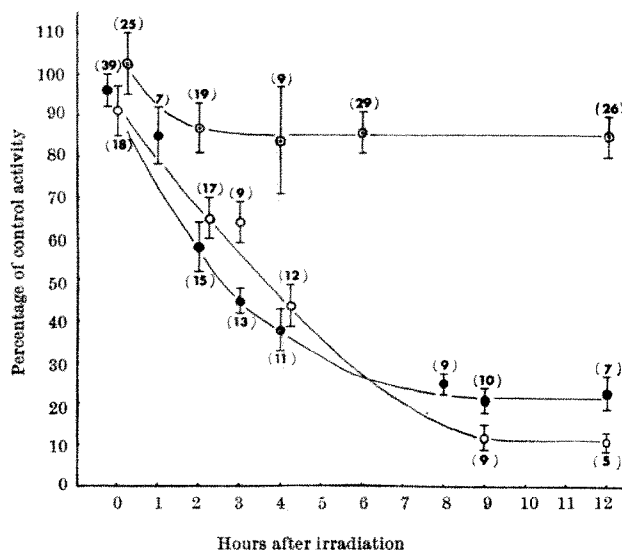


Fig. 2. Effect of irradiation on  $^3\text{H}$ -thymidine labelling. ○, Nucleolar irradiation; ●, extranucleolar nuclear irradiation; ○, cytoplasmic irradiation. Figures in parentheses show number of experimental cells.

In experiments on protein synthesis with phenylalanine incorporation, we found at most only a slight degree of inhibition for all irradiation sites, including nucleolar irradiation.

These results show that ultra-violet microbeam irradiation at one nucleolar or extranucleolar nuclear site rapidly caused an inhibition of RNA synthesis which is partly restored a short time after the irradiation. The inhibition of DNA synthesis, however, develops more slowly and is not restored within the observational period of 12 h. The experiment also shows that protein synthesis is resistant to irradiation of any cellular area at the dose used.

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### Evidence for Direct Physical Bonding between the Collagen Fibres and Apatite Crystals in Bone

THE exact relationship between the mineral crystals and the collagen fibres in bone has not been determined. While the present consensus is that the collagen fibres initiate mineralization<sup>1,2</sup>, and that the initial crystallites have a specific spatial relationship to the collagen fibres<sup>3</sup>, evidence of the exact physical relationship between the fibres and crystals is lacking. This question pertains chiefly to the initial crystallization phase, because subsequent secondary mineralization is believed to occur on the basis of inter-crystalline bonding.

In a previous communication we reported the detection of electron paramagnetic resonance signals from whole human bone as well as extracted collagen and apatite<sup>4</sup>. Apatite, prepared by refluxing with ethylene diamine<sup>5</sup> and washing in distilled water, gave a complex signal consisting of a  $g$ -2 resonance and several lower field resonances. The  $g$ -2 resonance was ascribed to an  $F$  centre and the remaining resonances to various types of structured water. Because the whole bone signal consisted of a singlet at  $g$ -2.001 with a line width of 10 gauss, the relationship between the complex resonance of extracted apatite and that of apatite *in situ* was obscure. In an attempt to clarify this, evidence was obtained which indicated the existence of a direct physical bond between the apatite crystals of the initial mineralization phase and the collagen fibres.

Our initial hypothesis was that the complex apatite signal was largely artefactual, derived from absorption of water molecules in the washing process. Accordingly, we examined apatite after refluxing in ethylene diamine and vacuum drying but without washing in water. A weak  $g$ -2 singlet of 10 gauss width was observed (Fig. 1a). This signal was found to follow a simple Curie dependence between 114° K and 295° K. Subsequent washing of this material for 24 h in methanol or ethanol did not alter the signal. Similar washing in distilled water, however, resulted in the appearance of the previously reported complex apatite signal (Fig. 1b). The magnitude of the total resonance was directly proportional to the length of washing time up to 24 h; however, line width and shape remained constant. Substitution of mammalian Ringer solution for the washing medium resulted in a much distorted  $g$ -2 resonance with marked broadening (Fig. 1c).



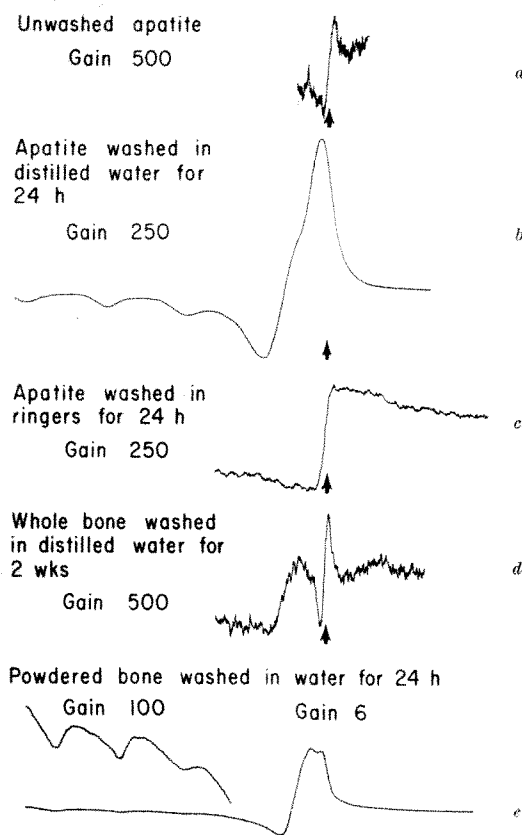


Fig. 1. Electron paramagnetic resonances of extracted apatite and bone. Instrument gain is as noted and the arrow denotes  $g=2.000$ . The broad resonance, just to the low field side of  $g=2$  in the water washed whole bone spectrum, is probably the result of water absorption by the collagen fibres, not by apatite. In the water washed, powdered whole bone spectrum (lowermost in illustration), the three, equally spaced low field resonances are shown at a gain of 100 while the whole trace is at gain 6. The higher gain tracing is sweep synchronized with the whole trace.

These results indicated that apatite, prepared by removal of the matrix collagen fibre, was capable of taking up a variety of inorganic ions from solution. The method of preparation used dissolves all the organic matrix leaving the mineral crystals undisturbed. In this fashion free surfaces are created at the original sites of attachment between the crystals of the initial mineralization phase and the organic matrix. Because collagen constitutes more than 95 per cent of the organic fraction of bone, it is reasonable to expect that the majority of the corresponding binding sites are located on the collagen fibres. The intercrystalline bonds of the secondary phase of mineralization are assumed to be unbroken. It therefore appeared most likely that the ions removed from solution by apatite prepared in this fashion were bonding to sites of previous attachment to the collagen fibres. If this attachment was a direct physical bond, then whole bone subjected to prolonged washing in distilled water should not demonstrate the complex signal. Powdered whole bone (in which a significant number of free surfaces are produced by disruption of the intercrystal bonds of the secondary calcification phases), however, should demonstrate the complex signal. This was found to be the case; whole bone washed for 2 weeks in distilled water displayed an unchanged  $g=2$  resonance (Fig. 1d) while powdered whole bone similarly washed for 24 h revealed a complex signal very similar to that of apatite (Fig. 1e). The broad low field resonance in Fig. 1d is probably a result of absorption of water directly on to collagen fibres<sup>1</sup>.

We conclude, therefore, that the experiments described can be interpreted as supporting the thesis that a direct

physical bond exists between the initial apatite crystallites and the collagen fibres. The constancy of the  $g=2$  singlet from apatite under all experimental conditions would seem to indicate its presence in apatite *in situ* as well. In this case, it would contribute to the  $g=2$  resonance displayed by whole bone.

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### Thermo-voltaic Radiation Dosimetry

THE most familiar process of solid state radiation dosimetry makes use of trapping processes in phosphorescent materials by monitoring thermoluminescent glow curves as a function of dosage received<sup>1</sup>. In previous papers<sup>2-4</sup> it has been shown that this process can be advantageously modified—at least in principle—in a number of ways. Its sensitivity, for example, can be increased by the application of high electric fields which accelerate thermally released electrons and thereby lead to impact ionization. Alternatively, it is possible to use “current-glow” or photo-stimulated currents<sup>3,4</sup> for dosimetric purposes. Because of the absence of light measurements, this leads to a considerable simplification of technique.

It has now been found that thermo-electric properties can be used for dosimetric purposes in a similar way. The principle is simple, though in practice a number of complications are observed. One would expect the incoming radiation to charge electron traps in proportion to the dosage received. Subsequent heating of the specimen in the presence of a thermal gradient should then yield a temporary thermopower which is lower than that associated with unirradiated specimens because of the release of free carriers from the traps. Once these carriers have recombined (whether thermally or radiatively), the original value should be restored.

In the exploratory work reported here, specimens of zinc sulphide in the form of a single crystal clamped between metal electrodes have been irradiated with ultra-violet light. One electrode is rapidly heated to produce a temperature gradient across the sample. In due course the temperature of the second electrode likewise increases, as a result of heat conduction through the crystal. Under final steady state conditions, the temperature difference is again small. The average temperature gradient thus passes through a maximum with time, and so does the corresponding thermo-voltage. Fig. 1 shows a set of results for three different dosages of ultra-violet. Irradiation by X-rays gives quite similar results. After each run, the specimen must be returned to its equilibrium condition. This is done by maintaining it at 400° C for 1–2 h. This procedure ensures repeatable results.

The process is essentially thermo-electric in character, but the conditions are complicated by at least two (probably related) factors: space charge regions arise

from local variations in the population of the filled trap and from the contact parameters. The last factor enters because opposite faces are not equivalent. The observed reversal of polarity is believed to be connected with these factors although the detailed mechanism is not yet fully understood.

As a dosimetric method, the procedures described here differ from those now in use inasmuch as the departures from zero dosage curves are most readily observed when the dosages are small. Saturation usually sets in at high dosages. It is not possible to compare the sensitivity of this method with that of other methods directly. Using zinc sulphide and photo-stimulated current dosimetry in the low temperature range ( $-100^{\circ}\text{C}$  to  $0^{\circ}\text{C}$ ), it has been shown that dosages down to  $0.01\text{ }\mu\text{W sec/cm}^2$  can be measured<sup>2</sup>. Above room temperature, the sensitivity of this process is extremely low, whereas the present procedures lead to still higher sensitivities of the order of  $1\text{ }\mu\text{W sec/cm}^2$ . Because it is only the range above room temperature which is of interest for practical purposes, this comparison favours the thermo-electric method. There are indications that its sensitivity at low temperature is comparable with that of photo-stimulated current dosimetry.

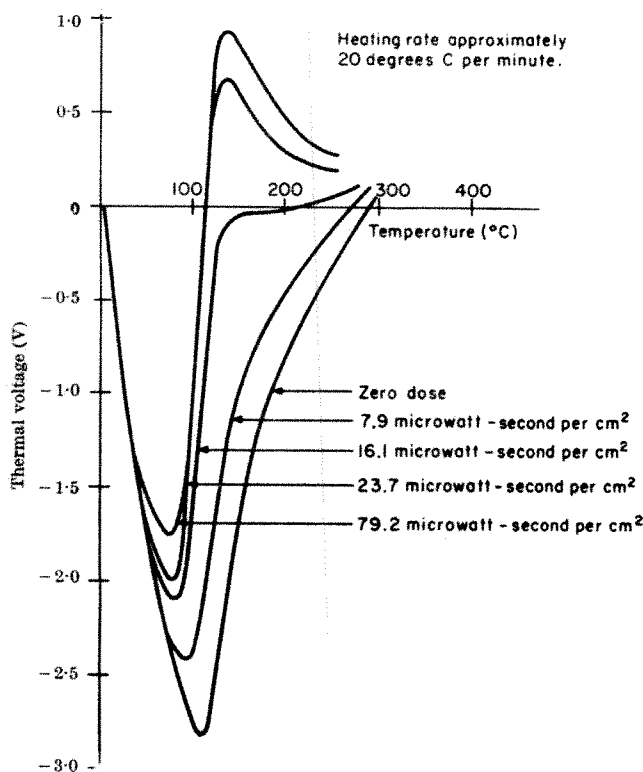


Fig. 1. Thermovoltage response versus hot electrode temperature as a function of ultra-violet dose. Zinc sulphide single crystal, undoped, containing a few p.p.m. of calcium, copper and magnesium. Electrodes on (0001) planes.

The absolute sensitivities recorded refer to specimens and heating programmes which have not been systematically optimized in any way. It is reasonable to expect improvements in sensitivity from appropriate adjustments of the available specimen parameters (including doping) and experimental procedures. Observations similar to those recorded here have been made on cadmium fluoride, lithium fluoride, strontium fluoride, calcium fluoride and beryllium fluoride. In zinc sulphide itself, the trap depth is insufficient to be capable of

storage at room temperature over periods longer than a few hours.

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## GENETICS

### Evidence of Mutation within the Rhesus Blood Group System

As a result of studying the blood groups of many mother and child pairs for medico-legal purposes at the State Institute of Blood Group Serology, Stockholm, several rare Rh phenotypes have been detected<sup>1</sup>. Subsequent family studies have shown their inheritance to be of a straightforward Mendelian type.

Recently another case was encountered in which a child was found to have a previously undescribed phenotype which could be explained by postulating that a mutation has taken place in the mother's reproductive cells. In Fig. 1, it can be seen that the mother, II-1, having an Rh phenotype of ccDEe, was presumed to be of *DcE/dce* genotype as her parents were of phenotypes ccDEE and ccddee. The three children were all of phenotype CcDEe, and were of the genotype *DcE/DcE* by virtue of negative reactions with anti-ce(f) sera. Full grouping of the members available for testing failed to demonstrate whether the same man fathered all three children (Fig. 1). The two putative fathers of the child III-3 had phenotypes of CCDee and CcDee and neither were excluded from paternity. It was established that the mother has passed on the same *DcE* gene complex to each child, as she possesses only one. The *DcE* gene complex is normally expressed in III-1 and III-2 but is much weaker in III-3. Since the *DcE* gene complex is most probably combined with a *DCe* gene complex, it is not possible to show whether the D antigen is suppressed, but the c and E antigens in III-3 were never demonstrable with saline antisera and showed marked weakness with incomplete antisera after ficin-treatment. In order to confirm the presence of c and E antigens in child III-3, the cells were shown to absorb and yield on elution anti-c and anti-E.

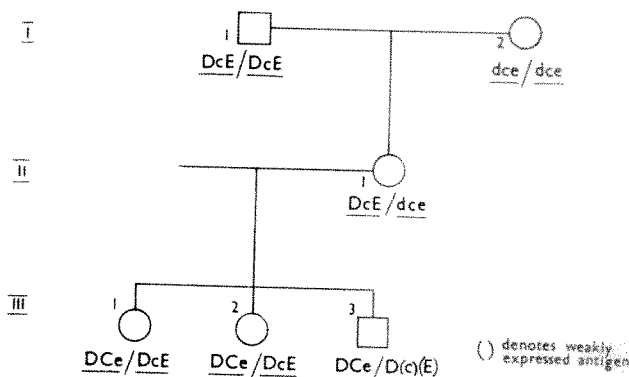


Fig. 1. Family study.

The serological results obtained in this family indicate that the *DcE* gene complex of the mother, II—1, is normally expressed as judged by reactions with anti-D and anti-E sera and that she has passed it on to her two older children, III—1 and III—2, as judged by reactions with both anti-c and anti-E sera. This same *DcE* gene complex has been modified in its expression on the third child's (III—3) red cells. All the other antigens, C, D and e, in child III—3 were quite normally expressed so that this case is not similar to those described by Levine *et al.*<sup>2</sup> and Giles and Bevan<sup>3</sup>, where all Rh antigens present in an individual were suppressed.

One can postulate that a gene mutation has occurred, and that the mother's *DcE* gene would be permanently expressed in the weak form.

Boettcher<sup>4</sup> devised a scheme which envisaged a series of biosynthetic pathways with a sequence of Rh genes acting on precursor substance. Each Rh gene is thought to control the specificity of a single enzyme. It is known<sup>5</sup> that mutations can affect enzyme activity completely or partially. If a mutation occurred along the genetic pathway between the D and C loci which resulted in partial reduction in enzyme activity for C (or c) and E genes, a gene complex of the type described above *D(c)(E)* might result. It must be stressed that it is not possible, in this study, to determine whether the D antigen was also affected by mutation, but if it were one would suggest that the mutation occurred earlier in the pathway before the D locus.

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### Immunochemical Detection of Antigens in Self-incompatibility Genotypes of Cabbage

RECENT advances in immunochemistry permit the characterization of tissue specific antigens, and thus provide highly sensitive techniques for the study of gene action<sup>1</sup>. Of the new techniques available, immunodiffusion offers the following advantages over other serological tests: (a) multiple antigens from particular tissues can be resolved so that the antigens characteristic of different genotypes can be assessed; (b) only a small amount of plant tissue is needed to provide the test antigen; and (c) immunodiffusion tests are simple and easy to set up. This communication deals with the use of immunodiffusion to detect antigenic differentiation in plant tissues, specifically in relation to the control of self-incompatibility by multiple alleles of an "S" locus in plants<sup>2-6</sup>.

From each of four different inbred homozygous genotypes of *Brassica oleracea*, variety 'Capitata', anthers and parts of the pistil, including the upper third of the ovary, the style and the stigma, were extracted in physiological saline. Inbred plants A and C represented two self-incompatible genotypes, while inbred plants D and E represented two self-compatible genotypes. Except for expression of incompatibility, inbred C and inbred D are phenotypically indistinguishable. Inbred D represents a presumed self-compatible mutant arising from inbred C after the latter had been selected for high intensity of self-incompatibility and inbred (by bud pollination) for more than ten generations.

From each inbred, 5 g of anthers and 5 g of pistils (fresh weight) were separately extracted with saline solution (0.8 per cent sodium chloride) in a mortar with a pestle, using sand which had been cleaned with acid. After centrifugation at 5,000g for 15 min, the supernatant was removed and the pulp was again extracted for two additional cycles. The total supernatant collected was adjusted to 45 ml. A total of 15 ml. of a specific extract was injected into each of twenty-four rabbits. The injections were given twice a week during 4 weeks. Every fourth injection was given subcutaneously and the others were given intravenously. One injection was given with complete Freund's adjuvant at the ratio of 1:1. Six rabbits of each incompatibility genotype were used, three for anther and three for pistil extracts.

Two weeks after the last injection, the blood was collected and the sera were obtained by allowing separation from whole blood and clarification by centrifugation at 1,000g. Merthiolate was added as a preservative to give a final concentration of 1:10,000 and the sera were frozen and stored at -10° C. Pre-immunization sera collected from each of the twenty-four rabbits gave negative results with both anther and pistil extracts. Preliminary double diffusion tests with individual antisera led to the selection of the most concentrated serum against anther and pistil antigens of each genotype. Double diffusion at 5° C or at room temperatures and immuno-electrophoretic

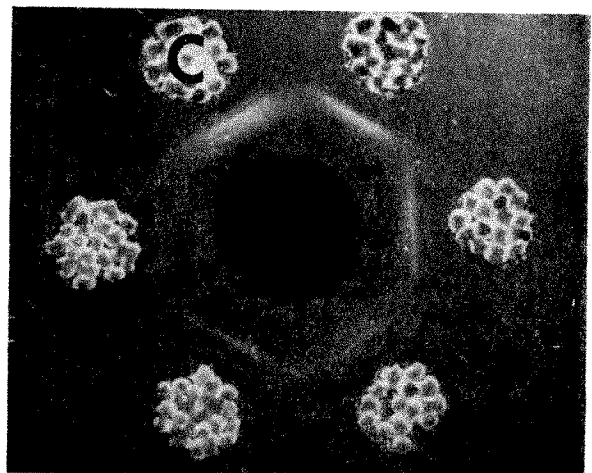


Fig. 1. Intact stigmas reacted in agar double diffusion with anti-pistil antiserum C (centre well). Stigma genotypes, upper left and clockwise: C, D, C x D, C, E, A. Unique bands are resolved opposite C and C x D.

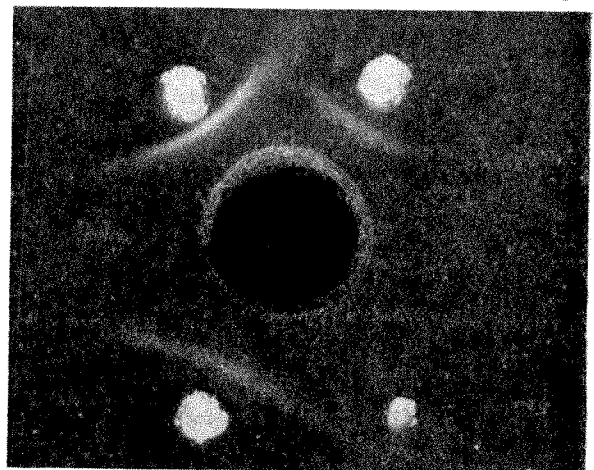


Fig. 2. Excised tissues of C pistil reacted in agar double diffusion with anti-pistil antiserum C (centre well). Left to right: stigma and ovary at the top, and stigma and style at the bottom. Tissue specific antigens are evident opposite the two individual stigmas and the ovary segment.



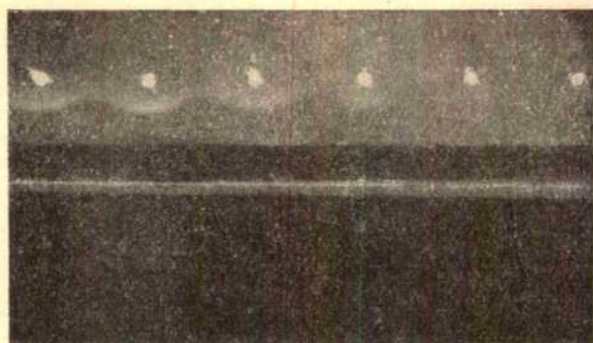


Fig. 3. Resolution, by reaction against anti-*C* serum, of freely diffusible antigens from six single stigmas from a sequence of developing flowers on a flowering raceme of inbred *C*. The age sequence from left to right is: 0, -1, -2, -3, -4, and -5, where 0 is anthesis and -5 is approximately 5 days before anthesis. 0, -1, -2 and -3 are self-incompatible and exhibit freely diffusible antigens while -4 and -5 are self-compatible and lack these antigens.

tests<sup>7</sup> failed to show consistent and unequivocal differences between anther homogenates obtained from the four genotypes and used as test antigens against homologous and heterologous sera. Anther homogenates were strongly antigenic in rabbits and a minimum number of seven precipitation bands were resolved in each genotype.

Excised mature anthers, used directly as test antigens, gave precipitation bands with anti-anther sera. One millimetre sections from anthers of different ages (*F*, *B*<sub>1</sub>, *B*<sub>2</sub>... *B*<sub>14</sub>) were excised and implanted as test antigens in agar double diffusion plates. (*F* refers to anthers from an open flower, while *B*<sub>1</sub> to *B*<sub>14</sub> refers to anthers from the first to the fourteenth consecutive bud above the youngest open flower on a raceme. Thus, *B*<sub>1</sub> refers to the oldest and *B*<sub>14</sub> to the youngest bud observed.) Distinct antigenic patterns were distinguishable for: (a) mature yellow anthers *F*, *B*<sub>1</sub> and *B*<sub>2</sub>; (b) yellow-green anthers of intermediate maturity (*B*<sub>3</sub> to *B*<sub>8</sub>); and (c) green immature anthers (*B*<sub>9</sub> to *B*<sub>14</sub>). Homogenates of samples from these three age categories of developing anthers also gave distinct antigenic patterns, and indicated that changes in soluble antigens accompany the development and differentiation of microspores. Acetocarmine squashes of individual anthers from each of the tested buds indicated that all the buds were in post-meiotic stages.

Reaction of pistil homogenates against anti-pistil sera gave fewer precipitation bands than did reaction of anther homogenates against anti-anther sera, and differences were observed among the pistil homogenates of the different incompatibility genotypes. Certain of these antigens diffused freely from unmutated stigmatic surfaces. As shown in Fig. 1, anti-pistil antiserum against inbred *C* (anti-*C*) indicated the presence of unique antigenic components in inbred *C* stigmas. Stigmas from none of six other incompatibility genotypes (including inbred *D*) gave the same number of bands as *C* stigmas when tested against anti-*C*. Precipitation bands became visible less than 24 h after reactants were placed on agar plates. Bands unique to the *C* genotype were evident only when stigmatic tissues were used as the source of antigens. The ovary exhibited tissue specific antigens (Fig. 2), but these were also present in the ovaries of other genotypes tested.

Diffusion of antigens from the unmutated stigmas presumably occurred through the papillary cells of the stigmatic surface. When the cut surface of the style was sealed with paraffin there was no effect on the diffusion of antigens from the excised stigmas. The role of the freely diffusible anther and stigmatic antigens is not known, but tests with developing stigmas suggest that the antigens may indeed play a part in self-incompatibility. Such tests were carried out with immature as well as mature stigmas of the self-incompatible inbred *C*. Buds of genetically self-incompatible cabbage plants exhibit self-compatibility

when pollinated in the intermediate and immature stages; at these stages they lack one or more of the unique and freely diffusible antigens present in stigmas of the self-incompatible buds and flowers (Fig. 3). Thus in a developmental sequence the appearance of self-incompatibility is correlated with the appearance of particular antigens.

The chemical nature of these stigmatic and anther antigens is not known, but some observations have been made. When the stigmatic homogenates are incubated at 60° C for 20 min and then used as test antigens, antigenic components are no longer detected by double diffusion. The stigmatic antigens migrate in an electric field as indicated by immuno-electrophoresis and are precipitated from solution by ammonium sulphate.

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## CYTOLOGY

### Histones in Prophase and their Possible Role in Nuclear Membrane Breakdown

THERE is much interest in the histones in the context of the hypothesis that their biological function is to exert specific genetic repression in differentiated cells<sup>1-3</sup>. There is, however, evidence which does not support this hypothesis<sup>4,5</sup> but which suggests that the histones could have a structural role in the organization of chromatin<sup>6</sup>.

There have recently been several reports that these basic polymers also have considerable activity in a second area of cell function; they affect a number of properties associated with cell membrane systems, including both the surface membrane<sup>7-8</sup> and the mitochondrial membrane<sup>9</sup>. In some of these investigations the histones are active at concentrations so low that it is difficult to avoid the implication that this activity has some physiological significance. For example, uptake of albumin into S180 cells in tissue culture, a process presumably dependent on activity of the surface membrane, is stimulated by the arginine-rich histone fraction at concentrations as low as 10<sup>-8</sup> molar (0.3 µg/ml.)<sup>7</sup>, and the surface membrane of these cells shows evidence of functional damage (as shown by abnormal dye and protein permeability) by this histone fraction at concentrations of 3 × 10<sup>-6</sup> molar (100 µg/ml.). The mechanism of these effects on membrane function is not yet understood and might be indeed quite complex; a simple process could be envisaged in which the adsorption of positively charged polymer molecules to the plasma membrane reduces the net negative charge of the cell surface<sup>8,9</sup>, and in which the resulting reduction in the electrostatic interactions would lead to an increased deformability similar to that resulting from charge reduction by neuraminidase treatment<sup>10</sup>. Such a process would form a basis for the stimulation by basic polyamino-acids of phagocytosis<sup>6</sup>, which involves an infolding of the membrane to form vesicles; and also for the stimulation of the uptake of macromolecules (nucleic acids<sup>11</sup>, albumin<sup>7</sup>, and inulin (work by Amundsen and Ryser, in preparation)) assuming that this uptake occurs by a process of pino- or micropino-cytosis. Higher levels of polymer adsorption lead to impaired exclusion of dye and protein molecules in S180 cells<sup>7</sup>, probably by causing structural changes in the surface membrane similar to those induced in the erythrocyte membrane by low concentrations (2 µg/ml.) of polylysine<sup>8</sup>, where it can be observed directly that the



homogeneous fine structure of the membrane is converted by the basic polymer to a much coarser texture with marked discontinuities.

One aspect of the possible significance of these actions of histones and basic polymers on cell membrane systems concerns the possibility that such an action could be involved in the breakdown of the nuclear membrane at prophase. This possibility follows from evidence which suggests that there is a release at prophase of some of the nuclear histones from their association with DNA. This evidence includes the following. (1) The conclusion, from autoradiographic analyses, that chromosomal proteins in hamster fibroblasts and root meristem cells of *Vicia faba* are not conserved during mitosis like the DNA, but become completely dispersed during relatively few divisions<sup>12,13</sup>. In *Amoeba proteus* a large proportion of the nuclear protein synthesized in interphase is dispersed throughout the cytoplasm at the subsequent mitosis<sup>12</sup>; a similar process has been reported in epithelial cells of rats<sup>14</sup>. (2) In the nuclei of tissues containing a high proportion of cells in interphase, the histone:DNA ratio has been shown to be  $\sim 2$  whereas in tissues with a high proportion of cells in mitosis it is close to 1 (ref. 15), approximating the value usually found for isolated chromatin<sup>16,17</sup>. This difference is suggested to represent a fraction of the total histone which is only loosely bound; the results would be compatible with the dispersion into the cytoplasm of a fraction of the total nuclear histone when the nucleus undergoes mitosis. Such a process was also put forward to explain the observation that in regenerating liver the ratio of one histone fraction (designated fraction 2) to DNA falls during a wave of mitoses<sup>18</sup>. (3) The observation that ribosomes from HeLa cells in metaphase are inactive in polypeptide synthesis relative to ribosomes from interphase cells, but that activity may be restored by very mild treatment with trypsin<sup>19</sup>; it was proposed that histones, released from the nucleus at the time of breakdown of the nuclear membrane, could be responsible for this inhibition of ribosome function. Ribosomes from non-synchronized chick fibroblasts can also be made inactive by addition *in vitro* of 1  $\mu\text{g}/\text{ml}$ . of calf thymus histone or salmon protamin (unpublished results of Amos).

These observations taken together seem to constitute strong evidence that a fraction of the histones of an interphase nucleus may be released from its association with DNA at prophase. This release could be concurrent with the changes in the configuration of the DNA as it condenses from its diffuse configuration in euchromatin to the dense configuration of prophase chromosomes, and be caused by the different structural (or functional) role of histone in these two states.

From this conclusion, together with the observations that histones may cause marked changes in the properties and the structure of cellular membranes, there follows the possibility that histones, thus released from the DNA, could be responsible for the structural changes in the nuclear membrane at prophase.

The quantity of histone in an interphase nucleus of a mammalian cell is approximately  $6 \times 10^{-6}$   $\mu\text{g}$  (approximately equivalent to the quantity<sup>20</sup> of DNA), so that if 10 per cent (for example) of the total histone were released at prophase within a spherical nucleus of diameter 10  $\mu$  (volume  $5 \times 10^{-10}$   $\text{cm}^3$ ), the concentration of free histone could reach 1,000  $\mu\text{g}/\text{ml}$ . If the nuclear membrane behaves similarly to the surface membrane in the sense of its reactivity with, and sensitivity to, basic polymers (it appears to carry a negative charge, like the surface membrane<sup>21</sup>), marked changes in its properties and structure would be predicted to follow the release of this quantity of histone, which is much above the critical level causing damage to the surface membrane<sup>7</sup>. The observations cited above suggest the release at prophase of a proportion of the total histone considerably greater than this figure of 10 per cent used for illustration, but the activity of histones on intracellular membranes would

undoubtedly be modified by the presence of other cellular constituents. It would seem possible that the resulting histone concentration could be high enough to cause the dispersion of the nuclear membrane material which occurs at prophase; the structural changes in the perinuclear zone, which have been shown to coincide with the breakdown of the nuclear envelope<sup>22</sup>, could have the same origin. A number of polybasic compounds of low molecular weight induce "blebbing" and dissolution of the nuclear membrane<sup>23</sup>. In view of the observations that the lysine-rich histones are most active in condensing chromatin<sup>24</sup>, whereas the arginine-rich histones show greater membrane activity<sup>7,9</sup>, the possibility could also be considered that there is a predominance of the arginine-rich fraction in the histone released at prophase.

On the basis of such a model, it could be further speculated that the marked changes in cytoplasmic structure accompanying mitosis could also be associated with the diffusion of histones from the nucleus. The possibility that these changes are caused by the release of (undefined) substances from the chromosomes in the early stages of mitosis has already been proposed and discussed in some detail<sup>25</sup>; other results led to the conclusion that the changes in the nuclear membrane in mitosis are generated within the nucleus itself rather than in the cytoplasm<sup>26</sup>. It was also suggested that the increased movements of the surface membrane during mitosis are caused by an agent released from the chromosomes<sup>27</sup>. It is clear that there are several aspects of the model discussed here which are susceptible to experimental test.

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### Deposition of Sporopollenin on Lamellae of Unit Membrane Dimensions

THERE are several reports of a lamellar inner part of the exine of pollen grains<sup>1-8</sup>. The number of species examined is small, but the sampling of plant families is wide enough to conclude that the deposition of sporopollenin in some kind of lamellar form is probably characteristic for at least the inner unornamented part of the exine of pollen grains.

In microspores of *Anthurium* sp., fixed in formalin<sup>9</sup> and stained with uranyl acetate and lead citrate<sup>10</sup>, we have direct evidence for the formation of some of the exine on lamellae of unit membrane dimensions. Sporopollenin accumulates on such membranes, often asymmetrically, to form the buttressing lamellae of the germinal apertures (Fig. 1). These buttressing lamellae grow to 0.3–0.4  $\mu$  in cross-sectional height, but they retain the line of low density characteristic of the unit membrane until near pollen maturity. In mature pollen only a staining discontinuity at the relative position of the uppermost line of low density around the apertures remains as an indication of the mode of origin (Fig. 2).



Fig. 1. Section of one side of a germinal aperture region in a microspore of *Anthurium*. The accumulation of sporopollenin on some of the unit membrane-like lamellae is asymmetric (arrow). Lines similar to the characteristic region of low density in the above lamellae are traceable (double-headed arrow) in all of the buttressing parts of the exine around the germinal aperture (P) including the one that is outermost. ( $\times$  c. 18,750.)

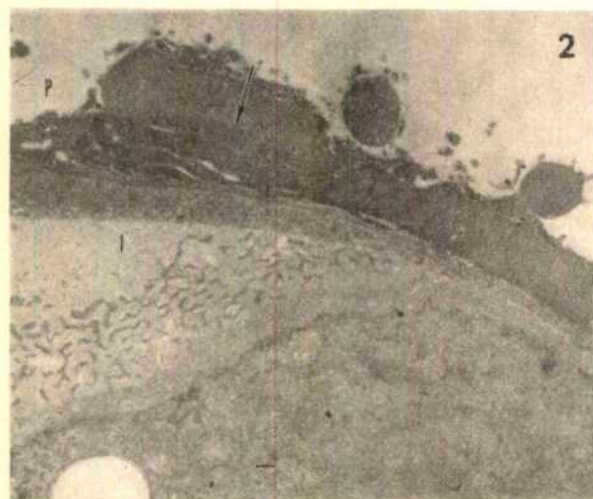


Fig. 2. Medial section of one side of a germinal aperture in a mature pollen grain of *Anthurium*. A staining discontinuity (arrow) can be traced in the outer part of the exine beside the pore (P). Strands of cytoplasm in the intine (I) are bounded by plasma membrane. ( $\times$  c. 15,000.)

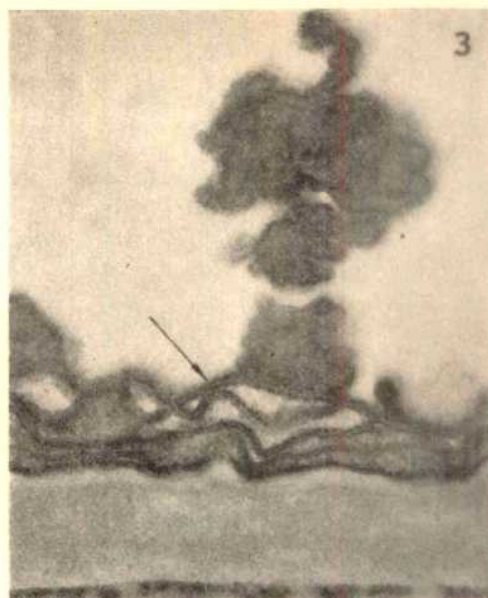


Fig. 3. Section from an immature *Scapania nemorosa* spore wall. About five unit membrane-like lamellae (arrow) form the exine. The piles of dense material that constitute the ornamentation over the lamellae may also form on membranes but the amount of sporopollenin already present obscures that information. At spore maturity the membranous origin of all parts of the wall is obscured. ( $\times$  c. 50,250.)

In the spores of the liverwort *Scapania nemorosa* most of the spore wall has a laminar or unit membrane origin (Fig. 3). The wall of immature spores of *Scapania* is composed of a layer of low density overlain by acetolysis-resistant material (sporopollenin). The resistant components consist of about five lamellae having some characteristics of unit membranes surmounted by stacks of disks bounded by a membrane (Fig. 3). The piles of disks are irregularly shaped and spaced and appear similar to the ornamentations on spores of mosses reported by von Wettstein<sup>11</sup> and McClymont and Larson<sup>12</sup>. Working with *Geothallus*, Doyle<sup>13</sup> determined that two of the four wall layers of the spores gave reactions which indicated the presence of waxy compounds on a carbohydrate framework. The inner layer of the above two was composed of a series of lamellae and both layers gave histochemical reactions indicative of sporopollenin.

Heslop-Harrison<sup>6,7</sup> and Larson and Lewis<sup>4</sup> have presented micrographs showing endoplasmic reticulum oriented parallel to the plasma membrane in regions of presumptive germinal apertures. These writers have strongly suggested that the position of elements of the endoplasmic reticulum influences the deposition of the exine. We do not know the source of the membranes, but lines of low density are about 40 Å in cross-sectional height and highly regular as would be expected of a paracrystalline molecular system such as the unit membrane. The minimal dimensions for the two dense outer areas measured 60 or 100–120 Å. The 60 Å dense bands may be a single strand of sporopollenin similar to Rowley's<sup>14</sup> description of stranded sporopollenin in the exine of *Poa annua* microspores or to the description of Afzelius<sup>1</sup> of aligned granules. The dense parts that were measured at 100–120 Å in thickness may consist of two layers of sporopollenin strands.

Afzelius<sup>15-17</sup> found lines of unit membrane dimensions in mature exines of *Acmopyle* and *Sequoia* pollen and of *Lycopodium* spores. In *Nuphar*, Rowley (unpublished data) observed low density lines of 35–40 Å width aligned both parallel and perpendicular to the ornamented surface of the exine. In *Nuphar* the low density lines extend through the entire length of 0.5  $\mu$  spinules.

The presence of unit membranes around isolated agglomerations of sporopollenin (Ubisch bodies), which are

found at considerable distances from microspores, is further evidence of a universal mode of sporopollenin deposition on unit membranes. While working with Professor G. Erdtman on pollen of *Populus tremula*, we observed membrane bound Ubisch bodies located between tapetal cells.

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## MICROBIOLOGY

### Sensitivity of *Trypanosoma equiperdum* to the Action of Tumour-inhibitory Antibiotics *in vivo*

THE marked sensitivity *in vitro*<sup>1</sup> of various metabolic pathways in *T. equiperdum* to actinomycin D, mitomycin C, porfiromycin, phleomycin, and pactamycin indicated that these tumour-inhibitory antibiotics may be trypanocidal *in vivo*. Our results show this to be so with all except pactamycin, and also that phleomycin and porfiromycin in particular cured a high percentage of infected mice at certain dosages.

Female *Ha/ICR* albino mice, 19–22 g, were inoculated intraperitoneally with about  $1 \times 10^6$  viable trypanosomes (by haemocytometer count) obtained from the blood of infected mice and suspended in isotonic saline containing 1 per cent glucose. The strain of *T. equiperdum* used in these experiments was obtained several years ago from Dr. David Weinman, Yale University School of Medicine, and the parasites have been propagated by mouse-to-mouse passage since that time. Untreated infected mice invariably died 52–70 h after inoculation. The efficacies of the maximal tolerated quantities of each antibiotic at repeated and single doses were compared. The time when each group of ten mice was first injected with an antibiotic is given in Table 1.

Using separate groups of similarly infected mice, blood collected periodically by orbital puncture was examined to determine changes in parasitaemia during the course of three consecutive single daily intraperitoneal injections of the antibiotics, treatment starting 24 h after inoculation. The haemocytometric technique for counting these blood forms is described in detail elsewhere<sup>2</sup>.

The trypanocidal property of phleomycin (8 mg/kg), porfiromycin (10 mg/kg), and mitomycin C (4 mg/kg) is

shown in Fig. 1. These antibiotics, when injected intraperitoneally into infected mice at 24, 48, and 72 h after inoculation, in each instance caused a significant reduction in parasitaemia within 6 h, which became most marked within 12 h. Thereafter, the surviving trypanosomal population began to increase, but at a rate slower than normal. Although the trypanocidal potency of phleomycin and porfiromycin apparently did not change with repeated injection, that of mitomycin C seemed to become progressively weaker under these conditions. Pactamycin (4 mg/kg) and actinomycin D (0.1 mg/kg) were not trypanocidal and altered the course of parasitaemia only slightly when injected intraperitoneally under the conditions already described. It has been reported<sup>3</sup> that a given dose of actinomycin D injected subcutaneously is more efficacious in tumour-bearing mice than when injected intraperitoneally; the effect of actinomycin D (0.1 mg/kg) given subcutaneously under the conditions already described was re-examined. It can be seen from Fig. 1 that daily doses of actinomycin D injected subcutaneously, although still not apparently trypanocidal, were capable of prolonging significantly the doubling time of blood-dwelling trypanosomal populations. Not shown in Fig. 1 but important in the subsequent adoption of optimal dosages were these findings: (1) the trypanocidal potency of phleomycin and porfiromycin injected intraperitoneally was much greater when the total maximally tolerated dose was divided into equal portions injected every 12 h than when larger portions were injected once daily or the entire amount at one time; (2) the trypanocidal potency of actinomycin D was revealed when the total maximally tolerated dose was injected subcutaneously at one time rather than in portions injected every 12 h or once daily.

The comparative efficacies of different antibiotic dosages in prolonging the life and curing mice infected with *T. equiperdum* are shown in Table 1. All regimens provided almost maximally tolerated total doses of each antibiotic, and it would appear that phleomycin was the most effective of those antibiotics tested in the treatment of this experimental trypanosomiasis. Its marked curative action when given intraperitoneally at intervals of 12 h starting shortly after the mice were inoculated with parasites still held when treatment under this regimen was delayed for 24 h. In advanced trypanosomiasis, when treatment was delayed for 48 h, phleomycin no longer effected a

Table 1. COMPARATIVE EFFICACIES OF DIFFERENT DOSAGE REGIMENS OF PHLEOMYCIN, PORFIROMYCIN, MITOMYCIN C, ACTINOMYCIN D, AND PACTAMYCIN IN THE TREATMENT OF MICE INFECTED WITH *Trypanosoma equiperdum*

Antibiotic	Repeated dose* (mg/kg)	Single dose† (mg/kg)	Time of first injection‡ (h)	Average survival time§ (h)	No. surviving after 3 weeks (N/20)
None	—	—	—	58 ± 1	0
Phleomycin	4	—	1	310	16
"	4	—	24	292	14
"	4	—	48	224 ± 24	0
"	—	24	1	120 ± 7	2
Porfiromycin	8	—	1	227	14
"	8	—	24	288 ± 16	4
"	8	—	48	65 ± 2	0
"	—	48	1	250 ± 15	0
Mitomycin C	2	—	1	240 ± 9	2
"	—	5	1	94 ± 3	0
Actinomycin D	0.1	—	1	80 ± 4	0
"	0.1 (s.c.)	—	1	120 ± 7	0
"	—	1 (s.c.)	1	169 ± 18	6
Pactamycin	2	—	1	71 ± 2	0
"	—	10	1	79 ± 4	0

*Ha/ICR* female albino mice, 19–22 g, were inoculated intraperitoneally with about  $1 \times 10^6$  viable trypanosomes, and were randomized into groups of ten. Each group was treated with an antibiotic given by either of two regimens: (1) at a dose listed in the column indicated by (\*), injected intraperitoneally (unless otherwise indicated) six consecutive times every 12 h, starting at a time after inoculation of parasites listed in the column indicated by (†); (2) at a dose listed in the column indicated by (†), injected intraperitoneally (unless otherwise indicated) just one 1 h after inoculation of the parasites. Controls received comparable volumes of isotonic saline solutions given by identical regimens. Standard errors of the average survival times calculated for each treated group (in duplicate) appear in the column indicated by (§). Such calculations were not performed when 70 per cent or more of the treated mice were cured.



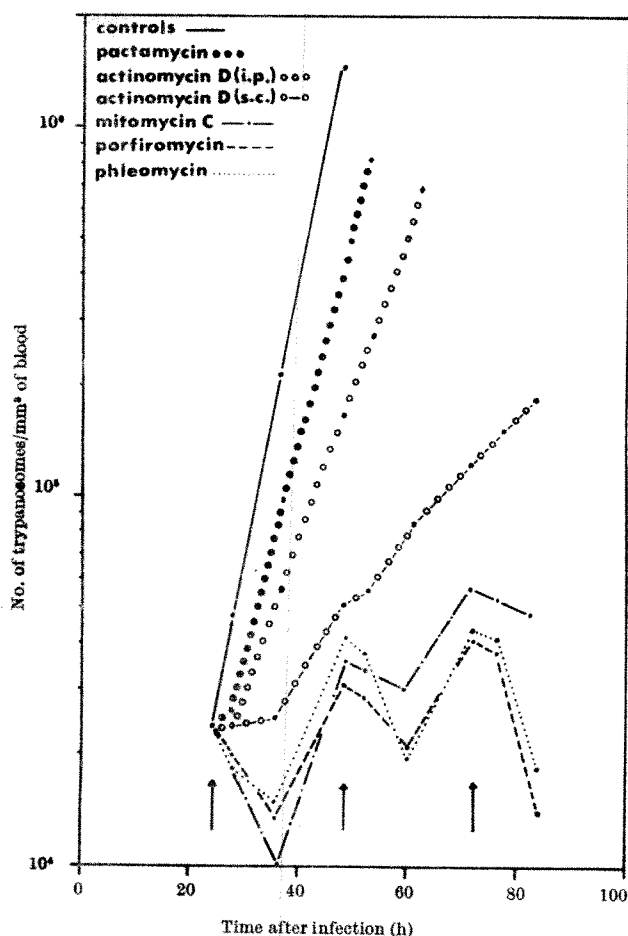


Fig. 1. *Ha/ICR* female albino mice, 19–22 g, were inoculated intraperitoneally with about  $1 \times 10^4$  viable trypanosomes, and were randomized into groups of ten. Each group treated with an antibiotic received intraperitoneal injections at 24, 48 and 72 h after inoculation of the parasites. Injections were by the intraperitoneal route unless otherwise indicated. The doses used were: phleomycin, 8 mg/kg; porfiromycin, 10 mg/kg; mitomycin C, 4 mg/kg; actinomycin D, 0.3 mg/kg; and pactamycin, 4 mg/kg.

cure, but it retained its ability to prolong markedly the lives of the hosts. The 12 h intraperitoneal dosage regimen was clearly superior to that using a single injection of the total maximum tolerated dose.

The mitosane antibiotics, mitomycin C and its methyl congener, porfiromycin, were qualitatively similar in their action against *T. equiperdum in vitro*<sup>1</sup>, and this was apparently also the case with regard to their effects on the course of parasitaemia *in vivo* (Fig. 1). While both mitosane antibiotics, at maximally tolerated doses, could markedly prolong the lives of mice infected with *T. equiperdum*, porfiromycin cured a much higher proportion of infected animals. The finding in this instance that porfiromycin has a more favourable therapeutic index than has mitomycin C is similar to that reported by others<sup>4</sup>. Like phleomycin, porfiromycin was more effective when given intraperitoneally at intervals of 12 h than when given by the intraperitoneal route in a single maximal tolerated dose. But, unlike phleomycin, porfiromycin lost most of its curative power when treatment was delayed for 24 h.

The results in Table 1 clearly indicate that a given dose of actinomycin D was more effective when injected subcutaneously than when injected intraperitoneally. Actinomycin D effected cures only when it was injected subcutaneously shortly after inoculation of the mice with parasites in a single maximally tolerated dose.

Despite its ability *in vitro* to interfere with several metabolic pathways of *T. equiperdum*, especially in the

area of carbohydrate metabolism<sup>1</sup>, pactamycin was almost inactive *in vivo* when given in a repeated or in a single dose shortly after inoculation of the mice with parasites. This discrepancy, as well as those already mentioned with regard to influence of the injection route on the efficacy of actinomycin D and the influence of chemical structure on the efficacy of the mitosane antibiotics, is probably related to pharmacological factors such as degree of serum binding and rate of inactivation by the liver.

It is of more than passing interest that phleomycin<sup>5</sup>, porfiromycin<sup>6</sup>, mitomycin C<sup>7</sup>, and actinomycin D<sup>8</sup>, which are more or less effective as curative agents in experimental trypanosomiasis caused by *T. equiperdum*, are derived from various species of *Streptomyces*. Thus, these antibiotics can be included in a growing family of trypanocidal substances, with disparate chemical structures, which are derived from the same genus<sup>9</sup>. Perhaps it is not too unrealistic to hope that further investigation will lead to the discovery of related trypanocidal antibiotics with therapeutic indices sufficiently favourable to be useful in veterinary or human medicine.

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### A D Serotype of Satellite Virus specifically associated with a D Serotype of Tobacco Necrosis Virus

Kassanis and Nixon<sup>1</sup> and Kassanis<sup>2</sup> reported that the Rothamsted strain of tobacco necrosis virus (RTNV) contains two serologically unrelated viruses with different sized particles; one with smaller particles (17 mμ) called "satellite virus" caused no lesions on beans and multiplied detectably only when present in mixed infections with the large particle (27 mμ) TNV. Tobacco mosaic, lucerne mosaic, carnation ringspot, and tomato bushy stunt viruses failed to make the small particles multiply<sup>1</sup>. All strains of TNV, except strain D, were reported to induce multiplication of satellite although some strains apparently were more effective than others<sup>3</sup>. Several strains of TNV were classified into two serotypes, A and D<sup>3</sup>. Strains A, B, C, F and S which supported multiplication of satellite are in serotype A. Strain E, which was also reported to support multiplication feebly, and strain D, which did not, are in serotype D. Babos and Kassanis<sup>3</sup> concluded that the ability of these strains to induce the multiplication of satellite was not related to their antigenic composition. We have found, however, that the satellites associated with different serotypes of TNV are serologically distinct, as are the virus strains with which they are associated.

In designations of serotypes of our isolates of TNV, we have followed Babos and Kassanis<sup>3</sup> and based our classification on reactions with antisera specific for A and D viruses kindly supplied by Dr. Kassanis. Moreover, the relative serological relationship of different cultures of



satellite is based on a comparison of the reaction of RTNV satellite (*A* serotype) with its specific antiserum and the reaction of a satellite associated with *AC* 36 TNV (American Type Culture Collection) which is a *D* serotype.

RTNV was increased in tobacco (*Nicotiana tabacum* L., Turkish) and *AC* 36 in pumpkin (*Cucurbita pepo* L., small sugar). Virus and satellite were partially purified by methods similar to those described by Kassanis and Nixon<sup>1</sup> and were further purified and separated by centrifugation in sucrose density gradients<sup>5</sup>. Contents of the gradient tubes were fractionated and recorded with an Isco 'Mod. D' density gradient fractionator<sup>6</sup>. Both virus and satellite were well separated in the first density gradient centrifugation, so recentrifugation of the separated components in density gradients produced a discrete band that appeared as a single peak which absorbed ultra-violet when gradient tubes were analysed and recorded with the Isco fractionator.

Purified virus and satellite appeared to be homogeneous as indicated by electron microscopy, the formation of single discrete bands in density gradients, and specific serological reactions with their respective antisera.

The satellite associated with *AC* 36 was approximately 18.0 m $\mu$  in diameter (Fig. 1) and sedimented at about the same rate in density gradients as the RTNV satellite. Thus it is similar to the RTNV satellite in both size and density.

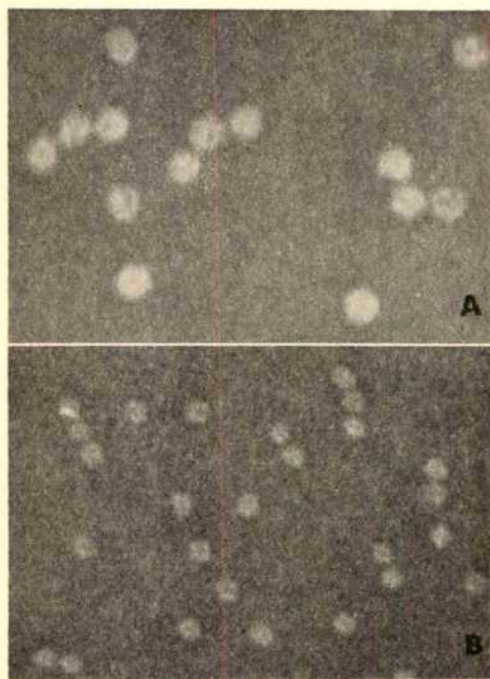


Fig. 1. Electron photomicrographs of (A) *AC* 36 TNV, and (B) *AC* 36 TNV satellite. Particles were negatively stained with phosphotungstic acid. ( $\times 225,000$ .)

Before use as injection antigens for production of antisera or as test antigens, virus and satellite were centrifuged in sucrose density gradients from one to four times depending on the degree of purity desired. Antisera for RTNV, RTNV-satellite, *AC* 36 virus and *AC* 36-satellite were prepared by a series of interveinal, intramuscular or subcutaneous injections with the respective purified antigens. The antigens were emulsified with Freund's incomplete adjuvant for the latter two types of injections. All serological comparisons were made by immunodiffusion precipitation tests in agar gel using methods described previously<sup>4</sup>. Dilution end point titres of the antisera expressed as reciprocals of dilutions to homologous and

heterologous antigens, respectively, were: RTNV satellite 8192 and 1024; RTNV virus, 512 and 64; *AC* 36 satellite, 8192 and 256; and *AC* 36 virus, 8192 and 256. The RTNV satellite antiserum received from Dr. Kassanis was reported to have a titre of 80 to homologous antigen; it reacted positively with *AC* 36 satellite, but its titre to this antigen was not determined.

Results from serological tests in which purified virus or satellite antigens were placed opposite their respective homologous antisera in a four-member pattern in agar gel (not illustrated) showed that the lines of precipitation formed by the respective homologous reactants crossed without apparent deflexion. These results indicate that virus and satellite are not serologically related or are, at most, only very distantly related; they support the conclusions of Kassanis and Nixon<sup>1</sup>.

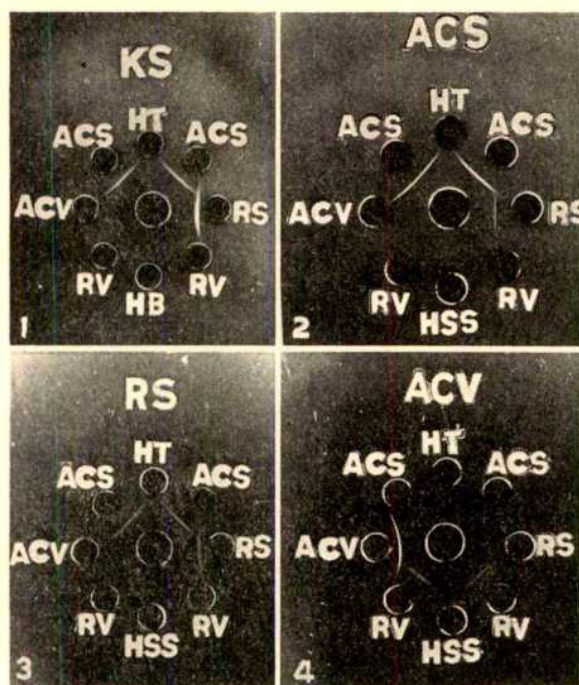


Fig. 2. Immunodiffusion precipitin patterns in agar gel produced by reaction of antisera for RTNV satellite, *AC* 36 satellite, and *AC* 36 TNV, with virus and satellite antigens. (1) *KS*-RTNV satellite antiserum from Kassanis; (2) *ACS*-*AC* 36 satellite antiserum; (3) *RS*-our RTNV satellite antiserum; and (4) *ACV*-*AC* 36 virus antiserum. Antisera were placed in the central wells and antigens in outer wells, designated as follows: *HT*, healthy tobacco; *ACS*, *AC* 36 satellite; *ACV*, *AC* 36 virus; *RS*, RTNV satellite; *RV*, RTNV; *HSS*, healthy small sugar pumpkin; and *HB*, healthy bean.

The reaction pattern produced when RTNV satellite antiserum received from Kassanis (*KS*, Fig. 2-1) was tested with RTNV satellite and *AC* 36 satellite antigens was identical with the pattern formed when our antiserum to RTNV satellite was tested with the same antigens (Fig. 2-3). The homologous lines of precipitation joined the lines formed by reaction with *AC* 36 satellite, but a distinct spur of precipitate was formed as an extension of the RTNV satellite line.

When the two antigens were similarly tested with antiserum for *AC* 36 satellite (Fig. 2-2) the lines of precipitate again joined, but the spur in this case was an extension of the *AC* 36 satellite line. This shows that the two satellite antigens are serologically related but mutually distinct serologically. The two *A* and *D* serotype viruses, RTNV and *AC* 36, have a similar serological relationship as demonstrated in Fig. 2-4. The line of precipitate formed by *AC* 36 and its specific antiserum joined the line formed by reaction with RTNV, but there was a distinct spur



which is an extension of the homologous line of precipitate. Reaction of the two viruses with RTNV antiserum (not illustrated) produced a similar pattern but with the spur forming as an extension of the RTNV line of precipitation. Thus the serological relationship of the two viruses is comparable with that of the two satellites in that they also are serologically related, but distinct.

Mixed inoculation of RTNV satellite with AC 36 virus resulted in production of only AC 36 satellite in the same amount and ratio to virus as when highly purified ( $5\times$  density gradient) AC 36 virus was inoculated alone.

This latter result indicates a need to determine with more certainty whether it is possible to free TNV cultures of satellite if they originally contained it. It should be pointed out here that certain TNV strains, for example Kassanis' TNVD, produce very little and usually no demonstrable satellite and this strain was reported by Babos and Kassanis<sup>3</sup> as not supporting production of satellite. Kassanis and Nixon<sup>1</sup> were unable to obtain TNVa or TNVb (serotype A) entirely free of satellite and suggested that a reasonable explanation for this failure was accidental contamination. Reichmann<sup>7</sup> stated that he had obtained satellite-free TNV as indicated by failure of satellite antiserum to react in agar gel double diffusion tests with extracts from TNV-infected plants. In contrast with Reichmann's claims, however, we have failed in many attempts to obtain a culture of RTNV that would not produce satellite when subcultured. For example, a culture of RTNV derived by successive isolation from five single isolated local lesions was increased and then purified by five successive isolations from sucrose density gradients. This culture was increased and shown by density gradient analysis and serological tests to contain satellite despite extreme precautions taken to prevent accidental contamination. This involved use of steam sterilized soil, pots, and saucers, which is a routine practice in our glasshouses, and isolation, in this instance, in a glasshouse free from other cultures of TNV. Furthermore, uninoculated controls were not infected with TNV.

If the interpretation by Kassanis and Nixon<sup>1</sup> and Kassanis<sup>2</sup> concerning the nature of satellite is correct, it should be possible to obtain cultures that do not produce satellite, but, with the exception of Reichmann<sup>7</sup>, no one has claimed to have done this. Kassanis and Nixon<sup>1</sup> suggested that TNV may not be able to function without satellite as an alternative explanation for their failure to obtain satellite-free cultures of TNV. We suggest that another, and possibly more likely, explanation is that the small satellite particles are always formed in some unknown fashion when the large particles are reproduced.

Our finding that the virus and satellite association is specifically related to serotypes of both entities plus the failures that we have experienced in eliminating satellite from TNV cultures indicates a need for further investigation concerning the nature and origin of satellites associated with TNV.

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## HAEMATOLOGY

### Erythrocyte Acid Phosphomonoesterase Activity in Newly Born Chinese deficient in Glucose-6-phosphate Dehydrogenase

In connexion with our investigations of the aetiological factors responsible for the high incidence of neonatal jaundice among Chinese infants in Taiwan, we have measured the concentrations of erythrocyte acid phosphomonoesterase in normal and Chinese newborn infants and those deficient in glucose-6-phosphate dehydrogenase (G-6-PD). Our experiments on the acid phosphomonoesterase enzyme were prompted by the studies of Oski *et al.*<sup>1</sup> and Choremis *et al.*<sup>2</sup>. In the results reported by Oski *et al.*<sup>1</sup> there was a difference between the acid phosphomonoesterase activities in erythrocytes of Negroes deficient in G-6-PD and those of Caucasians deficient in the enzyme. Specifically, Oski *et al.*<sup>1</sup> found that the acid phosphomonoesterase activities of Caucasians deficient in G-6-PD were reduced whereas those of Negroes deficient in G-6-PD were equal to those of normal Negroes and Caucasians. Oski *et al.*<sup>1</sup> were interested in comparing the biochemical profiles of Caucasians and Negroes deficient in G-6-PD because of the apparent difference in the nature of that defect in the two groups. For example, it has been suggested<sup>1</sup> that Negroes deficient in G-6-PD show a less acute haemolysis than that experienced by deficient Caucasians<sup>3</sup> after eating broad beans (*Vicia faba*). It has also been reported that not all individuals from the same ethnic group who are deficient in G-6-PD are equally susceptible to favism<sup>4-6</sup> nor do they experience equal degrees of severity of neonatal jaundice<sup>6</sup>. In these circumstances it is reasonable to seek differences among other enzyme systems the normal operations of which influence the levels of the intermediary metabolites involved as precursors or products of G-6-PD action. Acid phosphomonoesterase is an enzyme of this type.

In the present experiments, fourteen newly born male Chinese deficient in G-6-PD and 23 normal newborn Chinese, twelve male and eleven female, were tested for their erythrocyte G-6-PD and acid phosphomonoesterase activities. All were chosen from infants born at the National Taiwan University Hospital, Taipei City; the infants deficient in G-6-PD were located by our modification of the Motulsky and Campbell dye decolorization test<sup>6</sup>. The normal infants were taken at random, except that those with haemolytic disorders due to ABO or Rh incompatibilities were excluded. Quantitative estimations of G-6-PD were carried out by the method of Zinkham *et al.*<sup>4</sup> and the method of King *et al.*<sup>7</sup> was used to determine the acid phosphomonoesterase activity. Both activities were expressed as units per 100 ml. of packed erythrocytes.

The results of these determinations are summarized in Table 1. It is apparent that, among the present Chinese subjects, there was no difference between the acid phosphomonoesterase activities of the normal infants and those of infants with G-6-PD deficiency. These results, unlike those reported for Caucasians of mixed origins by Oski *et al.*<sup>1</sup> and for Greeks by Choremis *et al.*<sup>2</sup>, suggest that

Table 1. ACTIVITIES OF ERYTHROCYTE ACID PHOSPHOMONOESTERASE AND G-6-PD ENZYMES IN NEWBORN CHINESE INFANTS (units/100 ml. packed erythrocytes)

Type of infants	No.	G-6-PD activity			Acid phosphomonoesterase activity		
		Range	Mean	S.D.	Range	Mean	S.D.
Normal males	12	155-503	304	$\pm 108$	286-703	463	$\pm 101$
Normal females	11	223-318	275	$\pm 35$	266-624	408	$\pm 94$
Combined normals	23	155-503	290	$\pm 81$	266-703	437	$\pm 99$
G-6-PD deficient infants	14	0-50	14	$\pm 4$	345-647	488	$\pm 87$



there is no close association between deficiencies of erythrocyte G-6-PD and acid phosphomonoesterase enzymes in Chinese. Furthermore, deficiency of acid phosphomonoesterase activity seems unlikely to be involved as an aetiological factor of any importance in neonatal jaundice among Chinese.

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### Erythrocyte Volume Distribution during Recovery from Bone Marrow Arrest

Bröcher and Stohlman<sup>1</sup>, Borsook *et al.*<sup>2</sup>, and Seno *et al.*<sup>3</sup> have shown that macrocytosis results from erythropoietic stimulation by phenylhydrazine anaemia, bleeding or administration of erythropoietin. The macrocytic reticulocytes which result from skipped terminal division were shown to have a shortened life span and to be replaced by red cells of more normal size. This communication reports that radiation produces a similar macrocytosis in mice. We found during the experiment that the volume of mouse red cells decreases as they age.

Thirty female mice of *RF* strain, 6 weeks of age, were used. Twenty mice received a dose of cobalt-60 gamma-rays at a rate of 5 rads/h for 13.3 days (total 1,600 rads). Ten control mice were treated in the same manner but were not irradiated. We have previously found that this dose causes bone marrow arrest, probably within the first few days of exposure, and that 1,600 rads at this dose rate is the approximate  $LD_{50}$  dose for our *RF* strain<sup>4</sup>.

Blood samples were taken from four or five irradiated mice and two control mice at intervals of 2-4 days during a recovery period of 4 months. The orbital bleeding technique described by Riley<sup>5</sup> was used, and a blood volume of approximately 250  $\mu$ l. was taken from each mouse at each bleeding. Individual mice were allowed 9-14 days between bleedings. The red cell volume distribution and concentration were measured with a modified Coulter counter using an aperture 30  $\mu$  in diameter and 225  $\mu$  long and a 400-channel pulse-height analyser to allow a more accurate and detailed estimate of size distribution. Reticulocyte counts were made from slides of blood smears stained with methyl blue and Wright's stain.

The experimental size distributions were fitted with an iterative least-squares computer code<sup>6</sup>. A skewed normal distribution<sup>7</sup> was used, although the fit obtained with a normal distribution was almost as good. In cases where two populations were present, the sum of two normal distributions was used because the extra two parameters required for skewed distributions sometimes made convergence difficult.

Fig. 1 shows red cell volume distribution results at 15 and 34 days after irradiation and also for a control animal. Before the appearance of the second population the volume distributions were similar in shape to the controls, but they had a smaller mean volume and area

that is, red cell count). The second population of large cells appeared between the ninth and thirteenth days after irradiation; on the fifteenth day the mean volume of these cells was maximal. Microscope examination of the dry films showed them to be reticulocytes, and reticulocyte counts made from the slides agreed with those from the volume distributions. The reticulocytes could not be distinguished in the slides a few days later.

Fig. 2 summarizes the results of the computer fits to all the experimental data. The quantities plotted are the area (that is, the red cell count), the mean cell volume, and the fractional standard deviation of the fitted functions, all normalized to the control values. In the cases where the sum of two normal distributions was used in the fit, we have also plotted the mean cell volume for each population. The normalized area, which is the red cell count relative to the controls, was about 85 per cent of normal at the end of the exposure to gamma-rays, dropping to 50 per cent 10 days later. It then climbed rapidly as the second population appeared and returned slowly to near

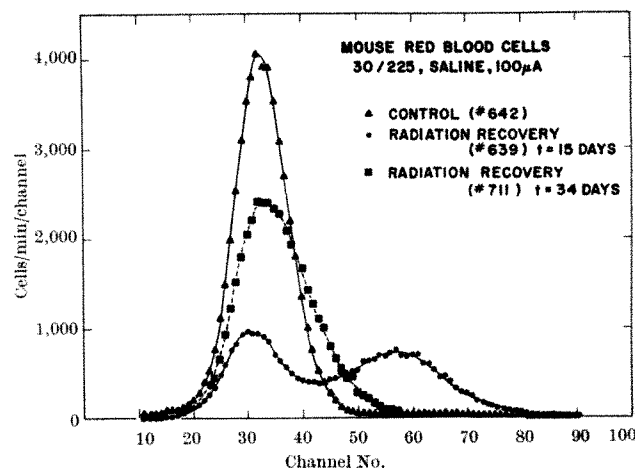


Fig. 1. Volume distribution of mouse red cells 15 and 34 days after irradiation compared with control; the area under the curve is proportional to red cell count.

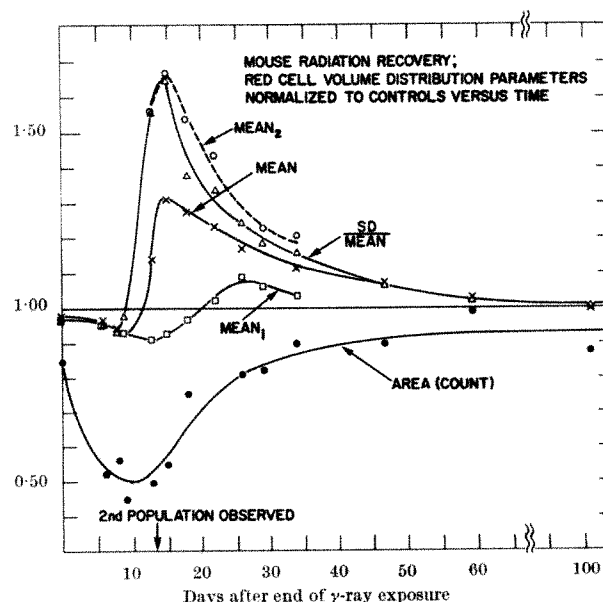


Fig. 2. Variation with time of red cell volume distribution parameters derived by computer after the end of exposure to gamma-rays, normalized to control values. The curve labels have the following significance: area is red blood cell count; mean, is mean cell volume of the first population; mean<sub>2</sub>, is mean cell volume of the second population; mean is mean cell volume of the overall population; and SD/mean is the ratio of standard deviation of the overall population to the mean cell volume of the overall population.

normal. The mean cell volume was also slightly (2 per cent) below normal initially, and fell steadily for about 10 days to 7 to 8 per cent below normal, then rose rapidly to 30 per cent above normal as the reticulocytes entered the circulation. Thereafter, there was a slow decline to normal over a period of 60–70 days. The width of the distribution, as indicated by the fractional standard deviation, showed similar changes but with a greater rise (to 65 per cent above normal) as the reticulocytes appeared. The mean cell volume of the second population reached a maximum value of 60–70 per cent above normal on the fifteenth day and then declined to 20 per cent above normal at day 33. The mean cell volume of the erythrocytes (first population) declined to 9 per cent below normal by the thirteenth day after the end of exposure, then increased to about 9 per cent above normal, and then decreased and slowly approached normal. At this point (34 days), it was no longer possible to resolve the distributions into two populations.

Before the appearance of macrocytic reticulocytes, the circulating red cells constituted an ageing population, because the supply of young erythrocytes had been blocked by the radiation. The red cell count dropped as would be expected, but the mean cell volume also decreased. In addition the population became more homogeneous, as was shown by the decrease in the ratio of standard deviation to mean cell volume. These observations indicate that the red cell volume in mice decreases as the red cells age, and adds to the list of properties already known to be dependent on age, such as density and osmotic fragility<sup>8</sup>. This experiment also shows that recovery from bone marrow arrest induced by radiation involves the same kind of changes in red blood cells that are induced by anaemia and erythropoietin administration.

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## IMMUNOLOGY

### Serum Proteins in the Germ-free Mouse after Oral Challenge with *Candida albicans*

THE serum of many conventional and so-called germ-free animals contains bactericidal activity against certain Gram-negative bacteria<sup>1</sup>. This activity is effective against those bacteria commonly found in the intestinal tract or bacteria related to them. Even the feeding of heat-killed bacteria to germ-free chicks results in specific agglutinin<sup>2</sup>. Thus it appears that antigens from the lumen of the gut may become transported to sites of antibody synthesis. It is well established that gamma globulin and possibly other serum globulins are decreased in germ-free animals<sup>3–5</sup>. All immunoglobulins were markedly decreased in the germ-free mouse<sup>6</sup>.

*Candida albicans* frequently occurs in the normal microbial flora of the alimentary tract of many animal species, although it is apparently not present in the laboratory mouse. In man there are many individuals with antibodies against *C. albicans* in the absence of frank candidiasis. The question, however, remains whether these antibodies are produced in response to subclinical infection, or whether antigens of *C. albicans* are transported from the gut to the antibody producing sites. These topics, as well as others relating to *C. albicans*, were recently reviewed<sup>7</sup>.

The present communication gives the results of an investigation of changes occurring in some serum proteins in the germ-free mouse after association with *C. albicans*.

*Candida albicans* ATCC 10231 was maintained on Sabouraud dextrose agar slants. This strain had an  $LD_{50}$  for mice after intravenous injection of about  $10^6$  viable cells. Isolates of micro-organisms from tissue or other sources were tested for their ability to produce mycelia, blastospores, and chlamydospores by inoculation and growth on corn meal agar for identification of *C. albicans*.

Germ-free mice of the ND 4 strain were originally obtained from Manor Farms Inc., Staatsburg, N.Y.; they were reared as a colony in our laboratory. A crude diet was fed after autoclaving for 25 min at 125° C. Animals were housed in 'Plexiglas' isolators<sup>8</sup>, but the following modifications were made in the equipment and procedures. The germicidal liquid lock was replaced with an interlock sterilized with peracetic acid, and an autoclavable transfer container<sup>9</sup>. Our colonies of germ-free mice have been maintained for almost 3 years.

The pooled blood serum from fifteen to twenty animals in each group was analysed for serum proteins with an analytical electrophoresis apparatus. Preparation of inoculum and inoculation of animals were done as follows. An overnight culture of *C. albicans* was grown at 37° C in Sabouraud dextrose broth in a glass ampoule which was then sealed and transferred into an appropriate isolator using the usual precautions to avoid extraneous contamination of animals. The ampoule was opened and the culture mixed with food, each animal receiving about  $10^6$  viable cells. Cultures were usually administered once only. Gnotobiotic animals were examined periodically for their microbial association as described<sup>10</sup>, but no extraneous contamination was observed in the experiments described here.

Tissue sections were prepared for histology from the following organs: stomach, small intestine, caecum, large intestine, liver, kidneys, pancreas, spleen, lungs. After fixing in neutral buffered formalin, duplicate tissue sections were stained with haematoxylin-eosin (HE) and periodic acid-Schiff stain (PAS). As a test for the presence or imminence of infection we looked for hyphal forms in stool specimens. Finally, the stained histological sections from each animal were examined.

The concentrations of serum proteins in germ-free and conventional mice are shown in Table 1. It is clear that the concentrations of gamma-globulin and beta-globulin in germ-free animals were substantially lower than in conventional mice. Concentrations of total serum protein were similar in both groups.

After association of germ-free mice with *C. albicans* the level of gamma-globulin increased with age, but did not achieve the level in conventional mice (Table 1 and Table

Table 1. SERUM PROTEINS IN CONVENTIONAL AND GERM-FREE MICE, AND IN THE GERM-FREE MOUSE AFTER ASSOCIATION WITH *Candida albicans*

Serum protein	Protein recovered (percentage of total serum protein)		
	Germ-free mice	Conventional mice	Mice with <i>C. albicans</i>
Albumin	57.4	38.3	53.0
$\alpha_1$ -Globulin	10.4	8.0	9.0
$\alpha_2$ -Globulin	17.1	6.2	9.4
$\beta$ -Globulin	14.9	35.8	23.3
$\gamma$ -Globulin	<0.5	11.7	6.0
Total protein in serum (g/100 ml.)	6.2	6.3	4.7

2). Moreover, there appeared to be a decrease in alpha-2-globulin with age in these animals (Table 2), whereas albumin and beta-globulin increased. After sera from germ-free, conventional and gnotobiotic (*C. albicans*) animals were mixed with *C. albicans* cells (and the remaining soluble proteins determined), total serum protein was decreased (Table 3). The most notable change was a decrease in the alpha-2-globulin in the gnotobiotic group. Each group of animals was autopsied and examined for infection. No infections were evident in the organs and tissues examined despite the fact that large numbers of budding yeasts ( $10^4$ – $10^8$ /g wet weight) were seen throughout the lumen of the gut of the gnotobiotic group (*C. albicans*). Many yeast cells appeared to be in close association with the mucosa of the intestine, although penetration of the mucosa was not evident; few hyphae or mycelia were seen. *Candida* species could not be recovered from conventional animals not fed *C. albicans*.

Table 2. SERUM PROTEINS IN GERM-FREE MICE OF DIFFERENT AGES AFTER ASSOCIATION WITH *Candida albicans*

Age of animals (months)	Total serum protein recovered (g/100 ml.)	Proteins recovered (percentage of total protein)				
		Albumin	alpha-1	alpha-2	beta	gamma
1	5.2	29	4	60	7	<1.0
4-6	6.9	56	7	24	11	2
12	4.7	53	9	9	23	6

Table 3. SERUM PROTEINS IN GNOTOBIOTIC AND CONVENTIONAL MICE AFTER SERUM ABSORPTION WITH *Candida albicans* CELLS

Microbial status of animals*	Total serum protein (g/100 ml.)	Serum protein (as percentage of total protein)				
		Albumin	Globulins			
			alpha-1	alpha-2	beta	gamma
		Before absorption				
Germ-free	5.3	49.5	12.4	5.7	29.5	2.9
<i>C. albicans</i>	4.7	52.3	9.3	9.3	23.3	5.8
Conventional	6.3	38.3	8.0	6.2	35.8	11.4
		After absorption				
Germ-free	4.0	47.7	11.5	5.3	31.8	3.2
<i>C. albicans</i>	3.5	54.5	10.2	4.6	25.0	6.4
Conventional	4.6	39.0	8.8	7.1	33.6	11.5

\* The animals were about 12 months of age.

Because living as well as dead bacteria and other micro-organisms present in the food of animals may stimulate the synthesis of immunoglobulin, our diet was examined before it was autoclaved: total microbial counts were  $10^4$ – $10^6$  cells/g diet (dry weight) and viable counts were  $10^2$ – $10^3$  colonies/g.

The decreased concentration of gamma-globulin in germ-free mice agrees with other reports for various animal species. Thus a mechanism exists for the transport of antigenic molecules from the alimentary tract to the sites of antibody synthesis. As with bacteria, the establishment of *C. albicans* in the gut induces synthesis of gamma-globulin and possibly other immunoglobulins. Thus a function of the intestinal microflora is to provide the stimulus for a system of defence against infection. In addition, this stimulus can be dead micro-organisms in the diet, as mentioned, or other antigens in crude diets such as we used. Even highly purified diets can serve as such a stimulus<sup>5</sup>. Absorption of antigen from the intestine in young germ-free pigs depended on the levels of antigen in the gut; also, unless intestinal bacteria penetrated the host tissues no antibodies against these bacteria could be detected<sup>14</sup>. In some respects, their results are in contrast with those of others<sup>1</sup> who found antibacterial antibodies in several species of germ-free animals; these antibodies were bactericidal against *Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhosa*. The serological patterns manifested by various species of germ-free animals have been described in several reviews and symposia some of which also deal with other aspects of gnotobiology<sup>12-17</sup>.

To sum up, values for total serum protein were similar in germ-free and conventional mice. The former had decreased levels of beta- and gamma-globulin and increased albumin. Association of *Candida albicans* with germ-free mice from birth to 12 months of age resulted in a progressive increase in gamma-globulin concentration, but this was less than that of the conventional animal at

12 months. Most abundant in these 1 month old mice was alpha-2-globulin and in 6 and 12 month old mice it was albumin. After absorption of serum with *C. albicans* cells the gamma-globulin level was decreased only in serum from bacteria-free mice associated with *C. albicans*. No infection was seen in these animals.

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## Allografts and Xenografts of Oviduct to the Cheek Pouch of the Syrian Hamster

ATTEMPTS<sup>1-3</sup> to transplant oviduct tissue have been relatively unsuccessful with one notable exception<sup>4</sup>. Successful transplantation of rabbit oviduct segments to the hamster cheek pouch has, however, been reported<sup>5</sup>. Further experiments have been conducted to refine the technique. Since this investigation, we have also used the cheek pouch successfully for transplantation and physiological studies of the uterus<sup>6,7</sup> and anterior pituitaries<sup>8</sup>. The transplantation terminology used here is that proposed by Snell<sup>9</sup>.

Oviducts were dissected from hamsters anaesthetized with 'Nembutal' after ligation of the utero-tubal junction and the ovarian end of the oviduct. Entire oviducts were dissected from rabbits killed by cervical dislocation or from dairy cows at the university abattoir. In each instance, the tissues were placed directly into a prophylactic bath<sup>6</sup> modified from that used by Billingham and Silvers<sup>10</sup>. Additional attempts were made to achieve a sterile technique during this procedure. Excess connective tissue was trimmed off and fine nylon threads were tied around the rabbit and cow oviducts in order to obtain doubly ligatured segments (3–12 mm long). Only segments of the middle region of the cow oviduct were used. Each host received two transplants (one to each pouch), both of which were from the same donor. Grafts were removed at various times up to 21 days after transplantation. After removal of the first transplants (first series), certain animals received allografts or xenografts as second transplants (second series). In the work with hamster and rabbit oviducts we tried to have at least two hosts, which were castrated males and females, treated with oestrogen or progesterone. Otherwise at least one host (male and female) was used with gonads intact or

as a castrate-untreated host. Oestradiol benzoate, 0.2 µg/0.1 ml. of corn oil, or progesterone, 2.0 mg/0.1 ml. of corn oil, was injected subcutaneously daily beginning 1 or 7 days after transplantation and continuing until the transplants were removed. Castrates treated with progesterone received a priming dose of oestradiol benzoate (0.2 µg) on day 1 or 7. Gross observations by transillumination were made at various intervals to evaluate the vascularity, health, turgidity and translucency of the transplants. Histological examination of each transplant was made.

In all, 168 transplants were recovered (150 first series plus 18 second series). Table 1 shows the number of grafts which survived in relation to treatment. Polymorphonuclear leucocytes were associated with less than 9 per cent of the grafts and interstitial red blood corpuscles were present in 22 per cent of the grafts. Infiltration of the peripheral portions of the transplants with mononuclear leucocytes was seen in the majority of grafts, especially those that were 12–21 days old. Survival of endosalpinx, evaluated histologically, was indicated when endosalpinx was present and intact around the entire lumen wall. There was a variable degree of connective tissue in all grafts whether or not the endosalpinx was degenerate. When viewed by transillumination, a rich blood supply to all portions of a graft was observed within two weeks of transplantation; also, all transplants possessing turgidity and translucency, the result of fluid production by the endosalpinx, showed survival of endosalpinx histologically. The absence of these two features, however, was not always related to degenerate endosalpinx.

Table 1. CONDITIONS OF ENDOSALPINX AFTER 12–21 DAYS AS ALLOGRAFTS AND XENOGRAFTS (FIRST SERIES) TO THE HAMSTER CHEEK POUCH

Donor tissue	Treatment group	No. of transplants		No. of transplants with non-degenerate endosalpinx	
		Male	Female	Male	Female
Hamster	Intact	2	6	2	6
	Untreated	2	2	2	2
	E-1	2	4	2	4
	E-7	2	8	2	4
	P-1	0	4	0	4
	P-7	4	4	1	2
Rabbit	Intact	2	10	2	7
	Untreated	2	2	1	0
	E-1	6	4	6	2
	E-7	5	2	3	2
	P-1	4	2	2	0
	P-7	2	4	1	3
Cow	Intact	0	7	0	0
	E-1	0	4	0	0
	P-1	0	2	0	2
	P-7	0	2	0	2

Intact, gonads intact; untreated, castrate and no injections; E-1, P-1 injections of EDB (E) or progesterone (P) begun on day 1; E-7, P-7, injections of EDB (E) or progesterone (P) begun on day 7; male, female, sex of host.

Except when otherwise stated, the following figures refer to first and second series allografts and first series xenografts that were 12–21 days old. Survival of endosalpinx was very low in 1–11 day old hamster and rabbit grafts: 8 per cent (1/12) and 13 per cent (5/38), respectively. In 12–21 day old grafts, the corresponding survival rates were 82 per cent (41/50) for hamster, 67 per cent (30/45) for rabbit and 15 per cent (2/13) for cow transplants. Rabbit ampullary tissue, however, gave the same survival rate (81 per cent, 29/36) as the allografts, whereas only 11 per cent (1/9) of the isthmus segments survived. In most instances, either the endosalpinx survived or was degenerate in both transplants to an animal. This was true in 92 per cent (21/26) of the hamster and 82 per cent (33/40) of the rabbit pairs. All second series allografts after first series allografts survived (6/6) while only 25 per cent (2/8) of the second series xenografts after first series xenografts survived. All allografts that followed xenografts survived (4/4).

Survival rates of hamster and rabbit endosalpinx in the intact animals were 100 per cent (8/8) and 75 per cent (9/12), respectively. The castrate-treated animals, with

hormone injections which started on day 1 and day 7, gave the following results: 100 per cent (10/10) and 50 per cent (9/18) for allografts; 62 per cent (10/16) and 69 per cent (9/13) for rabbit grafts. Treatment with oestradiol benzoate and progesterone gave these results: 80 per cent (16/20) and 75 per cent (15/20) for allografts; 77 per cent (13/17) and 50 per cent (6/12) for rabbit grafts. Survival in male hosts was 73 per cent (24/33) and 69 per cent (36/52) in female hosts.

Lumina of degenerate transplants (1–11 days old) were filled with atrophic epithelial cells. The few endosalpinx cells present were strongly basophilic and formed a simple, squamous layer. After 12 days, the epithelium varied from squamous to pseudostratified columnar and the taller cells showed secretory or luteal phase histology in response to hormone injections. We suggest that the degenerate epithelium of the 1–11 day old grafts was a result of inadequate circulation and that the epithelium 12–21 day old grafts resulted from proliferation and differentiation of intact squamous cells after an adequate blood supply had been established. It is probable that oviduct grafts can survive throughout the life of the host since long term (3–4 months) uterine allografts had intact endometrium and little or no appearance of tissue rejection<sup>8</sup>.

It was concluded that this transplantation procedure is a satisfactory technique for allografts of hamster oviduct and xenografts of rabbit ampullary oviduct segments, and that oviduct fluid is produced by endosalpinx in the absence of a nerve supply, or ovarian hormones, and even in the presence of testicular hormones. It is probable that this technique can be used to study transplants of other reproductive tract tissues such as rat and mouse oviducts, mouse uteri, and ovaries or portions thereof from various species.

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### Inhibition of Delayed Hypersensitivity in Guinea-pigs after Competition between Synthetic Antigens

It has been shown previously<sup>1–3</sup> that immunological responses to protein antigens can be inhibited by administration in advance of large quantities of unrelated proteins. A similar inhibition can also be produced when two antigens are given simultaneously in the same emulsion of Freund's adjuvant<sup>4,5</sup>. Competition between antigens occurs when synthetic antigenic determinants of different specificity attached to the same non-antigenic protein molecule are injected into rabbits<sup>6</sup>.

It seems, therefore, that competition of antigens is a rather general biological phenomenon<sup>5</sup>. It is not, however, established whether the competing material must be itself antigenic in the test animal or at what stage of the immunization process competition of antigens takes place.

All the experiments were performed on inbred guinea-pigs of (Sewall Wright) strain 2 of the National Institutes

of Health stock. The following substances produced and characterized by the Weizmann Institute were used. Poly-L-lysine conjugated with dinitrophenyl (DNP-PLL); an optical L-isomer of the synthetic polypeptide containing tyrosine, glutamic acid and lysine in molar proportions of 6 : 55 : 39 respectively (252-L-TGL) (ref. 7); an optical D-isomer of the same polypeptide containing the same proportion of amino-acids (6 T : 55 G : 39 L) (251-D-TGL) (ref. 7); a synthetic polypeptide containing tyrosine, alanine and lysine in molar proportions 1 : 7 : 24 : 7 : 1 respectively (33 T-A-L) (ref. 8).

Antigenicity of these products has been investigated<sup>7</sup>. Thus, 252-L-TGL is antigenic for non-inbred Hartley guinea-pigs whereas 251-D-TGL is not. DNP-PLL and 252-L-TGL are antigenic for the strain 2 guinea-pigs; the products 251-D-TGL and 33 T-A-L are not antigenic in this strain (unpublished results of Ben Efraim).

Delayed hypersensitivity was induced by injecting into the hind footpads of the animals 0.4 ml. of an emulsion containing equal parts of complete Freund's adjuvant ('Difco') and of a solution in saline of various quantities of one or two of the synthetic antigens. Evaluation of the intensity of delayed hypersensitivity was made by measuring the skin reactions after intradermal challenge injections of the same products 10 days after immunization. Skin reactions were inspected after 3, 6, 24 and 48 h. All reactions were of the delayed type negative at 3 and 6 h and positive at 24 and 48 h. In Table 1 reactions are expressed by the mean diameter in mm, 24 h after the challenge injection.

Table 1. EFFECT OF COMPETITION BETWEEN SYNTHETIC ANTIGENS ON THE DEVELOPMENT OF DELAYED HYPERSENSITIVITY

Group No.	Quantities ( $\mu$ g/animal) of the substances injected	Delayed cutaneous reactions		
		Product† injected	No. of guinea-pigs Total	Mean diameter (mm) Pos-ster
(1)	DNP-PLL 10 $\mu$ g	DNP-PLL	5	5 11.3
(2)	DNP-PLL 1 $\mu$ g	DNP-PLL	10	8 4.0
(3)	252-L-TGL 100 $\mu$ g	252-L-TGL	11	11 14.5
(4)	252-L-TGL 10 $\mu$ g	252-L-TGL	10	10 9.0
(5)	252-L-TGL 1 $\mu$ g	252-L-TGL	5	0 0
(6)	251-D-TGL 600 $\mu$ g	251-D-TGL	5	0 0
(7)	33 T-A-L 600 $\mu$ g	33 T-A-L	5	0 0
(8)	DNP-PLL 600 $\mu$ g + 252-L-TGL 10 $\mu$ g	DNP-PLL	5	5 13.9
		252-L-TGL	5	1 1.4
(9)	DNP-PLL 10 $\mu$ g + 252-L-TGL 600 $\mu$ g	DNP-PLL	5	4 4.4
		252-L-TGL	5	4 10.0
(10)	251-D-TGL 600 $\mu$ g + 252-L-TGL 10 $\mu$ g	251-D-TGL	5	0 0
		252-L-TGL	5	0 0
(11)	251-D-TGL 600 $\mu$ g + 252-L-TGL 100 $\mu$ g	251-D-TGL	5	0 0
		252-L-TGL	5	5 10.4
(12)	251-D-TGL 600 $\mu$ g + DNP-PLL 10 $\mu$ g	251-D-TGL	5	0 0
		DNP-PLL	5	5 11.4
(13)	33 T-A-L 600 $\mu$ g + 252-L-TGL 10 $\mu$ g	33 T-A-L	4	0 0
		252-L-TGL	4	4 14.2
(14)	Freund's adjuvant alone	DNP-PLL	5	0 0
		252-L-TGL	5	0 0

\* Diameter more than 5 mm.

† Concentration of DNP-PLL was 10  $\mu$ g/0.1 ml.; concentration of 252-L-TGL, 251-D-TGL and 33 T-A-L was 50  $\mu$ g/0.1 ml.

The products DNP-PLL and 252-L-TGL are powerful antigens, because 10  $\mu$ g of each of them incorporated in Freund's complete adjuvant produced by 10 days a state of delayed hypersensitivity which was evidenced by good delayed cutaneous reactions (groups 1 and 4). On the contrary, the product 251-D-TGL and the product 33 T-A-L, when injected in a dose of 600  $\mu$ g, failed to induce any detectable state of delayed hypersensitivity: all guinea-pigs tested 10 days after the injection of these substances remained negative (groups 6 and 7).

The second part of this table (groups 8 to 13) shows the results of the experiments in which guinea-pigs were injected with an emulsion containing two of the products, in various proportions, incorporated in Freund's adjuvant. The findings of these experiments may be summarized as follows. (a) When a large quantity of the antigen DNP-PLL is mixed with an immunizing dose of 252-L-TGL, the development of delayed hypersensitivity to the second antigen is inhibited (group 8). (b) When a large quantity of 252-L-TGL is injected with a small dose of DNP-PLL,

development of delayed hypersensitivity towards both of these antigens is impaired (group 9). (c) When a large quantity of the non-immunizing product 252-D-TGL is mixed with a minimum immunizing dose of 252-L-TGL, development of hypersensitivity to this antigen is entirely inhibited (group 10), but it is only partially inhibited if the immunizing quantity of 252-L-TGL is larger (100  $\mu$ g) (group 11). If the same product 251-D-TGL is injected with the DNP-PLL (10  $\mu$ g), the development of hypersensitivity to this antigen is not affected (group 12). (d) When a large quantity of the non-immunizing product 33-T-A-L was mixed with 10  $\mu$ g of the antigen 252-L-TGL, the development of delayed hypersensitivity to this antigen is not affected (group 13). It seemed to be rather enhanced, for these animals reacted as strongly as those of group 3 which were immunized with 100  $\mu$ g of 252-L-TGL.

The guinea-pigs of the last group (14) were injected with the same quantity of Freund's adjuvant alone and tested 10 days later with the antigen used. There was no reaction, which demonstrated that delayed cutaneous reactions to these antigens were highly specific.

Specific amino-acid polymers are very convenient for examination of the phenomenon of competition of antigens, because antigenic determinants are chemically well characterized and confusion from possible cross-reactions can be avoided. Furthermore, it could be possible to decide whether competing antigens must be chemically related (although immunologically non-cross-reacting) or if any antigen could compete with another.

Our results show that synthetic antigens may compete with each other as well as the protein antigens previously investigated<sup>1-4</sup>. It is noteworthy that 600  $\mu$ g of DNP-PLL, which showed a stronger antigenicity than 252-L-TGL (of groups 4, 2 and 5), produced a severe inhibition of reactivity to the latter (group 8), whereas when the injected quantities of these antigens were reversed (group 9), the inhibition of the reaction to DNP-PLL was only partial.

More interesting are the results produced by the products 251-D-TGL and 33 T-A-L. These substances when injected alone do not produce any detectable state of delayed hypersensitivity (groups 6 and 7), and thus cannot be considered as antigens. Nevertheless, when 600  $\mu$ g of the D isomer, 251-D-TGL, is injected together with 10  $\mu$ g of its L-isomer (group 10), it entirely inhibits skin reactivity to the latter. It would seem, therefore, that certain non-antigenic substances mixed with antigens could compete and inhibit immunization. It is now known, however, that when some oligopeptides of D-isomers are attached to non-antigenic protein molecules, they can stimulate immuno-competent cells, that is, they can be recognized as antigens by these cells, and induce production of specific antibodies, which do not cross-react with the L-isomers of these oligopeptides<sup>9,10</sup>. If the whole molecule was constituted by D amino-acids there was no antibody formation or development of delayed hypersensitivity (group 6), but this may be because the immunization process was blocked in some stage other than antigen recognition. Consequently, the finding that the D-isomer can inhibit immunization to its L-isomer suggests that the immunologically competent cells may recognize both substances as "antigens".

The same does not apply in the case of the product 33 T-A-L. This substance is not antigenic in the strain 2 guinea-pigs, although it is a good antigen in rabbits<sup>7</sup>. Our results show that when a large quantity of 33 T-A-L is injected with a minimum immunizing dose of 252-L-TGL, immunization of the animals towards this antigen is not affected (group 13).

It can be concluded, on the basis of these results, that competition in immunization only occurs between substances recognized as antigens, and that this competition takes place during the state of recognition by the immunologically competent cells.



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## PHYSIOLOGY

### Movement of Glucose and Sodium Chloride from the Oropharyngeal Cavity to the Brain

OROPHARYNGEAL stimuli evoked during tasting, chewing and swallowing of food have been shown to influence the rate and degree of ingestion<sup>1,2</sup>. The "satiety mechanism" seems, however, to be operative only if ingesta enter the stomach. It has been reported that animals which have been depleted of sodium or water consume sufficient quantities to compensate for deficits. The observation of animals satiating their appetite in a few minutes, or by a single ingestive act, before absorption from the intestine is likely has led several investigators to postulate some sort of oropharyngeal metering<sup>3,4</sup>.

During the oral phase of ingestion, information from nutrients may be transmitted directly from the oropharyngeal cavity to brain structures associated with the mediation of consumption. Whether the mediation of the ingestive response occurs through neural, humoral or a combination of pathways has not been determined. Furthermore, little consideration has been given to the possibility that rapid transmission of humoral information from the oropharyngeal cavity to the central nervous system (CNS) could be of consequence for ingestion before feedback from post-ingestion occurs. Support for this idea is provided by the observation that absorption of chemical compounds has been shown to occur from the oropharyngeal cavity into the circulatory system<sup>5,6</sup>. Moreover, greater activity has been found in the brain samples of deficient rats administered magnesium-28 intraperitoneally than in replete animals, which suggests a selective movement of depleted nutrients to the CNS<sup>7</sup>.

In experiments to determine whether glucose and sodium chloride would pass from a ligatured oropharyngeal cavity to the brain, a total of eighteen male Holtzman rats, weighing 380–470 g, maintained on an *ad libitum* food and water regimen, were used. The animals were anaesthetized with chloral hydrate (400 mg/kg, intraperitoneally). The oesophagus and trachea were securely ligatured medial to the submaxillary glands. A tracheal cannula was inserted posterior to the ligature, and the animals were mounted in an upright position by means of a head holder.

In experiment 1 glucose labelled with carbon-14 (5  $\mu$ c./0.5 ml.) mixed in a 2 per cent solution of glucose, and oleic acid labelled with carbon-14 (0.25  $\mu$ c./0.05 ml.) in a 2 per cent solution of oleic acid, were used. In experiment 2 glucose labelled with carbon-14 (6.25  $\mu$ c./0.25 ml.) was

used, and in experiment 3 sodium-24 (14.8  $\mu$ c./0.25 ml.) in a 0.96 per cent sodium chloride solution was used. Samples of 250  $\lambda$  of the isotope (250  $\lambda$ ) solutions were placed into the oropharyngeal cavity. Several animals received the isotope solutions into the initial portion of the duodenum.

Blood samples were obtained by cardiac puncture just before decapitation, at the end of the period of isotope application. During the removal of the brain from the cranium, the head was maintained with the mouth held in a downward position to prevent contamination from any radioactive material remaining in the oropharyngeal cavity.

In the experiments with labelled glucose the complete liver was removed and analysed. The times were noted to the nearest second for the period of isotope application. The exposure time refers to the time period beginning with the introduction of the isotope until the removal of the tissue samples. The counting procedure used for the labelled glucose involved a modification of the dry flask combustion technique<sup>8</sup>. An NMC model 4A proportional gas flow counter was employed (counter efficiency of 34 per cent) to count the labelled barium carbonate planchets. Each sample was counted ten times for 500 sec. Corrections were used for background, counter efficiency and self-absorption.

The application procedure for the labelled sodium chloride was similar to treatment with labelled glucose. After the death of the animals the "wet" sample weights were obtained and counting was recorded with a 200-channel RIDL pulse height analyser in conjunction with a 3' x 3' well-type thallium doped crystal of sodium iodide as the detector<sup>9</sup>. Counts of the samples were taken for either 20- or 30-min periods, depending on the level of activity. The area under the 1.38-MeV sodium-24 peak was compared with a standard in order to determine the activity in each sample. Corrections of the activity were made for background and crystal efficiency. The samples were dried and the weights obtained. The initial experiment involved the use of labelled glucose and labelled oleic acid. An oral application time of 5 min was used. Table 1 shows the treatments, exposure time, per cent uptake, and the disintegrations/sec/g of wet sample weight.

Table 1. ACTIVITY OF LABELLED GLUCOSE AND LABELLED OLEIC ACID OBTAINED WITH A 5 MIN OROPHARYNGEAL APPLICATION

Treatment	Measurement	Brain	Liver	Blood
Glucose	D.p.s./g*	113.6	0.03	1.01
	Exposure time (min)	14.0	10.0	6.00
	Percentage uptake	$1.98 \times 10^{-2}$	Nil	$0.05 \times 10^{-2}$
	D.p.s./g	79.0	0.09	0.19
	Exposure time (min)	15.0	9.0	7.0
	Percentage uptake	$2.37 \times 10^{-2}$	$0.09 \times 10^{-2}$	$0.33 \times 10^{-2}$
Oleic acid	D.p.s./g	Nil	Nil	0.061
	Exposure time (min)	13.0	8.0	6.00
	Percentage uptake	Nil	Nil	$0.21 \times 10^{-2}$
	D.p.s./g	2.4	0.27	0.30
	Exposure time (min)	14.0	9.0	5.00
	Percentage uptake	$0.10 \times 10^{-2}$	$0.10 \times 10^{-2}$	$0.21 \times 10^{-2}$
Control for background activity	D.p.s./g	Nil	Nil	Nil
	Exposure time (min)			
	Percentage uptake			

\* Obtained on a wet weight basis: dry weights were not taken.

Substantial activity appeared in the brain with the oral administration of labelled glucose, but little or no activity with the labelled oleic acid. It should, however, be noted that the level of activity in the oleic acid was lower than that of the glucose. Relatively little activity was detected in the wet samples of the liver or blood after the oral administration of the isotope.

In a second experiment the glucose treatments were repeated with exposure for 1, 3 and 7 min. Additional controls employing duodenal administration were added. The exposure time of the blood samples indicates the period

Table 2. ACTIVITY OF LABELLED GLUCOSE OBTAINED WITH A 1, 3, OR 7 MIN OROPHARYNGEAL OR DUODENAL APPLICATION

Treatment	Measurements	Brain	Liver	Blood
Oral	D.p.s./g*	71.5	13.1	Nil
	Exposure time (min)	5.0	3.0	1.0
	Percentage uptake	$0.41 \times 10^{-2}$	Nil	Nil
	N = 3	29.2	21.1	Nil
	Exposure time (min)	5.8	2.3	3.0
	Percentage uptake	$0.91 \times 10^{-2}$	$0.30 \times 10^{-2}$	Nil
	D.p.s./g	236.0	33.7	200
	Exposure time (min)	9.3	8.1	7.0
	Percentage uptake	$1.37 \times 10^{-2}$	$0.22 \times 10^{-2}$	$0.80 \times 10^{-2}$
	D.p.s./g	34.6	415	Nil
Duodenal	N = 1			
	Exposure time (min)	4.0	2.8	1.0
	Percentage uptake	$0.31 \times 10^{-2}$	$4.80 \times 10^{-2}$	Nil
Control for background activity	N = 1			
	D.p.s./g	Nil	Nil	Nil
	Exposure time (min)			
	Percentage uptake			

\* Obtained on a dry weight basis.

of application. Table 2 summarizes the data. The activity in the liver was substantial in the case of intestinal administration of the sugar. Only after oropharyngeal administration of the labelled glucose was substantial activity in the brain detected.

A third experiment was undertaken using labelled sodium to determine whether the movement of labelled glucose or its metabolites to the brain was limited to this nutrient or represented a more general phenomenon. Operative procedures similar to those previously described were carried out and the results are presented in Table 3.

Table 3. ACTIVITY OF LABELLED SODIUM CHLORIDE OBTAINED WITH 3 AND 8 MIN OROPHARYNGEAL OR DUODENAL APPLICATION

Treatment	Measurements	Brain
Oral	D.p.s./g*	1,866.2
	Exposure time (min)	5.0
	Percentage uptake	$3.66 \times 10^{-2}$
	N = 4	1,713.9
	Exposure time (min)	5.5
	Percentage uptake	$3.63 \times 10^{-2}$
	D.p.s./g	1,239.0
	Exposure time (min)	10.0
	Percentage uptake	$3.29 \times 10^{-2}$
	D.p.s./g	1,203.6
Duodenal	Exposure time (min)	10.0
	Percentage uptake	$3.50 \times 10^{-2}$
	D.p.s./g	298.0
	Exposure time (min)	5.5
	Percentage uptake	$0.92 \times 10^{-2}$
	N = 2	318.9
	Exposure time (min)	9.0
	Percentage uptake	$0.93 \times 10^{-2}$
	D.p.s./g	Nil
	Exposure time (min)	
Control for background activity	N = 1	
	D.p.s./g	Nil
	Exposure time (min)	
	Percentage uptake	

\* Obtained on a dry weight basis.

Examination of the activity described in Table 3 indicates that sodium-24 passes from the mouth to the brain. Furthermore, for the time periods investigated, activity of sodium-24 in the brain samples was substantially greater when administered by mouth than through the duodenum.

Activity from labelled glucose and labelled sodium chloride was detectable in the brain after the isotopes were placed into the ligatured oropharyngeal cavity. The levels in the brain exceeded those found in the circulation. Some activity from these substances was detected in the brain after introduction into the intestine, but it was of a substantially smaller magnitude. The activity of carbon-14 which was measured could represent an accumulation of glucose or glucose metabolites. The experiments with labelled sodium chloride indicated also that, after oropharyngeal placement, this isotope moved very rapidly to the brain in greater concentrations than after intestinal administration.

The data obtained do not indicate the pathway of the isotopes. The glucose and sodium chloride could have been absorbed and moved through the circulatory system, to the heart and then back to the brain. An alternative would be a direct passage through special pathways from the oropharyngeal cavity to the brain. The low level of activities detected in the blood gives some support to the idea of a direct pathway.

The movement of small quantities of nutrients from the oropharyngeal cavity to the brain could be of special significance in the detection and monitoring of ingesta before feedback from post-ingestion can occur. The volume of food and fluid consumed by mammals has been shown to be mediated partly by subcortical structures located at the base of the brain<sup>10</sup>.

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### Concept of Micro-environment related to the Regional Cytology of Lymph Nodes

It is a matter of common observation that in the lymph node of the older animal each of the subdivisions of the lymphoid pulp, namely, centron<sup>1</sup>, cortex and medullary cord, maintains a different type of population. The centron may have a high population of blast cells; the cortex remains predominantly composed of small lymphocytes, and the medullary cord specializes in plasma cells.

These area peculiarities of the lymphoid pulp are maintained in spite of the high probability that lymphoid cells are continually moving from region to region. It is true that fixed macrophage barriers seem to exist between these regions<sup>2</sup>, which are capable of trapping lymph-borne particles<sup>1,2</sup>, but these loose structures can scarcely be regarded as a bar to the amoeboid movement of lymphoid cells. A further complication is the constant stream of small lymphocytes emerging from venules<sup>4</sup>.

If the area populations are so constantly maintained in spite of almost certain cellular contamination from outside, a logical way to account for the findings is to suppose that these regions all have a different stromal micro-environment.

According to this concept, the centron, cortex and medullary cord are each little worlds of their own. Each environment governs the population of lymphoid cells which reside in it or have recently invaded the region. A complex kinetic equilibrium is produced, to which cell division, interconversion and inter-regional movement all contribute. The end result is a stable population, which is different for each region.

Can it be shown that these areas of pulp have different micro-environments? If two matters—relative antigen concentration and blood supply—are considered, a reasonable case can be made out for such differences.

First, in respect of relative antigen concentration, Nossal *et al.*<sup>5</sup>, in their investigations of the distribution of afferent lymph-borne antigen marked by radio-iodine in various regions of the lymph node, have shown a high concentration of antigen in the centron macrophages, a low concentration in the cortex, and a high concentration in all medullary structures (Fig. 1).

To this antigen environmental parameter can be added another, namely, blood supply. I have re-investigated<sup>8</sup> this, using aortic injection of 10 per cent gelatine in indian ink to show vascular patterns in para-aortic lymph nodes. The technique, and the patterns produced by it, have been discussed in detail elsewhere<sup>6</sup>.

Two important points arose from these vascular studies. First, the centron is an almost exclusively capillary body. Afferent arterioles arborize into capillaries in the immediately neighbouring cortex, but do not penetrate the centron itself. Large cortical venules can be observed in the immediate vicinity, but rarely within the centron sphere (Fig. 2).

Second, the medullary cords nearly always contain a large, central, very thin-walled venule (Fig. 2). Arterioles are found only occasionally in the cords; venules far outnumber them.

The combination of the two environmental parameters is summarized in Table 1.

Table 1

	Local antigen concentration	Local blood supply	Predominant cell
Centron	High	Capillary	Blast cell
Cortex	Low	Arterio-venous	Small lymphocyte
Medullary cord	High	Venous	Plasma cell

Examination of Table 1 leads to the thought that there may be a mitogenic factor in arterial blood, which diffuses progressively from capillaries and venules. This factor can be supposed to operate only in synergism with a high local antigen environment. In the cortex it is inoperative for this reason, and the population in this area, therefore, remains composed of small lymphocytes.

In the presence of a local high concentration of antigen, the diffusing haematogenous factor produces two types of result, depending on its local perivascular concentration. If this concentration is high, the effect is to encourage cell division, producing large numbers of local blast cells with a high mitotic count. If its concentration is low, the effect is to encourage the production of protein, with the resultant local appearance of plasma cells. This vascular factor thus explains the different lymphoid population of centron and medullary cord, in spite of the fact that they are both high antigen areas.

A gradient of diffusible factor, higher at the arterial, lower at the venous end, could occur around a long venule, such as the straight central venule of the medullary cord. This might account for Sainte Marie's observation<sup>7</sup> that plasma cells which he classifies as immature



Fig. 2. Section of cortex and medullary cords of 200 g rat with well formed follicles. The vessels have been injected with 10 per cent gelatine in indian ink, as mentioned in the text. This is essentially the venular pattern. In this preparation, capillaries and arterioles are not seen. Note (a) complete absence of major venules in follicles; (b) anastomosing, wide-bore venules in cortex; (c) bottom left, a straight venule passing through the centre of a medullary cord. Haematoxylin and eosin. Gelatine section cut at  $20\mu$  ( $\times 90$ ).

are found more at the outer end of the cord than towards the hilum. My experience with various types of antigen stimulation has confirmed that this outer-inner maturation gradient in the cord is a frequent finding.

So much for the existence of a hypothetical diffusion factor from the blood. What is certain is that centron, cortex and medullary cord do seem to have different micro-environments. This cannot be without effect on their contained lymphoid populations.

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## Physiological Adaptation and Partial Dormancy in *Ascaris* Eggs

PHYSIOLOGICAL adaptation of the rates of life processes in response to changes in environmental temperature is now known to be commonplace in poikilothermic organisms<sup>1</sup>. Animals, plants and micro-organisms experimentally subjected to a change in temperature within normal viable limits initially show modified rates of metabolic functions which, in the absence of "overshoot", accord with predictions based on chemical kinetics. In many cases, however, these rates either decrease (rise in temperature: " $T+$ " adaptation) after the first phase or increase (fall in temperature: " $T-$ " adaptation) slowly over a period of days or weeks, eventually attaining a new constant value which tends towards the rate characteristic for the original temperature. The nearer the correspondence the more highly adaptable is the organism in that particular respect<sup>2</sup>. Such slow modifications are generally considered to be true physiological adaptation (that is, "capacity adaptation") in contrast to the rapid changes (overshoots) which are completed in a matter of minutes or hours after the change in temperature.

Overshoot phenomena may be related to neuromuscular physiology<sup>3</sup> or, at the cellular level, to the rate of attainment of new steady states dependent on diffusion processes<sup>4</sup>. The biological significance of true physiological



Fig. 1. Section of cortex of rat lymph node, containing afferent lymph-borne flagellar antigen marked by radio-iodine. The antigen has concentrated in the region of two follicles. The rest of the cortex contains virtually no antigen. The medullary structures, seen as a zone at the bottom of the preparation, are densely marked. Preparation supplied by courtesy of Professor Nossal, Walter and Eliza Hall Institute, Melbourne. Methyl green-pyronin. Paraffin section cut at  $7\mu$  ( $\times 90$ ).

adaptation, Precht's<sup>2</sup> "resistance" adaptation apart, is less easy to evaluate. Studies so far have mostly concerned actively metabolizing organisms in which it seems likely that adjustments of this kind further a better integrated rate pattern throughout a complex series of processes.

This communication suggests that physiological adaptation of the capacity type to temperature change may acquire a new significance in the evolution of some dormant mechanisms, and that stages of organisms having metabolic characteristics approaching dormancy may provide ideal tools for the study of physiological adaptation.

In living things with no immediate energy requirement, whose energy sources are endogenous and finite, and whose success is proportional to survival time, selection in favour of reduced metabolic rate would be expected. A mechanism ready to hand for such selection is the  $T^+$  adaptation outlined earlier—which would be selected at the expense of the converse, that is  $T^-$  adaptation. The suggestion obviously presupposes independent physiological and genetic control for the two processes; a possibility not contradicted by the available evidence.

Structures likely to be subjected to these selection pressures include protozoan cysts and the eggs and larvae of some metazoan parasites. Useful experimental material is likely to be found among these forms, because metabolism, though reduced, can be measured by conventional means<sup>5,6</sup>.

A test for temperature adaptation is the measurement of a rate function at the same temperature of two populations of an organism acclimated to two different temperatures. A difference between rates under these conditions is evidence for the thesis, provided no reproduction has occurred and no physiological damage has resulted from the temperature treatment itself.

The infective eggs of the pig parasite, *Ascaris suum* (Nematoda), exhibit partial dormancy in that they have a low  $Q_{O_2}$  (ref. 5) and can survive for long periods without exogenous energy sources<sup>7</sup>. Dormancy is broken when the temperature rises above 36°C, together with changes in pH, carbon dioxide concentration and redox potential<sup>8</sup>.

Table 1.  $Q_{O_2}$  AT 35° C OF ACCLIMATED INFECTIVE *Ascaris* EGGS

Hours of measurement	Eggs acclimated to 25° C ( $\mu\text{l./mg/h}^*$ )	Eggs acclimated to 30° C ( $\mu\text{l./mg/h}^*$ )
First	0.351 $\pm$ 0.026	0.201 $\pm$ 0.015
Second	0.399 $\pm$ 0.019	0.224 $\pm$ 0.037
Third	0.357 $\pm$ 0.018	0.233 $\pm$ 0.022
Fourth	0.365 $\pm$ 0.031	0.223 $\pm$ 0.045
Fifth	0.393 $\pm$ 0.010	0.229 $\pm$ 0.028
Means	0.374 $\pm$ 0.020	0.221 $\pm$ 0.029

\* Combined data from three experiments: means and standard deviations. Individual determinations made in triplicate 5 ml. Warburg flasks containing 70–100 mg dry weight of freshly washed eggs in 1 ml. 0.1 normal sulphuric acid per flask.

Warburg measurement of the oxygen uptake at 35°C of "de-coated"<sup>9</sup> infective *Ascaris* eggs acclimated previously to 25°C and 30°C for 11 days shows adaptation to be well developed (Table 1). The  $Q_{O_2}$  at three other temperatures confirms these observations and shows translation and rotation of the  $R-T$  plots<sup>1</sup> (Fig. 1). The eggs used in these measurements were incubated to the infective stage at 25°C for 39 days in 0.1 normal sulphuric acid before acclimation. All temperature treatments were carried out in the same solution in water baths at constant temperature equipped with shakers operating at 100 oscillations/min. The eggs suffered no damage from acclimation experiments, as was shown by hatching trials using the physiological methods of Fairbairn<sup>8</sup>. Approximately 90 per cent of the fertile eggs (2–3 per cent were infertile) hatched, and there was no significant difference after acclimation to either temperature.

Chemical analyses were also made of eggs before and after acclimation to assess the economic advantage conferred by the adaptation demonstrated in metabolic rate.

No significant difference emerges between eggs acclimated to the two temperatures in regard to total dry weight, nitrogen as protein, total lipid, or carbohydrate (for methods, see ref. 6). A significant increase in the content of alkali-stable carbohydrate (ACHO—glycogen plus trehalose), however, occurs after acclimation to both temperatures compared with eggs before acclimation (Fig. 2). Such synthesis probably utilizes lipid carbon<sup>10</sup>.

Assuming that adaptive decrease in metabolism is linear with time, and that carbohydrate synthesis is proportional to the utilization of energy reserves, an approximate estimate of economic advantage can be made. Values of the ratio

$$\frac{\text{percentage increase in ACHO}}{\text{mean } Q_{O_2}}$$

were 140 and 120 for eggs acclimated to 25°C and 30°C respectively. From the mean value of 130 one can predict the approximate synthesis that would have occurred in the absence of adaptation, using the  $Q_{O_2}$  estimates for the non-acclimated eggs, that is,  $0.135 \times 130 = 18$  per cent (25°C) and  $0.251 \times 130 = 33$  per cent (30°C). The measured increases were 14 per cent (25°C), a "saving" of 22 per cent, and 18 per cent (30°C), a saving of 45 per cent. There is every reason to believe adaptation to be possible to even lower metabolic rates in such organisms, so that the economies shown to occur in 11 days' acclimation are slight compared with what would accrue over prolonged periods.

The analyses do not reveal the concurrent loss of lipid that would be expected, probably because the gravimetric lipid measurements were not precise enough to detect it (see Fig. 2). In any case, decrease in lipid would be expected to be only 50–60 per cent by weight of the carbohydrate synthesized from it.

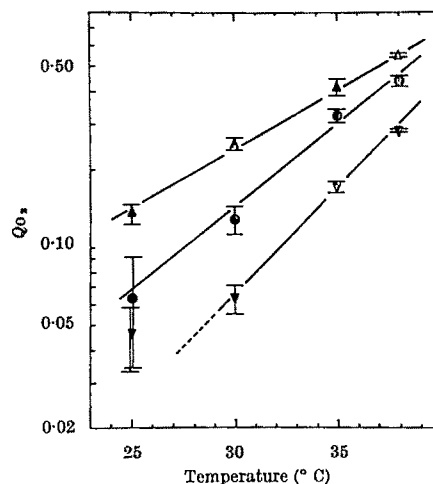


Fig. 1. Berthelot plot of  $Q_{O_2}$  measurements ( $\mu\text{l./mg/h}$ ) of infective *Ascaris* eggs before (▲) and after acclimation to 25°C (●) and 30°C (▼) for 11 days. The data are from one experiment in which 10 min readings were made for 1 h at each temperature. Each point and vertical line is the mean  $\pm$  S.D. of triplicate 5 ml. Warburg flasks containing 60–70 mg dry weight of freshly washed eggs in 1 ml. 0.1 normal sulphuric acid per flask. The same samples were successively treated to stepwise increases in temperature in the Warburg bath and equilibrated for 15 min between each hour of measurement.

It is obvious that the adaptation shown to be possible in *Ascaris* eggs cannot be fitted into the categories described by Precht<sup>2</sup>, but it would be surprising if it could be. If one assumes an equilibrium between  $T^+$  and  $T^-$  processes to be the "normal" situation in metabolically active organisms, a selection pressure which emphasizes the former at the expense of the latter would tend to eliminate the equilibrium state and therefore result in a continuously declining metabolic rate at any temperature. This is consistent with the fact that *Ascaris* eggs, even though they were kept for 39 days at 25°C during their development to the infective stage, still showed a signi-

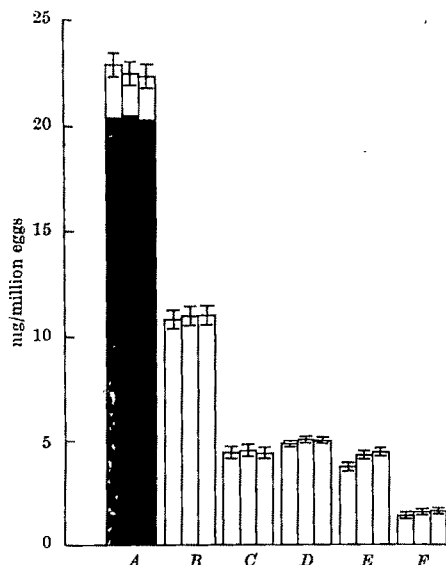


Fig. 2. Chemical composition of eggs before acclimation (left hand column of each set of three) and after 11 days acclimation to 25° C (centre columns) and 30° C (right hand columns). A, Total dry weight (black portion is sum of total solids measured independently); B, nitrogen as protein (that is  $N \times 6.25$ ); C, total lipid; D, total carbohydrate; E, alkali-stable carbohydrate (glycogen plus trehalose); F, glycogen. Numbers of eggs were determined with an automatic cell counter. Combined data from four experiments with 95 per cent confidence limits.

significant decline in  $Q_{O_2}$  when kept subsequently at the same temperature. Despite the "abnormal" nature of such adaptation, however, I suggest that infective *Ascaris* eggs, and similar living things, are particularly suitable for the analysis of these phenomena for the following reasons: a, there are no short-term fluctuations in metabolism related to behavioural phenomena to complicate measurements; b, reproduction, cell division and growth do not occur; c, there are no exogenous nutritive sources; and, d, the very abnormality may assist analysis if, as is proposed, only one of the two main aspects of temperature adaptation is present. Such an approach may also provide a new insight into the study of dormant mechanisms.

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### Reduction-Oxidation Potential of Blood as a Function of Partial Pressure of Oxygen

ATTEMPTS to establish the reduction-oxidation potential of blood as a useful index in clinical medicine<sup>1</sup> have met with little success. Possibly the redox measurement of blood, despite its reasonable reliability<sup>2</sup>, is too crude an index for the changes which are likely to occur in the

many and complex interacting electron transport systems of the blood. On the other hand, the reasons for failure may be largely technical. Redox measurements of the blood have usually been carried out under unknown or uncontrolled conditions of pH and temperature, and in the presence of unknown partial pressures of oxygen in the blood. These variables could account for relatively marked changes in the redox measurement<sup>2</sup>.

The present investigation demonstrates the relationship between the redox measurements of the blood and the partial pressure of oxygen in the blood under known conditions of pH and temperature. As in our previous work<sup>3</sup>, the potential was measured by means of a gold electrode using a saturated calomel electrode as a reference cell. The only significant change introduced was the use of unbuffered citrated blood, and the measurement throughout the experiment of the partial pressure of oxygen and of pH. (The partial pressure of oxygen was measured by means of an oxygen macro electrode sealed into the blood chamber and connected to a physiological gas analyser. The pH was determined by removing anaerobically small portions of the blood sample for measurement in a Beckman 'Micro Blood pH Assembly'.) A magnetic stirrer kept the blood circulating. The temperature of the blood was constant at 37.4° C. Oxygen was expelled by bubbling pure argon through the blood.

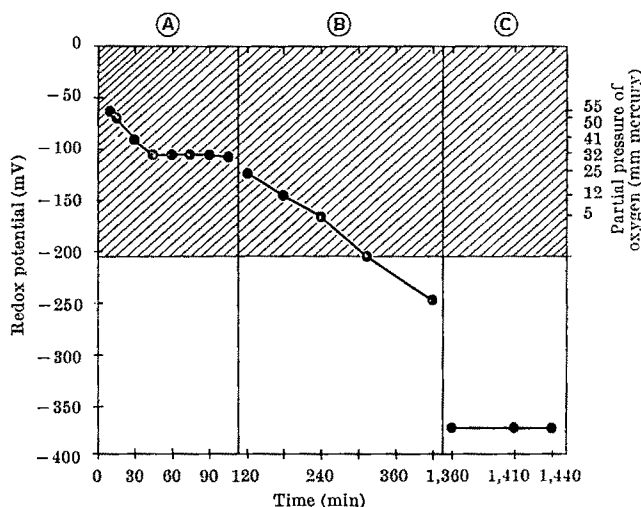


Fig. 1. Relationship in the blood between reduction-oxidation potentials and partial pressures of oxygen during a period of time necessary to establish a steady final redox state.

Fig. 1 illustrates a representative finding of the relationship between the redox potentials and the partial pressure of oxygen over a period of 24 h. The pH of the blood was 6.8.

Section A of the graph shows that there is a positive, though not quite linear, relationship between redox measurements and partial pressure of oxygen. A reduction of the partial pressure of oxygen from 55 to 32 mm of mercury was associated with a concomitant steady reduction in the redox potential from -62 mV to -102 mV—a change of 1.7 mV/mm of mercury of the partial pressure of oxygen. Section A also shows that when the partial pressure of oxygen was held constant at 32 mm of mercury, the redox potential remained steady at -102 mV.

Section B of the graph shows that when deoxygenation of the blood was resumed, the redox potential again began to decline. When the partial pressure of oxygen reached zero, the redox potential was -202 mV. The redox potential of the blood was not, however, arrested at the point where the partial pressure of oxygen became zero. The even decline of the redox potential continued, and at about the same rate as before, until it reached a final



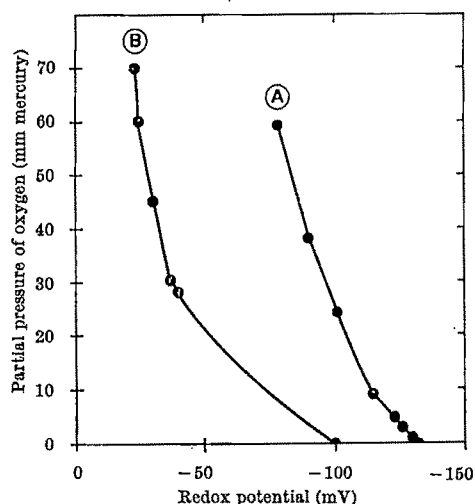


Fig. 2. Relationship between redox potentials and partial pressures of oxygen of the same sample of blood in two consecutive experiments. (A) Represents first experiment. Second experiment (B) was carried out after re-oxygenation of the blood. The final steady redox potential in both (A) and (B) was  $-416$  mV.

steady state of  $-370$  mV (section C). From the beginning of the experiment, it took approximately 20 h before the redox potential attained its final steady state.

Similar experiments were carried out with other samples of blood, and with the same sample of blood under altered conditions (such as previous freezing of the blood sample). All produced similar results. The relationship between the partial pressure of oxygen and redox potential was, however, found to vary considerably, even when the final steady redox states were the same. This is illustrated in Fig. 2 which shows the relationship between redox measurements and partial pressures of oxygen of the same sample of blood submitted to two consecutive experiments. The same final steady state was reached in both instances (redox potential  $-416$  mV), but the relationship between redox measurements and partial pressure of oxygen was quite different.

In summary, our experiments indicate that (a) there is a positive, but not quite linear, relationship between redox measurements and partial pressure of oxygen; (b) this relationship is not constant and varies greatly with certain experimental manipulations of the blood sample; (c) a steady partial pressure of oxygen is associated with a steady redox measurement; (d) complete deoxygenation of the blood initiates a steady, progressive decline in redox values which ordinarily do not reach a steady state for at least 10 h; and (e) the value of the final steady state cannot be accurately predicted from a redox measurement taken in the presence of oxygen. It would seem that redox measurements of the blood need to be made in conditions of complete deoxygenation and in known conditions of temperature and pH. Redox investigations carried out *in vivo*, or in the presence of oxygen, are likely to be of doubtful value.

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## Differential Identification of Micro-organisms by Analysis of Phosphorescent Decay

THIS communication reports evidence for the existence of unique and characteristic components in the phosphorescent decay of individual strains, species and morphologies of various micro-organisms.

If observation of the decay is restricted to a particular energy of transition, that is, a particular emission wavelength, then for an array of radiators in the structure the physical origins of which will in general be different, the total intensity of radiation at any given time,  $t$ , will be expressed by a function of the form

$$I_T = I_0 \exp(-\lambda_0 t) + I_1 \exp(-\lambda_1 t) + \dots + I_n \exp(-\lambda_n t) \equiv \sum_i I_{i0} e^{-\lambda_i t} \quad (1)$$

and the form of this function will be valid irrespective of alternative decay paths or parent-daughter relationships between adjacent states if no restrictions are placed on the coefficients.

The biological samples in this experiment were prepared from five bacterial strains; namely, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Neisseria meningitidis* Group A (strain 1027A), *N. meningitidis* Group B (strain L-1), and *N. meningitidis* Group B (strain 2091B).

All the samples were grown in modified Franz medium for 20–22 h at  $37^\circ$  C. After this period of growth the collected cultures were washed five times in a solution of 0.15 molar sodium chloride and then were collected by centrifugation. The *B. subtilis* and *S. epidermidis* were studied both as living organisms and as autoclaved whole cells, while the *Neisseria* cultures were killed by 1 per

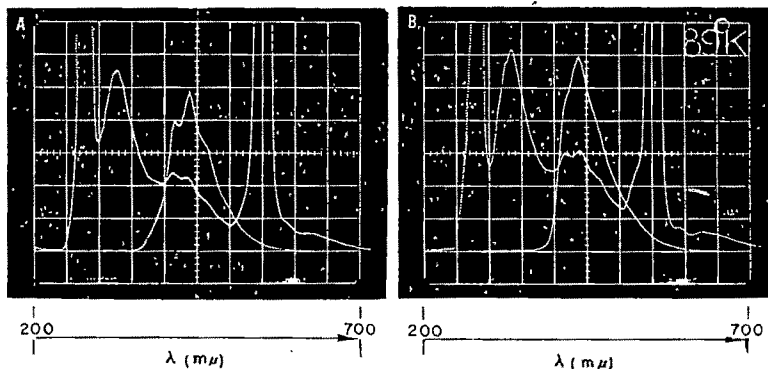


Fig. 1. Plot of emission intensity versus emission wavelength for two intact organisms: A, *Bacillus subtilis*; B, *Staphylococcus epidermidis*.

cent  $\beta$ -propiolactone in which they were placed for 2 h at room temperature, followed by about 24 h at  $0^\circ$ – $4^\circ$  C.

In addition to the study of the structures of washed whole cells from each sample, preparations of the *Neisseria* strains were morphologically disrupted by alternate freezing and thawing at pH 7.0. The freed cell walls were cleaned by washing in saline solution, in phosphate-buffered saline, and finally in distilled water. The cell sap, on the other hand, was filtered through a membrane filter with a pore size of  $0.45\mu$ . The protein content of the cell wall suspensions and the cell sap was determined by the Lowry method and adjusted to uniform values before the analysis was begun.

Fig. 1 shows the total emission spectra for two whole cell samples. In both cases the x scale is linear,  $x_0 = 200$  mμ and each division is 50 mμ. The curve with multiple peaks is the total spectrum in which the first peak represents elastic scatter of exciting radiation, the second peak fluorescence and the third phosphorescence. The superimposed second curve shows the phosphorescent emission bands alone obtained by using a shutter to exclude scatter and fluorescent emission.

Table 1. EXCITATION AND EMISSION WAVELENGTHS CORRESPONDING TO  $F/P$  AND PHOSPHORESCENT MAXIMA AND VALUES OF  $F/P$  RATIOS

Samples at 89° K	Excitation ( $\lambda$ )*			Emission ( $\lambda$ )*			$kx_p$	$F/P$
	$F/P$ (m $\mu$ )	$P$ (m $\mu$ )	$F_{max}$ (m $\mu$ )	$x_{p1}$	$x_{p2}$	$x_{p3}$		
Whole cells								
2a. <i>B. subtilis</i>	270	286	330	~395	412	438	~465	3.66
2b. <i>S. epidermidis</i>	270	300	335	~395	412	438	460	2.65
2c. <i>N. meningitidis</i>	270	289	325	~395	412	440	~470	4.52
1027A								
2d. <i>N. meningitidis</i>	270	290	335	~395	412	438	465	5.09
L-1								
<i>N. meningitidis</i>								
Substituents								
3a. 1027A wall†	270	289	334	~395	420	447	470	5.00
3b. 1027A sap	270	308	355			445		32.0
3c. L-1 wall	270	290	325	~395	410	434	460	4.88
3d. L-1 sap	270	310	356			436		27.8
3e. 2091B wall	270	290	325	~395	410	435	455	4.02
3f. 2091B sap	270	310	360			447		38.0

\* Uncorrected for variation of photomultiplier sensitivity with respect to changing  $\lambda$ .

† Red-shifted by 10 m $\mu$  as a result of an uncorrected monochromator.

Table 1 lists for each sample the excitation wavelength for the total emission and phosphorescent curves, the wavelength corresponding to the point of maximum fluorescence ( $x_f$ ), the wavelength corresponding to the point of maximum phosphorescence ( $x_p$ ) and the ratio of the maximum fluorescent intensity to the maximum phosphorescent intensity ( $F/P$  ratio). The spectra and maxima presented are not corrected for variation in the sensitivity of the photomultiplier to wavelength.

Fig. 2 shows the phosphorescent decay curves for the same samples plotted on a semilogarithmic scale. The dotted line envelope is the overall decay curve, the solid

lines show the individual components obtained by piecemeal subtraction. Alongside each component is its half-life, defined by the time required for the population of the state concerned to decrease to half its initial value.

Table 2 lists the values of  $\lambda_i$  for each component and each sample as well as the quantity  $T_{L \times 10^{-3}}$  defined by

$$T_{L \times 10^{-3}} = \frac{\ln 1000}{\lambda_i}$$

The analysis of total emission and phosphorescent spectra of the four micro-organisms and their substituents was highly reproducible from preparation to preparation in single organisms, and also showed distinguishing marks of emitting domains from one organism to another. The relative coincidence of the overall phosphorescent emission curves was obtained by calculating a constant  $kx_p'$  given by

$$kx_p' = \frac{x_{p_3} - x_{p_2}}{x_{p_3} - x_{p_1}} \quad (2)$$

The  $x_p$  values for cell saps of all strains of *N. meningitidis* were uniformly located at 445 m $\mu$  and appeared to be occurring singly although there was some indication of a shoulder at ~410 m $\mu$ . This very weak phosphorescent emission occurred after excitation at 310 m $\mu$ . An excitation maximum at 310 m $\mu$  for cell sap with an  $x_p$  of 445 m $\mu$  is uncommon and, so far as could be determined, is not typical of centres associated with protein.

We now draw attention to Fig. 1 and the emission spectra of whole cells of *B. subtilis* (Fig. 1A) and *S. epidermidis* (Fig. 1B). The general forms of the total

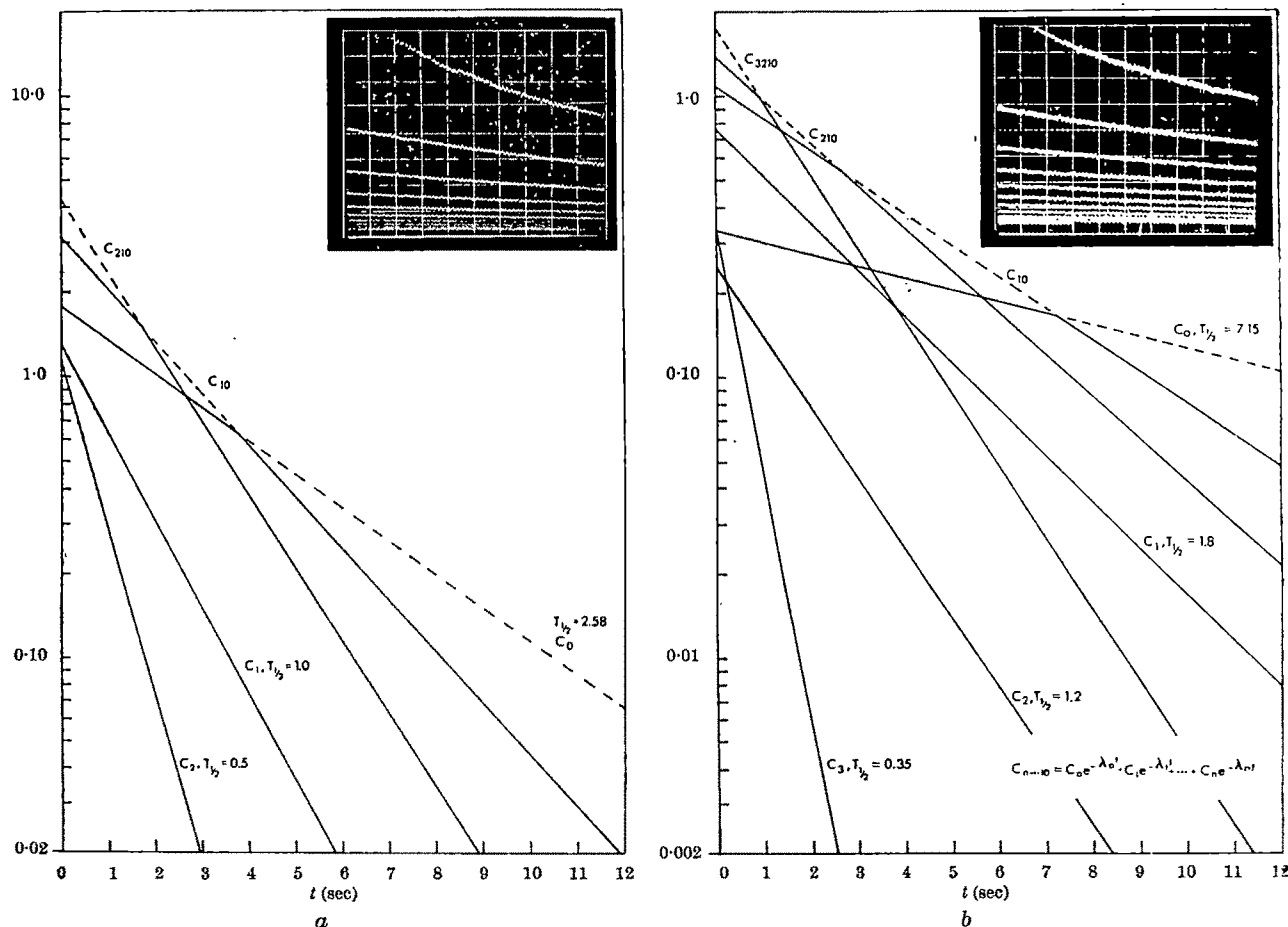


Fig. 2. Plot of the natural logarithm of emission intensity versus time for various samples. The ordinate is in arbitrary units. a, *B. subtilis*; b, *S. epidermidis*.

Table 2. PHOSPHORESCENT DECAY CONSTANTS OF SAMPLES AT 89° K

Organism Substituent Group	<i>B. subtilis</i>	<i>S. epidermidis</i>				<i>N. meningitidis</i>				
	Whole cell	Whole cell		Cell wall			Cell sap			Whole cell
			<i>A</i>		<i>B</i>	<i>A</i>		<i>B</i>	<i>A</i>	<i>B</i>
Strain			1027 <i>A</i>	<i>L</i> -1	2091 <i>B</i>	1027 <i>A</i>	<i>L</i> -1	2091 <i>B</i>	1027 <i>A</i>	2091 <i>B</i>
$\lambda_0$	0.097	0.269	0.277	0.257	0.266	0.42	1.260	0.272	0.239	0.248
$T_0L \times 10^{-3}$	71.24	25.69	24.95	26.89	25.97	10.45	5.48	25.39	28.90	27.85
$\lambda_1$	0.385	0.693	1.595	1.155	1.260	1.540		1.260	0.533	0.866
$T_1L \times 10^{-3}$	17.95	9.97	4.33	5.98	5.48	4.49		5.48	12.96	7.98
$\lambda_2$	0.578	1.386							1.066	
$T_2L \times 10^{-3}$	11.95	4.98							6.48	
$\lambda_3$	1.98									
$T_3L \times 10^{-3}$	3.49									

and phosphorescent emission curves are different, if not indeed unique, from one species or strain to another. It is entirely possible that cellular substituents, such as cell sap which has its excitation maxima at 310 mμ, might reabsorb fluorescent radiation of the prime luminescent centre ( $x_f \sim 325$  mμ) and dissipate this energy in a characteristic fashion simultaneously with other discrete centres, thus giving a typical total emission curve for the species as an expression of the sum of all available processes concerned.

The alteration, for example, in the phosphorescent emissivity of *B. subtilis* was of considerable interest in view of its marked morphological difference from the other micro-organisms studied.

It will be seen from the decay curves (Figs. 2a and b) that the samples investigated differ both in the number of exponential terms required to produce the function,  $I_T$ , and in the decay constants,  $\lambda_i$ , associated with each term.

Figs. 2a and b show the decay curves of *B. subtilis* and *S. epidermidis*, respectively. We have found these functions to be characteristic of these two organisms, indeed constant, in a manner independent of whether these organisms were alive or autoclaved with steam as well as independent of the culture medium, preparation technique and concentration. It has been possible, as a result, to pick out samples of each of them blindly from a control batch. A salient feature of the two curves is the four component nature of the decay of *Bacillus subtilis* and the three component character of the *Staphylococcus epidermidis*. In addition, it can be seen that the components have significantly different decay constants associated with them (Table 2).

Thus, because of the nature of the function,  $I_T$ , it would appear possible to form a vector of the individual coefficients and decay constants of the form

$$S \equiv \{\lambda_0, \lambda_1, \dots, \lambda_n, I_0, I_1, \dots, I_n, x_{f1}, x_{p1}, I_{f\max}, I_{p\max}\}$$

such that

$$\sum_i I_i \exp(-\lambda_i t) \equiv I_T$$

and

$$\sum_i I_i \equiv 1$$

and where  $x_f$  and  $x_p$  are the wavelengths in the total emission spectra corresponding to maximum fluorescent and phosphorescent intensity respectively and  $I_f$  and  $I_p$  are those maximum intensities. It is recognized that the terms  $I_{f\max}$  and  $I_{p\max}$  depend on concentration and as such, under the present methods, will not be constants. It is clear, however, that these components can be used in calculation of quantities such as  $F/P$  ratios and the constant  $K_{xp}$  described here, which are, in fact, independent of the variable parameters of the analysis.

The data presented indicate that the molecular domains and the manner in which they dissipate absorbed energy can form a unique signature,  $S$ , of the specific organism or component under examination. Furthermore, comparison of such  $S$  vectors may permit identification of at least groups and perhaps individual components and organisms.

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### Absence of Atherosclerosis in the Cerebral Arteries of Chickens fed an Atherogenic Diet

THERE are innumerable reports of the formation of atherosclerotic plaques in the aorta and coronary vessels of chickens fed atherogenic diets. Little work has been done, however, on the development of atherosclerosis in the cerebral arteries. A recent review of comparative atherosclerosis<sup>1</sup> reported that spontaneous atherosclerosis was not found in the cerebral arteries of chickens, turkeys, rabbits, baboons and pigeons; whereas it was observed in swine and older dogs. Cerebral atherosclerosis did not develop in rabbits fed cholesterol, unless their cerebral circulation was impaired by ligation of either the carotid artery, the jugular vein or both<sup>2</sup>. Investigation of the cerebral vessels of chickens was of interest because of the paucity of information available, especially as related to birds fed atherogenic diets.

Male and female chickens were fed either an atherogenic or a non-atherogenic diet for 2 years. The composition of the diets has been described previously<sup>3</sup>. The birds were killed and the aorta was removed by dissection from the junction at the heart and its bifurcation into the iliac arteries.

The aorta was divided further into thoracic and abdominal sections and evaluated macroscopically as to severity of atherosclerosis (graded 1-4) and extent of aortic area involved (percentage). The atherosclerotic score was the multiple of these two parameters. Table 1 gives the mean aortic atherosclerotic scores for the birds, and reaffirms the fact that cholesterol-fed chickens develop atherosclerosis (an aortic score of 300 represents a very severe atherosclerotic condition). These birds would have been expected to exhibit coronary atherosclerosis if their hearts had been prepared for examination, because a previous investigation<sup>4</sup> had shown that feeding this atherogenic diet resulted in the development of coronary atherosclerosis. The brains of these birds were removed and prepared as follows. Transverse sections were made through the brain and meninges at three selected sites so that a representative sample of arteries, both small and large, would be transected. The sections were all stained with Verhoeff van Gieson and haematoxylin and eosin and examined "blind". This examination was later repeated, again blind. In both cases the results were identical, and they are shown in Table 1. The lesions were not graded because the majority of arteries were normal, and the few vascular lesions observed were so

Table 1. ATHEROSCLEROTIC SCORE IN THE AORTAE AND CEREBRAL ARTERIES OF CHICKENS FED AN ATHEROGENIC OR NON-ATHEROGENIC DIET FOR 2 YEARS

Sex	No. of birds	Mean atherosclerotic score $\pm$ S.E.*			No. of birds	Non-atherogenic diet		
		Atherogenic diet	A.A.	C.A.		T.A.	A.A.	C.A.
Males	5	34 $\pm$ 7	400 $\pm$ 0	Trace (2) Normal (3)	7	2 $\pm$ 0	43 $\pm$ 9	Trace (2) Normal (5)
Females	8	107 $\pm$ 31	363 $\pm$ 20	Trace (1) Normal (7)	9	9 $\pm$ 6	107 $\pm$ 42	Trace (1) Normal (8)

\* Atherosclerotic score  $\pm$  standard error, severity (1-4) times area involved (per cent).

T.A., Thoracic aorta; A.A., abdominal aorta; C.A., cerebral arteries (the lesions were too small to warrant special grading). White Leghorn chickens were used.

small as to warrant no special grading. When present, these lesions could not have given rise to any circulatory disturbance, and they are probably best considered as insignificant.

The results are interesting in that they point out the difference that exists between arterial vessels in the chicken. The cerebral arteries of a chicken fed an atherogenic diet appear to be resistant to the development of atherosclerosis, but the aorta and the coronary arteries, as previously reported, are susceptible to the disease. The reason for this resistance is difficult to explain. In the intimal layer of the cerebral artery, however, there may exist a barrier to the diffusion of cholesterol and other lipids through the arterial wall to the brain. This suggested explanation finds support from the evidence that birds fed an atherogenic diet containing cholesterol did not show an increase in concentration of brain cholesterol, whereas other body tissues showed significant increases in cholesterol level<sup>4,5</sup>. An answer to the question whether the cerebral artery in the chicken does not develop atherosclerosis is as important as whether the aorta and coronary vessels do become atherosclerotic; this information is necessary for a proper understanding of the aetiology of atherosclerosis.

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## BIOCHEMISTRY

### Metabolism of Propranolol ('Inderal'\*), a Potent, Specific $\beta$ -Adrenergic Receptor Blocking Agent

THIS is a preliminary report on the metabolism of propranolol<sup>1</sup> (I) in several species, including man.

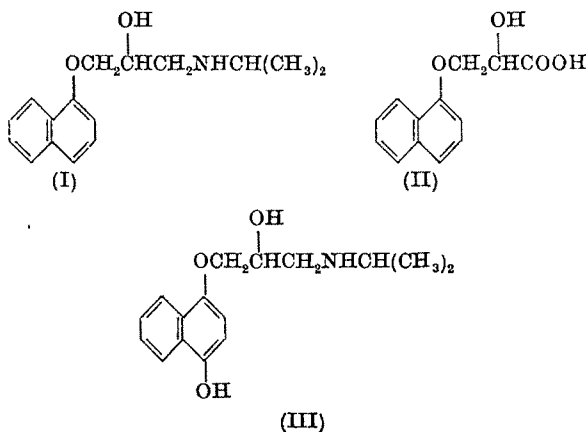
When propranolol labelled with carbon-14 at C-1 in the naphthalene ring was injected subcutaneously into the rat, mouse, guinea-pig or rabbit at a dose of 1 mg/kg, the majority of the radioactivity was excreted in the urine, mainly during the first 24 h after the injection. In the case of the rat, half the administered radioactivity appeared in the urine during the 24 h immediately after dosing, and only traces of radioactivity were detected in the expired air.

The radioactive metabolites were separated by electrophoresis of the rat urine in 0.05 molar sodium dihydrogen phosphate. There were two main groups, namely, acidic (approximately 30 per cent) and amphoteric (approximately 70 per cent) compounds; only small amounts of basic compounds were found.

A pure acidic metabolite was isolated from the urine of injected rabbits by ion exchange chromatography and

counter-current distribution. It was shown to be identical with a synthetic sample<sup>2</sup> of 2-hydroxy-3-(1'-naphthylthioxy)-propionic acid (II) by melting point and mixed melting point determinations, ultra-violet and infra-red spectroscopy, and thin layer chromatography in two systems.

Two amphoteric compounds were also separated from the urine of injected rats, mice and rabbits by ion exchange and gel filtration chromatography. These compounds were shown to be glucuronides of propranolol (I) and its 4-hydroxy derivative (III). The nature of these was demonstrated by the acidic hydrolysis of the former to propranolol and enzymatic hydrolysis (using  $\beta$ -glucuronidase taken from *Helix pomatia*) of the latter, to a compound shown to be identical with a synthetic sample<sup>3</sup> of 1-(4'-hydroxy-1'-naphthylthioxy)-3-isopropylamino-2-propanol (III) in infra-red and ultra-violet spectroscopy and thin layer and paper chromatography. The two glucuronides were also excreted in the bile of the rat and guinea-pig. Small amounts of propranolol and its 4-hydroxy derivative were detected in the urine of all the species examined.



The same metabolites were obtained after administering propranolol to mice, rats, guinea-pigs, rabbits and man. The main pathways of the metabolism of propranolol are thus demonstrated to be (a) hydroxylation (followed by conjugation with D-glucuronic acid) and (b) side-chain oxidation.

Work on further aspects of this problem including the absorption, distribution, metabolism, excretion and the possible entero-hepatic circulation of propranolol, together with the possibly differing metabolism of its two optical isomers, is in progress and will be reported separately.

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\* The term 'Inderal' is a registered trade mark, the property of Imperial Chemical Industries, Ltd.

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<sup>1</sup> Crowther, A. F., and Smith, L. H., U.K. Patent No. 994,918.

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### Deaggregation of Chlorophyll *a* by Xanthophylls

It is known<sup>1-3</sup> that the chlorophylls (but not their pheophytins) will aggregate in a variety of non-polar solvents (for example, benzene and carbon tetrachloride), with characteristic spectra for the respective monomers and dimers. These aggregates are concentration-dependent in the usual mass action sense up to a concentration of at least  $10^{-2}$  moles/l. They may be deaggregated by solvents which co-ordinate with the magnesium, for example, alcohols, and the extent of deaggregation may be calculated from the characteristic spectra.

We have found that aggregated solutions of chlorophyll *a* in carbon tetrachloride can be deaggregated by approximately equimolar amounts of leaf xanthophylls, but not by carotenes. The deaggregation shows itself spectroscopically by loss of the shoulder band on the red side of the red absorption band and an increase in intensity of the latter, along with a shift from 668 to 665 nm (Fig. 1). The corresponding spectra for  $\beta$ -carotene show no change over two orders of magnitude of concentration, the strongest having a carotene/chlorophyll ratio of approximately two (Fig. 2).

What is probably an isomerization of neoxanthin to two additional compounds resulted from rechromatography in petroleum ether on a sugar column. Of the three, only the middle compound on the chromatograph could deaggregate. The specificity and sensitivity of the different xanthophylls for deaggregation of both chlorophylls are being investigated.

Without knowing the matrix in which the chlorophylls occur *in vivo*, it might be expected that at the calculated natural concentration (approximately  $10^{-1}$  molar) they may be in a highly aggregated state. Our experiments lead us to presume that xanthophylls in chloroplasts regulate the extent of aggregation of the chlorophylls. The overwhelming concentration of the chlorophylls (for example,  $\sim 115$  chlorophyll *a* + *b*/17 xanthophylls)<sup>4</sup> precludes any but a minor fraction of aggregation control by this class of compounds. Chlorophyll *a* forms at least two different kinds of dimer, however, and it is clear that the accessory components of the chloroplast can regulate the detailed geometry controlling energy transfer in an energy trapping and biochemical function of the chlorophylls.

As a corollary, it should be recognized that difference spectra can result not only from changes in the state of oxidation of a pigment, but also from its state of aggregation or from a conformational change within a particular aggregated state.

This work was performed when one of us (S. A.) was a summer guest in the laboratory of Dr. C. Ponnamperna.

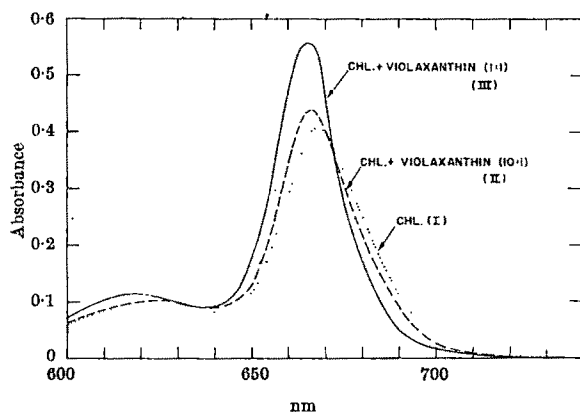


Fig. 1. Deaggregation of chlorophyll *a* by violaxanthin. .... Absorption spectrum of chlorophyll *a* aggregate (I) (dimer) in carbon tetrachloride (1 mg/ml.); ----, I plus violaxanthin (II) in the molecular ratio of approximately 10 : 1; —, I plus violaxanthin (III) in the molecular ratio of approximately 1 : 1. III is approximately identical to the curve for chlorophyll *a* monomer.

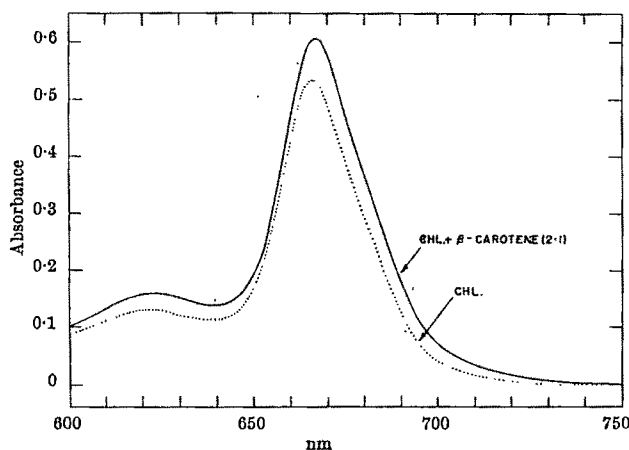


Fig. 2. Inability of  $\beta$ -carotene to deaggregate chlorophyll *a* dimers, indicated by lack of increase of absorbance and hypsochromic shift.

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### Protein Synthesis by Amoebal Ribosomes

ALTHOUGH some of the first observations localizing the site of protein biosynthesis to microsomes were made with *Tetrahymena*<sup>1</sup>, interest in protozoa has generally been overshadowed by that shown for bacteria, yeast or rat liver, as appropriate cells in which to study the mechanisms of protein synthesis. None the less, the possibilities of variation in the organization of polysomes or in genetic code assignments make these organisms of prime interest. The availability of axenic cultures of *Entamoeba histolytica*<sup>2</sup>, an important pathogen for man (amoebic dysentery and amoebic liver abscess), prompted us to study the polyribosomes of this organism. At present, only the trophozoite can be cultivated axenically.

*E. histolytica* (National Institutes of Health strain 200) were grown at 35° C in a liquid medium consisting of peptones, glucose, serum, a liver digest and vitamin supplement. For the purposes of mass cultivation, 125 ml. Erlenmeyer flasks with screw caps containing 100 ml. medium were used. Yields of  $10-15 \times 10^6$  organisms were collected from each flask at the height of a logarithmic growth phase (96 h) after inoculation of each flask with  $1 \times 10^6$  organisms. Amoebae obtained from twenty growth flasks were chilled and washed twice using Littlefield's medium (0.25 molar *tris* buffer, pH 7.6, 0.005 molar magnesium acetate, and 0.025 molar potassium chloride).

The washed cells were resuspended and broken by 13-14 strokes with a Dounce homogenizer. This process disrupted most of the cells and released intact nuclei. The nuclei and remaining whole cells were sedimented at 2,000*g*, and the homogenization steps repeated. Purified nuclei were obtained from the nuclear pellet by suspending



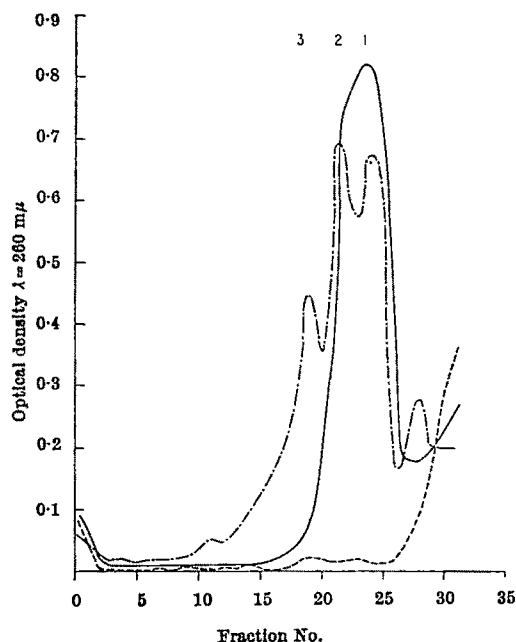


Fig. 1. Optical density profile of *E. histolytica* ribosomes. A two membered mixing chamber was used to prepare 10–34 per cent sucrose gradients. Two portions of *E. histolytica* ribosomes were treated with RNase at concentrations of 0.1  $\mu\text{g/ml}$  or 10  $\mu\text{g/ml}$  for 10 min at 37°. Polysome profiles were resolved by centrifugation at 25,000 r.p.m. for 2.5 h, using an 'SW 25-1' rotor. Adsorption at 260  $m\mu$  was monitored by passing the gradient, pumped at a rate of 1.0 ml/min, through a Gifford flow cell with a 5 mm light path. —, Control; ---, RNase (0.1  $\mu\text{g/ml}$ ); ·····, RNase (10  $\mu\text{g/ml}$ ).

it in *RSB* (0.01 molar *tris*, pH 7.4, 0.01 molar potassium chloride and 0.003 molar magnesium chloride) and centrifuging this suspension (45 min, 100,000*g*) through successive layers of 0.5 and 2.1 molar sucrose.

The supernatant fluid from the 2,000*g* pellet was clarified by centrifugation at 20,000*g* for 15 min. This solution was centrifuged for 4 h at 105,000*g* to yield a pellet which, when examined by electron microscopy, consisted of glycogen and ribosomal particles (200–300 Å) attached to an endoplasmic reticulum. For the preparation of polyribosomes, sodium deoxycholate was added to the 20,000*g* supernatant fluid to a concentration of 1.2 per cent. This solution was placed over layers of 0.5 and 2.1 molar sucrose and centrifuged for 4 h at 105,000*g* at 2° C. The microsomal or ribosomal pellets were suspended in *RSB* to give a final concentration of 1.5–2 mg ribosomal protein/ml. The Lowry method for determining protein was used<sup>3</sup>.

After passing the post-microsomal supernatant fluid through 0.22  $\mu$  'Millipore' filters, an enzyme fraction was prepared by lowering the pH to 5.0 with 0.1 molar acetic acid. The resultant precipitate was sedimented at 20,000*g* for 20 min and washed twice with water previously distilled over glass. Medium A was added to adjust the protein concentration to 10–12 mg/ml. All samples were stored at –170° C in liquid nitrogen.

Table 1 shows the activities of three fractions in promoting the incorporation of an amino-acid labelled with carbon-14 into a product precipitable with acid. The assay system made use of the filter paper technique of Mans and Novelli<sup>4</sup>: 100  $\mu\text{l}$ . samples were removed from the incubation mixtures at 5 min, 10 min and 20 min and pipetted on to 2.3 cm disks of Whatman No. 3 filter paper. The disks were washed in 5 per cent trichloroacetic acid (TCA) and 0.25 per cent sodium tungstate at 4° C. After washing for 45 min, the disks were heated to 90° C for 7 min in fresh 5 per cent TCA. They were then washed in cold 5 per cent TCA, twice in ethanol, dried, and counted in a liquid scintillation spectrometer. The activity of the ribosomal preparation was equal to

Table 1. POLY U STIMULATION OF CARBON-14-PHENYLALANINE INCORPORATION

		Specific activity		Ratio (to ribosomes)
		c.p.m.	m $\mu\text{moles}$	
1	Nuclear fraction	$0.051 \times 10^4$	0.0066	0.033
2	Microsomal fraction	$0.394 \times 10^4$	0.050	0.25
3	Ribosomal fraction	$1.57 \times 10^4$	0.20	1.0

The reaction mixture contained in a total volume of 0.5 ml.: 130  $\mu\text{moles}$  of sucrose, 20  $\mu\text{moles}$  of *tris* buffer pH 7.6, 20  $\mu\text{moles}$  of ammonium chloride, 28  $\mu\text{moles}$  of potassium chloride, 4.7  $\mu\text{moles}$  of magnesium acetate, 2.4  $\mu\text{moles}$  of  $\beta$ -mercaptoethanol, 0.48  $\mu\text{mole}$  of ATP, 0.12  $\mu\text{mole}$  GTP, 2.5  $\mu\text{moles}$  of phosphoenolpyruvate, 28  $\mu\text{g}$  of phosphoenolpyruvate kinase, 100  $\mu\text{g}$  of Poly U, 1  $\mu\text{c}$ . of carbon-14-labelled phenylalanine 1.0 mg of pH 5 enzyme protein and 0.25 mg of ribosomal (or nuclear) protein. Specific activities are expressed as c.p.m. or m $\mu\text{moles}$  of carbon-14-labelled phenylalanine incorporated into acid precipitable phenylalanine/mg of ribosomal (or nuclear) protein/60 min.

that previously observed with rat liver, when either cytoplasmic or nuclear ribosomes were used<sup>5</sup>.

*E. histolytica* ribosomes were fractionated on 10–34 per cent sucrose gradients (Fig. 1) and sedimentation values calculated with the *L* cell monomeric ribosome ( $\sim 74S$ ) as a marker, using the procedure of Martin and Ames<sup>6</sup>. The protozoan monomer migrated the same distance in the density gradient as the corresponding mammalian particle. Two additional peaks, representing a dimer and trimer, were also observed. When plotted as log *S* against log number of apparent monomers, a slope was obtained similar to that obtained by Pfuderer *et al.*<sup>7</sup> with liver polysomes. There was a shift to the monomer and dimer region with the addition of as little as 0.1  $\mu\text{g/ml}$ . RNase. Ribonucleoprotein particles with sedimentation properties similar to the monomer and dimer have been recorded by Scherbaum<sup>8</sup> in *Tetrahymena pyriformis*. An optical density peak, lighter than the monomeric ribosome, was found at concentrations of magnesium ions (3 mmoles/l.) which would normally suppress the disruption of monomers into their sub-units. This component may correspond to the 40S sub-unit reported in normal HeLa cells at lower concentrations of magnesium by Girard *et al.*<sup>9</sup>, or in HeLa cells infected with vaccinia at normal concentrations of magnesium by Joklik and Becker (personal communication). In the HeLa cell, the particle may represent a ribosomal precursor to which template RNA attaches before forming a polysome.

*E. histolytica* ribosomes were examined for a capacity to promote the incorporation of labelled amino-acids under a variety of conditions. The slope of the initial incorporation velocity  $V_0$  varied with the ribosomal concentration at a constant enzyme concentration (Fig. 2). Corresponding trials with synthetic polynucleotides indicated that 100  $\mu\text{g}$  of polynucleotide/reaction mixture achieved the optimal activity. When stimulated by polyuridine (Poly U), polyphenylalanine synthesis was

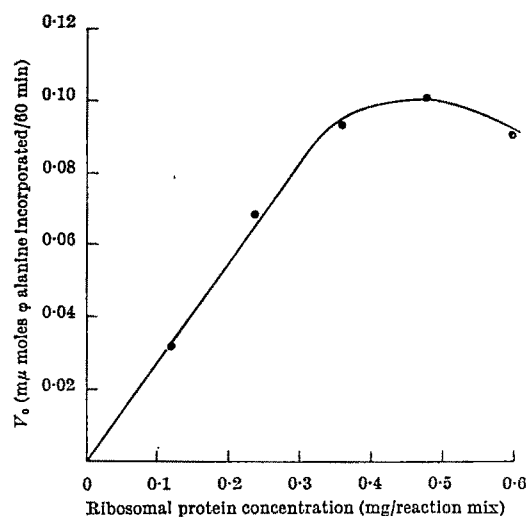


Fig. 2. Dependence of the rate of amino-acid incorporation on ribosome concentration.  $V_0$  is calculated from the initial reaction rate expressed as m $\mu\text{moles}$  of acid precipitable phenylalanine/60 min.

Table 2. POLY U STIMULATION OF CARBON-14 PHENYLALANINE INCORPORATION

Expt. No.		c.p.m.	mμmoles	Per cent inhibition
1	Complete system	$1.65 \times 10^5$	0.21	
2	" - pH 5.0 fraction	$0.086 \times 10^5$	0.011	95
3	" - Ribosomal fraction	-	-	100
4	" + RNase (10 μg/ml.)	-	-	100
5	" + DNase (30 μg/ml.)	$1.57 \times 10^5$	0.20	-
6	" + Puromycin (10 μmole/ml.)	$0.393 \times 10^5$	0.05	76
7	" + Tetracycline (25 μmole/ml.)	$0.55 \times 10^5$	0.07	65

The assay conditions are similar to those of Table 1 except that a single component was removed from, or added to, the reaction tube at zero time. Specific activity is expressed as mμmoles or c.p.m. of acid precipitable phenylalanine/mg ribosomal protein/60 min.

enhanced by a factor of from twelve to fifteen and increased linearly for 25 min. When three homopolymers (Poly U, Poly A and Poly C) were examined for their capacity to encode their corresponding amino-acids according to the assignments of Brimacombe *et al.*<sup>10</sup>, an enhanced formation of polypeptide was noted in the predicted direction.

Table 2 indicates the amount of product when a single component was removed from, or added to, the reaction mixture at the beginning of the experiment. The formation of the product depended on enzyme catalysis and the presence of ribosomes. Tetracycline, an antibiotic to which the mammalian protein synthesizing mechanism has appeared refractory, depressed polypeptide formation in this system at a low concentration. The *in vivo* activity of this drug in amoebiasis is well established. Recently, Suarez and Nathans<sup>11</sup> have shown that this antibiotic inhibits bacterial protein synthesis by competitively blocking one of the amino-acyl tRNA binding sites on the 50S ribosomal sub-unit. Although the drug might decrease nutrients available to non-axenic amoeba through a reduction of bacterial polypeptides, these results suggest that tetracycline may have a direct action on this protozoan.

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### Ribosome and Polyribosome Disappearance during *in vivo* Erythroid Maturation

THE maturation of the mammalian erythroid cell is marked by loss of the ability to synthesize protein<sup>1</sup>. The predominant site of protein synthesis within the cell is the polyribosome, an aggregate of two or more single ribosomes<sup>2</sup>. Although the decline in protein synthesis which accompanies erythroid maturation is apparently related both to a reduction in the cell content of polyribosomes and

to a decrease in their functional capacity<sup>3-5</sup>, there has been disagreement over the rates of polyribosome and single ribosome disappearance. It has been claimed, on the basis of *in vitro*<sup>3</sup> and *in vivo*<sup>5,6</sup> investigations, that erythroid maturation is accompanied by a selective loss of heavier polyribosomes, which results in an increasing proportion of smaller aggregates and single ribosomes as the reticulocyte ages. Other investigations have shown that there is a relatively constant proportion of polyribosomes in reticulocytes of different ages<sup>4,7</sup>. We have developed a system which allows longitudinal investigation of a population of reticulocytes as they mature *in vivo*, unaffected by continued production of new erythroid cells. Investigations of ribosome behaviour in this system indicate that with maturation all classes of ribosomes disappear at proportional rates, and that in particular there is no orderly shift of polyribosomes from larger to smaller aggregates.

The RNA of the newly formed rabbit reticulocytes induced by subcutaneous administration of phenylhydrazine was labelled by intravenous administration of 5-10 mc. of phosphorus-32 at the time of the final phenylhydrazine injection and 40 h before collection of the cells. The haemoglobin of the reticulocytes was labelled with iron-59 administered intravenously, given 24 h before cell collection. Donor cells, consisting of more than 80 per cent reticulocytes, were washed and infused into a recipient rabbit pretreated for 2 days with a daily intravenous dose of 1 mg of colchicine in order to suppress autogenous red cell production. Daily colchicine administration continued for the duration of the experiments. At intervals up to 72 h, blood samples were obtained from the recipient to determine the fate of the infused reticulocytes and their content of RNA and ribosomes. Reticulocytes were enumerated by supravital staining with new methylene blue dye. Ribosomes were recovered from stroma-free haemolysates by sedimentation at 150,000g and characterized by their sedimentation properties in sucrose density gradients<sup>2</sup>. Radioactivity incorporated into the ribosomes was determined by techniques described in detail elsewhere<sup>2</sup>, and that of haemoglobin in a well-type scintillation detector.

The sequence of events which follows infusion of the donor cells into the recipient animal is shown in Fig. 1. Within 15 min the circulating reticulocytes in the recipient had increased to 38 per cent from the colchicine-suppressed level of 2 per cent, and then progressively decreased to 4 per cent over the next 20 h. That the decrease in circulating reticulocytes was morphological evidence of maturation, and not of cell sequestration and destruction, is indicated by failure of the specific radioactivity of circu-

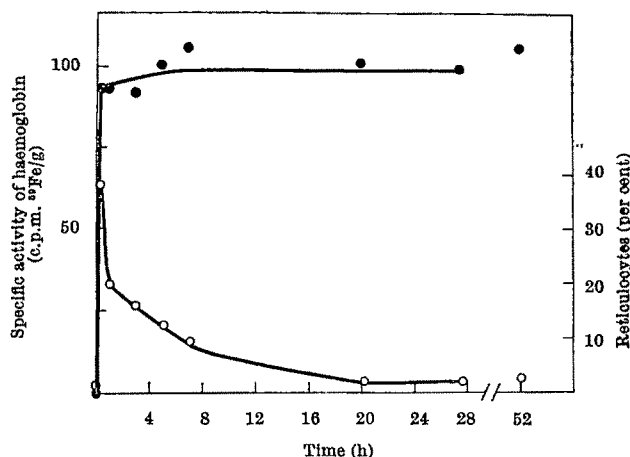


Fig. 1. Reticulocytosis and specific activity of <sup>59</sup>Fe-labelled haemoglobin in the recipient animal following infusion of donor reticulocytes. In this experiment the recipient blood volume calculated by dilution of <sup>59</sup>Fe was 331 ml. and by dilution of infused reticulocytes, 309 ml. (●, specific activity of haemoglobin; ○, reticulocytosis).

lating haemoglobin in the recipient to decline. The slight rise in the specific activity of haemoglobin was consistently noted and is probably caused by senescent loss of the recipient's unlabelled cells. Previous infusion investigations with phenylhydrazine-induced reticulocytes have indicated a dual population of cells, with as many as 55 per cent of the cells rapidly removed from circulation<sup>8</sup>. This problem was avoided by timing the collection of blood so that less than 5 per cent of the donor cells contained Heinz bodies.

The ribosome content of the infused cells progressively decreased with maturation (Fig. 2). The specific activity of the <sup>32</sup>P-labelled ribosomes was constant, indicating no significant delivery of unlabelled new reticulocytes to the peripheral blood during this time. The average life span of the infused cell population as reticulocytes, on the basis of ribosome content (Table 1), varied between 11 and 15 h in the five animals investigated.

Table 1. RIBOSOME RECOVERY AND POLYRIBOSOME PROPORTION DURING *in vivo* MATURATION

Time	Reticulo- cytosis per cent	*Ribosome recovery (mg/ml. cells)	†Per cent ribosomes existing as aggregates of				
			1	2	3	4	> 4
Experiment I							
Injected cells	95	1.54	42	19	30		
10 min	38	0.091	36	23	41		
760 min	20	0.058	34	24	42		
180 min	16	0.035	39	23	38		
300 min	12.5	0.028	37	22	41		
420 min	8.4	0.039	39	23	39		
Experiment II							
Injected cells	81	1.84	83	30	16	9	12
15 min	15	0.17	32	26	20	10	12
90 min	11.4	0.16	81	20	16	14	19
165 min	11	0.11	25	31	22	13	15
315 min	8.5	0.087	34	28	19	12	16
420 min	7.0	0.021	not determined				
Experiment III							
Injected cells	90	5.69	35	25	16	12	12
15 min	14	0.42	32	25	21	9	13
60 min	12	0.49	31	21	15	16	17
240 min	10	0.29	32	26	10	16	16
510 min	8	0.095	not determined				

\* Determined as described for Fig. 2.

† Determined by incorporation of phosphorus-32 into ribosomes sedimenting in appropriate zones of a sucrose density gradient under the conditions described for Fig. 3.

The behaviour of the various classes of ribosomes during *in vivo* maturation was examined by analysis of sucrose density gradient patterns of isolated ribosomes. The polyribosome content of maturing reticulocytes in three experiments is shown in Table 1. The proportion of ribosomes existing as single ribosomes and as aggregates of 2, 3, 4 and 5 or more ribosomes was determined by the proportion of phosphorus-32 incorporated into appropriate

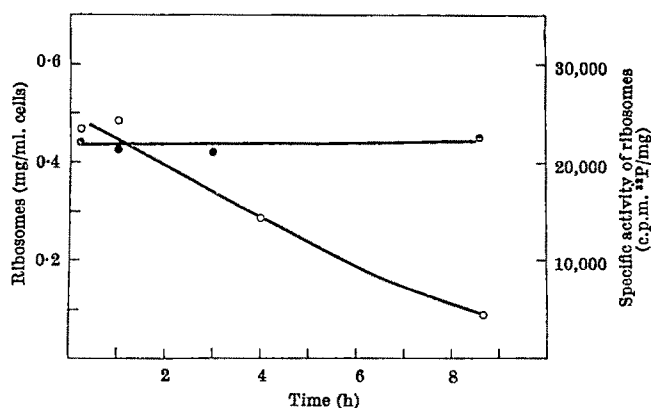


Fig. 2. <sup>32</sup>P-labelled ribosome content and specific activity of peripheral blood in the recipient following infusion of donor reticulocytes. Ribosomes were isolated from stroma-free lysates of red cells by centrifugation at 150,000g for 2 h. Milligrams of ribosomes recovered were calculated assuming a coefficient of extinction of 11 O.D. units/mg at 260 mμ. Disappearance of ribosomes based on c.p.m. of incorporated phosphorus-32 gave a curve identical to that of optical density measurements. (○, ribosome content of cells; ●, ribosome specific activity).

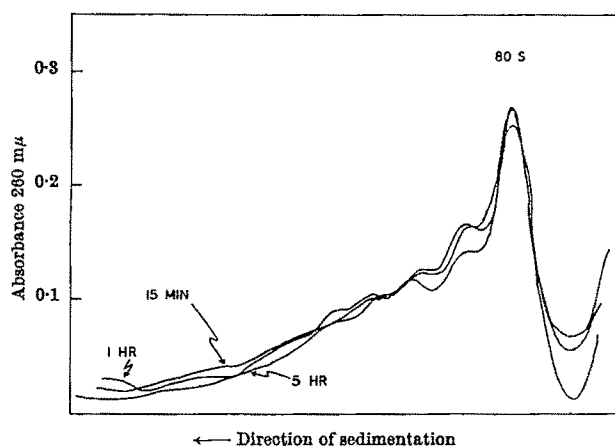


Fig. 3. Sucrose density gradient patterns of <sup>32</sup>P-labelled ribosomes following infusion of donor reticulocytes into the recipient. Ribosomes were recovered from stroma-free cell lysates at the times indicated and equal amounts sedimented in a 'Spinco SW-25-3' rotor through a linear gradient of 5–20 per cent of sucrose in 1.5 × 10<sup>-3</sup> magnesium chloride, 1 × 10<sup>-3</sup> molar *tris*, pH 7.5, for 2.5 h at 25,000 r.p.m. at 4° C. Fractions for determination of radioactivity were collected after the distribution of ribosomes in the gradient had been determined by absorbance at 260 mμ in a 'Gilford model 2000' recording spectrophotometer.

regions of the gradients. In experiment 1, aggregates of more than three ribosomes could not be adequately resolved. Examples of gradient patterns, obtained at 15 min, 1 h and 5 h in one of the experiments, are shown in Fig. 3. During the period of observation, when reticulocytosis decreased from 15 to 8 per cent and ribosome content fell from 0.17 to 0.09 mg/ml. of packed cells, the size distribution of the ribosomes remained constant. The results show that as a population of reticulocytes matures *in vivo* all classes of ribosomes disappear at proportional rates.

Previous analyses of the biochemical events which occur during erythroid maturation have utilized separation of cells on the basis of density, which is related to cell age, and longitudinal investigations during *in vitro* incubation. Rowley<sup>5</sup> and Glowacki and Millette<sup>4</sup> separated reticulocytes of various ages on albumin density gradients and arrived at opposite conclusions concerning ribosome disappearance. Rowley, in agreement with investigations of reticulocyte maturation *in vitro*<sup>3</sup>, concluded that maturation was accompanied by a preferential loss of polyribosomes. Glowacki and Millette<sup>4</sup> found no decrease in polyribosome proportion in progressively more mature reticulocytes. The proportion of monosomes in the very oldest cells appeared slightly increased, but there was no change in the size distribution of the remaining polyribosomes. Danon and his co-workers<sup>6</sup> investigated maturing reticulocytes *in vivo* in animals which had been treated with actinomycin in order to suppress continued erythroid production. His results, showing an orderly loss of larger polyribosomes with maturation, may be explained by the known impairment of energy metabolism by actinomycin<sup>9</sup>. It has been shown that other agents which interfere with energy utilization cause a reversible orderly disaggregation of polyribosomes in the intact cell<sup>10</sup>.

Our investigations meet two essential requirements for investigating aspects of erythroid maturation. These requirements are that the investigation should be done under physiological conditions and that maturation should occur without continued input of new cells. The data show conclusively that during erythroid maturation in the intact animal all classes of ribosomes disappear at proportional rates. Although the population of reticulocytes which we observed was heterogeneous with respect to age, this does not invalidate the findings because the cells were progressively maturing without continued delivery of younger cells. The absence of an orderly and progressive breakdown of polyribosomes during erythroid maturation

under physiological conditions is consistent with electron micrograph evidence of the presence of polyribosomes in cells containing very few ribosomes<sup>8</sup>. These findings are not necessarily in disagreement with *in vitro* investigations<sup>9</sup> where examinations were limited to initial and very late stages of maturation. Our data do not rule out a selective breakdown of polyribosomes which might occur as a terminal event in the life span of the reticulocyte.

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### Glyoxylate as a Substrate for Lactate Dehydrogenase

If 2-oxobutyrates are substituted for pyruvate as substrate for lactate dehydrogenase (LD), it is found to be more readily reduced by the electrophoretically faster moving LD isoenzymes than by the slower moving LD isoenzymes<sup>1,2</sup>. Because glyoxylate may also serve as a substrate for LD (ref. 3), we investigated the effect of LD isoenzyme fractions on this compound and compared the findings with those obtained with pyruvate and 2-oxobutyrates.

Crystalline suspensions of the electrophoretically fastest migrating (LD1 from pig heart) and slowest migrating (LD5 from rabbit muscle) LD isoenzymes were appropriately diluted with distilled water just before use. The substrates glyoxylate and 2-oxobutyrates were prepared as 1.0 molar and 0.1 molar solutions, respectively, in 0.067 molar Sørensen phosphate buffer (pH 7.4) after previous neutralization with saturated potassium hydroxide solution. Portions of equal volume were stored at -18° C until required. Sodium pyruvate was used freshly dissolved in phosphate buffer.

Enzyme activities were determined with a reaction mixture containing 2.7 ml. of 0.067 molar Sørensen phosphate buffer pH 7.4: 0.1 ml. of reduced nicotinamide-adenine dinucleotide (NADH<sub>2</sub>) freshly prepared at a concentration of 2.5 mg NADH<sub>2</sub>/ml. phosphate buffer and 0.1 ml. of enzyme preparation. After a suitable period of equilibration the reaction was started by the addition of 0.1 ml. of substrate solution, and enzyme activities determined by measuring the rate of decrease in optical density at 340 mμ and 25° C.

Inhibitor studies were also carried out with oxamate (0.02–0.5 mmoles/l.) or oxalate (0.01–0.2 mmoles/l.) incorporated into the reaction mixture immediately before the substrate solution was added. For investigations

of the inhibition by urea, enzyme and inhibitor were incubated in the reaction mixture for 15 min at 25° C before the substrate was added.

The concentration of glyoxylate required for maximal LD5 activity (33 mmoles/l.) was found to be considerably higher than that required for maximal activity of LD1 (6.67 mmoles/l.). High concentrations of glyoxylate had an inhibitory effect, which was most marked with LD1. In both these respects glyoxylate behaved similarly to pyruvate and to 2-oxobutyrates; however, the apparent enzyme substrate constants (*K<sub>s</sub>*) for LD1 and LD5 were much higher with glyoxylate as substrate (about  $5 \times 10^{-3}$  moles/l. for LD1 and  $30 \times 10^{-3}$  moles/l. for LD5) than the corresponding values with pyruvate or 2-oxobutyrates<sup>2</sup>.

Comparison was made between the activities of LD1 and LD5 using 0.7 mmolar pyruvate<sup>4</sup> and either 3.3 mmolar 2-oxobutyrates<sup>1</sup> or 6.67 mmolar glyoxylate (final concentration). Differentiation between the two isoenzymes, using glyoxylate, was less than that obtained with 2-oxobutyrates as substrate (Table 1).

Table 1. ACTIVITIES OF LD1 AND LD5 WITH 2-OXOBUTYRATE AND GLYOXYLATE RELATIVE TO THEIR ACTIVITIES WITH PYRUVATE

Isoenzyme	Activity ratios	
	2-Oxobutyrates/pyruvate	Glyoxylate/pyruvate
LD1	1.03	0.81
LD5	0.23	0.41

Inhibitor studies confirmed that oxamate acts as a competitive inhibitor for LD with both pyruvate and 2-oxobutyrates; the inhibition was more marked with LD1 than LD5 (refs. 5 and 6). With glyoxylate the inhibition was again found to be competitive in type and more marked with LD1. Oxamate inhibition with glyoxylate was similar in degree to that observed with 2-oxobutyrates but greater than that seen with pyruvate.

Oxalate, which is known to act as a non-competitive inhibitor with pyruvate and 2-oxobutyrates<sup>4,6</sup>, was also a non-competitive inhibitor with glyoxylate. A similar degree of inhibition was seen with all three substrates, and was more marked with LD1 than with LD5.

Urea inhibition of the LD isoenzymes with glyoxylate as substrate was similar to that found with pyruvate and 2-oxobutyrates in being more marked with LD5 and LD1 (refs. 7 and 8). When the ratios of enzyme activities with and without inhibitors were plotted against the concentration of urea, the curves obtained were of similar shape, showing marked inflexions. With glyoxylate inflexions occurred at about 1.0 molar urea for LD5 and about 2.0 molar for LD1. The inflexions with 2-oxobutyrates and pyruvate occurred at a similar urea concentration.

Despite their difference in structure both glyoxylate and 2-oxobutyrates have been shown to behave with remarkable similarity towards the LD isoenzymes. The type of inhibition of the LD isoenzymes was similar with all three substrates, and the degree of inhibition similar with glyoxylate and 2-oxobutyrates. Both glyoxylate and 2-oxobutyrates showed greater activity with LD1 than with LD5, although 2-oxobutyrates was a superior differential substrate in this respect. It is not anticipated, therefore, that glyoxylate as a substrate for LD will prove of clinical value in the way that 2-oxobutyrates as a substrate for LD ("alpha-hydroxybutyrate dehydrogenase") has found diagnostic application in cardiac disorders<sup>9,10</sup>.

Observations with LDX, the isoenzyme derived from human spermatozoa, have shown that this isoenzyme more readily reduces 2-oxobutyrates than would be anticipated from its electrophoretic mobility<sup>11</sup>. Preliminary investigations have shown similar findings with glyoxylate, which emphasizes the similarity between glyoxylate and 2-oxobutyrates as substrates for LD despite their dissimilar structures. This finding serves as evidence against a suggested specific metabolic role for 2-oxobutyrates in the metabolism of spermatozoa<sup>11</sup>.

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### Stimulation of Insulin Secretion by Theophylline

RECENT reports that epinephrine and glucagon, hormones which affect the generation of cyclic 3',5'-AMP, also influence the release of pancreatic insulin<sup>1-5</sup> have led us to examine the effect of this cyclic nucleotide on insulin secretion. In the present experiments the effects on insulin secretion of theophylline, a methyl xanthine derivative which inhibits cyclic nucleotide phosphodiesterase<sup>6</sup> and increases the intracellular steady state level of cyclic 3',5'-AMP, alone and in combination with epinephrine and alpha and beta adrenergic blocking agents, have been examined in the rat.

Male Charles River rats, weighing 200-400 g and fasted for 24-48 h, were adrenalectomized under ether anaesthesia 3 h before use to exclude acute variations in catecholamine secretion during the experimental procedure. Under pentobarbitone anaesthesia (3 mg/100 g intraperitoneally), a polyethylene catheter was inserted through the jugular vein into the right atrium for theophylline injection (15 mg in 0.5 ml. saline) and blood sampling. Two control blood samples were obtained before injecting theophylline. 0.4 ml. blood samples were afterwards withdrawn at frequent intervals, centrifuged and portions of plasma taken for insulin radioimmunoassay<sup>7</sup> and glucose determination<sup>8</sup>. Rat plasma insulin levels are given in terms of porcine insulin equivalents. The rates of disappearance of plasma insulin were deter-

mined using porcine insulin labelled with iodine-125 (ref. 9).

In the acutely adrenalectomized rat fasted for 48 h, theophylline produced a prompt rise in plasma insulin which could be detected within 1 min (Fig. 1). Plasma insulin concentrations up to ten times as high as the basal values were reached 5-10 min after injection and remained elevated for 20-30 min. The plasma insulin response following theophylline is not a consequence of the associated slight rise in blood sugar because (a) raising blood sugar to even greater levels by glucose infusion elicited only a minimal insulin response (Fig. 1) and (b) the increase in plasma insulin induced by theophylline persisted even after the blood sugar had returned to control concentrations.

We have used the area circumscribed by the plasma insulin response curve, expressed in  $\mu\text{U}\cdot\text{min}$ , as an index of insulin secretion. Although this expression is not a precise measure of pancreatic secretion rates, it is a valid technique for comparing insulin secretion under varying conditions provided the rate of insulin degradation remains unchanged. The half-life of plasma insulin did not differ significantly between control animals ( $33 \pm 4.4$  min) and rats treated with theophylline ( $32 \pm 2.8$  min). Insulin secretion stimulated by theophylline ( $487 \pm 42 \mu\text{U}\cdot\text{min}$ ) was more than three times that seen after glucose infusion ( $148 \pm 26 \mu\text{U}\cdot\text{min}$ ). When insulin secretion is expressed in terms of the rise in blood glucose during the 30 min experimental period, the insulin/glucose ratio is  $16.2 \mu\text{U}/\text{mg}$  glucose after theophylline and  $0.24 \mu\text{U}/\text{mg}$  glucose after infusion of glucose, indicating that the insulin response induced by theophylline is sixty-nine times greater than that expected from the rise in plasma glucose.

Within 1 min of the injection of theophylline, portal vein insulin increased from  $136 \pm 16$  to  $328 \pm 28 \mu\text{U}/\text{ml}$ . (Fig. 2). This prompt response followed by a subsequent fall and more prolonged increase in plasma insulin is consistent with an immediate effect of an initial bolus of theophylline which then underwent uniform mixing in the plasma compartment.

Recent studies by Porte<sup>11</sup> suggest that alpha adrenergic receptors mediate the inhibitory effect of epinephrine on insulin secretion, whereas beta adrenergic receptor

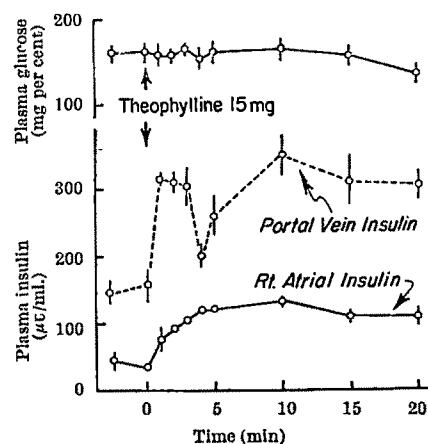


Fig. 2. Effect of theophylline on the plasma insulin levels of portal and right atrial venous blood in 15 h fasted, adrenalectomized rats. Theophylline was given intravenously in a single injection of 15 mg in 0.5 ml. physiological saline. Each point represents the mean  $\pm$  S.E.M. of five rats.

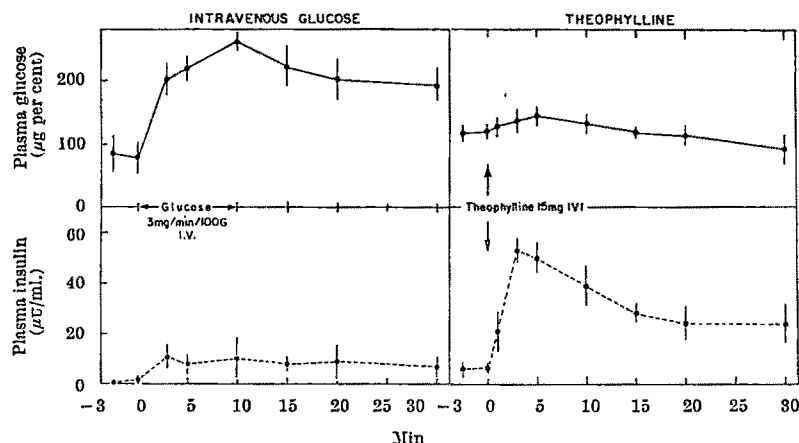


Fig. 1. Effect of infusion of theophylline and intravenous glucose on plasma insulin responses of 48 h fasted, adrenalectomized rats. Theophylline was given intravenously in a single injection of 15 mg in 0.5 ml. physiological saline. Glucose was infused at a rate of 3 mg/min/100 g body weight for 10 min. Each point represents the mean  $\pm$  S.E.M. of four to eight rats.



stimulation increases release of the insulin. Theophylline, injected 10 min after the beginning of an epinephrine infusion ( $0.1 \mu\text{g}/\text{min}/\text{kg}$ ), provoked a prompt 800 per cent rise in plasma insulin. In the presence of alpha adrenergic blockade and epinephrine infusion, theophylline provoked an extraordinary increase in plasma insulin content ( $3,000\text{--}4,000$  per cent rise to  $348 \pm 15 \mu\text{U}/\text{ml.}$ ) which continued throughout the experimental period and resulted in a rapid fall in plasma glucose (Fig. 3). During epinephrine infusion and beta adrenergic receptor blockade, theophylline injection produced no detectable plasma insulin response. These results suggest that alpha and beta adrenergic receptors in the islet beta cells mediate divergent effects of epinephrine on cyclic  $3',5'$ -AMP synthesis; alpha receptor inhibiting and beta receptor increasing the generation of cyclic nucleotide. Specific studies of alpha receptor stimulation on cyclic AMP generation have not been reported, but Murad *et al.*<sup>12</sup> have shown that beta receptor blockade prevented epinephrine stimulation of adrenyl cyclase activity in the dog heart.

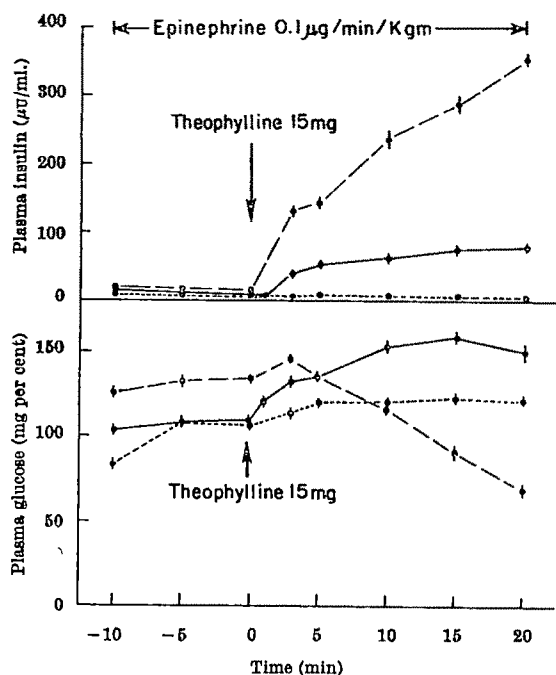


Fig. 3. The effect of theophylline on the plasma insulin responses of 48 h fasted, acutely adrenalectomized rats during an epinephrine infusion with and without alpha and beta adrenergic receptor inhibition. Epinephrine was infused at the rate of  $0.1 \mu\text{g}/\text{min}/\text{kg}$  body weight. Alpha receptor blockade was established with phentolamine ( $10 \text{ mg}/\text{kg}$  intraperitoneally) and beta receptor blockade with propranolol ( $1 \text{ mg}/\text{kg}$  given intravenously) given immediately before starting the epinephrine infusion. Theophylline,  $15 \text{ mg}$  in  $0.5 \text{ ml.}$  of physiological saline, was injected intravenously 10 min after beginning the epinephrine infusion. Each point represents the mean  $\pm$  S.E.M. of five rats. —, Control; ---, phentolamine; ···, propranolol.

Cyclic  $3',5'$ -AMP is involved in a wide variety of apparently divergent processes ranging from activation of enzymes such as phosphorylase<sup>13</sup>, UDPG glucogen alpha-4-glucosyltransferase<sup>13</sup> and tryptophan pyrrolase<sup>14</sup> to increasing adipose tissue lipolysis<sup>15,16</sup>, steroid hydroxylation<sup>17</sup>, uterine protein synthesis<sup>18,19</sup> and permeability of the toad bladder to water<sup>20</sup>. The results of this experiment indicate still another function for this nucleotide; namely, activation of a system involved in insulin secretion. The possibility that this effect may be of a more generalized nature in that the cyclic nucleotide participates in the secretory mechanisms of other peptide hormones merits further investigation.

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### Photo-induced Benzyl Substitution of Flavins by Phenylacetate: a Possible Model for Flavoprotein Catalysis

PHOTOREDUCTION of flavins is known to occur with amino-acids<sup>1,2</sup> or, intramolecularly, with the ribityl side chain of the flavin<sup>3,4</sup> as hydrogen donors. We wish to report here a new photochemical reaction of flavins which could also explain the mechanism of flavin and flavoenzyme catalysis as a whole.

We have found that, under anaerobic conditions, flavoquinones of general type I (scheme I) are rapidly and irreversibly bleached by visible light in the presence of phenylacetate anions. During this reaction a quantity of carbon dioxide stoichiometric with the flavin reduced is liberated, as measured manometrically. At higher concentrations of flavin and with suitable flavoquinone models, for example, 3-methylumiflavin (I,  $R_1 = R_2 = \text{CH}_3$ ), the photoreduced product precipitates quantitatively from aqueous reaction mixture and can be filtered off in the presence of traces of  $\text{S}_2\text{O}_4^{2-}$  as anti-oxidant. The product contains the benzyl residue of the phenylacetate in an apparently covalent bond to the flavin nucleus. Analysis established the product as a mixture of isomeric flavohydroquinones IIa and IIb, separable by their different solubility in dilute aqueous ammonia: IIa (melting point,  $225^\circ\text{--}230^\circ\text{C}$ ;  $\lambda_{\text{max}}$ ,  $342 \text{ m}\mu$  at pH 5) undergoes salt formation in this medium and is reprecipitated by acetic acid, preferably in the presence of  $\text{S}_2\text{O}_4^{2-}$ . The ammonia insoluble IIb (decomposition temperature,  $230^\circ\text{C}$ ;  $\lambda_{\text{max}}$ ,  $364 \text{ m}\mu$  at pH 5) can be purified by recrystallization from non-polar solvents. When the temperature of photoreaction is increased to  $50^\circ\text{C}$ , IIb is obtained exclusively, in a yield of 95 per cent.

Both compounds are stable in the dry, crystalline state and were shown by elementary analysis to be structural isomers  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_4$  (for I,  $R_1 = R_2 = \text{CH}_3$ ).

The formation of both compounds IIa and IIb is not rapidly reversible by air, as is the case for unsubstituted

flavohydroquinones. Instead, compound IIa reacts relatively slowly with air, whereby an olive green intermediate is formed which by electron spin resonance spectrometry was found to be a radical species. Its yield was found by double integration to reach 50 per cent and more of total flavin present. The radical disappeared only after several hours of aeration, to yield a third leucoflavin (IIc) which, unlike IIa or IIb, appeared to be stable towards oxygen even in the light. When the reaction  $\text{IIa} \rightarrow \text{IIc}$  was conducted by iodine instead of oxygen, the consumption of exactly two electron equivalents was measured. IIb, on the other hand, was found to be easily photo-oxidized, rendering I in a yield of 100 per cent. In the absence of light it was stable towards oxygen and in the absence of oxygen it was stable towards light. Formation of an intermediate radical was not observed. When the photo-oxidation was carried out with iodine instead of oxygen the consumption of 2.0 equivalents of iodine was measured. IIc, in turn, could be quantitatively reconverted to I by acid and to IIa by reduction with  $\text{S}_2\text{O}_4^{2-}$ .

The structure of the products IIa-c was established by combined spectrometry, ultra-violet, infra-red, nuclear magnetic resonance and electron spin resonance, acidimetry, and by comparison with known flavin derivatives. Scheme 1 (shown in Fig. 1) for flavin photoreduction and reoxidation was derived. The following evidence is given for the structures shown in Fig. 1. IIa shows the acidic NH characteristic for leucoflavins<sup>6</sup>, for example, FMNH<sub>2</sub>, with a  $pK_a$  of 7.2. The radical, which is green in the neutral (see here), and red in the protonated state, gives a complex electron spin resonance hyperfine pattern (unpublished work of Palmer, Massey and Hemmerich) unaffected by  $\text{H}_2\text{O}/\text{D}_2\text{O}$ -exchange, indicating lack of proton substituent at N(5) (refs. 6 and 7). The hyperfine pattern, however, reflects the deuteration of the benzyl residue, that is, replacement of  $\text{C}_6\text{H}_5\text{CH}_2\text{COO}^-$  by  $\text{C}_6\text{H}_5\text{CD}_2\text{COO}^-$  in the photoreduction, which indicates hyperconjugation between benzylmethylene and a position of high spin density in the heteroaromatic nucleus<sup>6,7</sup>.

The red radical cation from IIa has the same electron spin resonance hyperfine pattern as known 5-benzyl-leucoflavins<sup>6</sup> and the position of its absorption band at 510 m $\mu$  is characteristic of 5-alkylation<sup>8</sup>, compared with the  $\lambda_{\text{max}}$  of 490 m $\mu$  of 5-unsubstituted analogues<sup>6</sup>. IIa itself exhibits all CH-groups of the flavoquinone (I) in nuclear magnetic resonance, and in addition five unresolved phenyl-CH plus a benzyl  $\text{CH}_2$  at 4.35 p.p.m. ( $\text{CDCl}_3$ ). The latter signal is reduced by about 90 per cent in size in the phenyl-deuteroacetate photo-product.

IIb shows no  $pK_a$  and the same nuclear magnetic resonance signals, except that  $\text{CH}_2$  appears at 3.0 p.p.m. ( $\text{CDCl}_3$ ) as an AB-system. This clearly shows that the benzyl residue is fixed to an asymmetric carbon atom. The two methylene protons are therefore not equivalent. Again the  $\text{C}_6\text{H}_5\text{CD}_2$ -analogue is deficient in this signal. The free N(5)H is also seen in the nuclear magnetic resonance of IIb at 4.7 p.p.m. ( $\text{CDCl}_3$ ) and is exchangeable by  $\text{D}_2\text{O}$  as is to be expected.

IIa shows two strong CO bands at 1,700 and 1,640  $\text{cm}^{-1}$  in the infra-red ( $\text{CH}_2\text{Cl}_2$ ) and a very broad amide NH-stretching in the 3  $\mu$  region, whereas IIb shows only one strong CO band at 1,670  $\text{cm}^{-1}$  ( $\text{CH}_2\text{Cl}_2$ ), a weaker one at 1,725  $\text{cm}^{-1}$ , and the very sharp pyrrole type NH at 3,350  $\text{cm}^{-1}$ .

IIc, finally,  $\text{C}_{21}\text{H}_{22}\text{O}_3\text{N}_4$  after elementary analysis, displays in the near ultra-violet two peaks at 308 and 356  $\mu$  in methyl alcohol. In the infra-red, it shows a strong CO band at 1,640  $\text{cm}^{-1}$  and a weaker one at 1,725  $\text{cm}^{-1}$ , but no pyrrole-NH. The proton nuclear magnetic resonance spectrum shows all protons of IIa, but the two C- $\text{CH}_3$  groups now have distinctly different chemical shifts, indicating chemical non-equivalence, and therefore higher asymmetry of  $\pi$ -electron distribution, compared with IIa. The benzyl- $\text{CH}_2$  protons are now found at 4.65 p.p.m. ( $\text{CDCl}_3$ ) and a new broad signal caused by one  $\text{D}_2\text{O}$ -exchangeable proton is found at 1.65 p.p.m. ( $\text{CDCl}_3$ ). Thus, the structure of IIC appears to be that of a pseudo base derived from a 5-alkyl flavoquinone quaternary salt. In agreement with this, IIC is converted at  $\text{pH} < 2$  to a purple coloured compound ( $\lambda_{\text{max}}$ , 580 m $\mu$ ) which decomposes within minutes to give free flavoquinone (I) or, in the absence of oxygen, free flavosemiquinone cation.

Photoreduction of flavins was found with the following substrates: phenylacetic acid, mandelic acid, phenylpropionic acid, phenylglycine, indolyl-3-acetic acid, naphthylacetic acid. Photodecarboxylation of phenylacetate was found with the following flavins: FAD, FMN, riboflavin, lumiflavin, isoriboflavin, as well as various kinds of 2,3,4-substituted flavins, alloxazines and pteridines.

The reaction described here, in addition to the recently described reaction of glucose oxidase<sup>9</sup> and other flavo-proteins with bisulphite, suggests that the chemical reactivity of N(5) may be of importance in flavoprotein catalysis. Recent years have seen the report of the flavoprotein nature of several enzymes which possess no known redox function (oxynitrilase<sup>10</sup>, glyoxylate carboligase<sup>11</sup>). Thus the flavin coenzymes may serve a dual function in enzyme reactions; in addition to their well known redox properties they may catalyse reactions through chemical substitution reactions. Indeed, it is even possible that the redox function may result by group transfer rather than electron or H-transfer. It is remarkable that flavoproteins in general act only on compounds containing CH-bonds which are "activated" by substituents with large +M effects, such as DPNH, thioesters (acyl coenzyme A), amino-acids and aldehydes. No simple alcohol oxidation reaction catalysed by flavo-proteins is known; in the known cases where flavins are involved in oxidation of alcoholic groups (for example, glycolic acid oxidase,  $\alpha$ -hydroxyacid oxidases) the CH-bond is also "activated" by the carboxyl substituent,

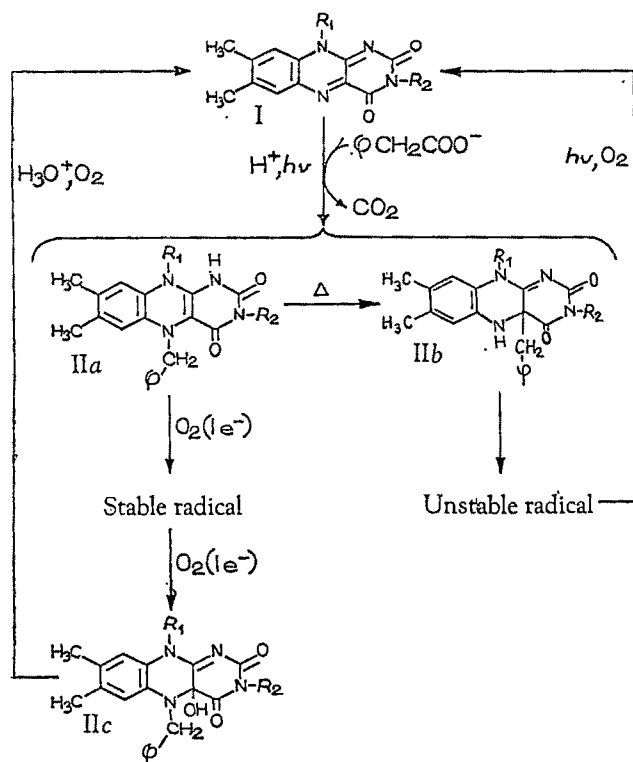


Fig. 1. Scheme 1 for photoreduction and reoxidation of flavin.

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### Synthesis of Serotonin by Pineal Glands of the Rat in Organ Culture

THE pineal gland of the rat has been shown to contain large quantities of the indole serotonin<sup>1</sup>. This organ can also metabolize serotonin by a unique pathway to form the hormone melatonin (5-methoxy *N*-acetyltryptamine)<sup>2</sup>. The content of serotonin<sup>1,3</sup> and the activity of the melatonin-forming enzyme<sup>4</sup> in the pineal have been found to vary with a 24 h cycle. These rhythms are controlled by information transmitted to the pineal through its sympathetic nerves<sup>5</sup>.

Little is known about the biochemical control of serotonin synthesis in the intact pineal gland. The investigation of this and other regulatory processes in the pineal may be facilitated by the development of an *in vitro* system in which serotonin synthesis persists. Previous investigators have grown mammalian pineal glands in organ and tissue culture<sup>6-10</sup>, and have shown that the cells which appear bear a histological resemblance to pineal parenchymal cells<sup>9</sup>. The biochemical properties of pineal cells in culture, however, have not been examined yet. The present communication describes a technique for cultivation of the pineal glands of adult rats, such that serotonin is synthesized from the amino-acids tryptophan and 5-hydroxytryptophan with a rate that increases during the first 4 days in culture. Additional investigations provide evidence for the continuing activity of both the enzymes required for serotonin synthesis (tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase), and for a rate-limiting role of tryptophan hydroxylase in the formation of this amine.

Sprague-Dawley female rats weighing 160-180 g were killed by neck fracture, and the pineal glands were quickly removed<sup>2,4</sup> and transferred to a Petri dish, where they were covered with neutralized Hanks solution. The gland was then divided into two approximately equal parts. These fragments were clotted to the walls of a Wasserman tube (which had previously been coated with a thin film of chicken plasma) by the application of chick embryo extract to the glass surface<sup>11</sup>. Fifteen to thirty minutes later, when the clot was sufficiently firm, 0.5 ml. of nutrient medium was added to the culture; the tube was sealed with a rubber stopper and incubated in a roller wheel at 37° C. The nutrient medium consisted of 75 per

cent Puck N 16 medium, 5 per cent Evans NCTC 109 medium, 10 per cent foetal calf serum and 10 per cent heat-inactivated (56 per cent C for 30 min) horse serum. The constituents of the medium were obtained as standard preparations, except for the N 16 and NCTC 109, which were prepared without phenol red. (This indicator dye had been found in preliminary experiments to interfere with the assay for serotonin.) The nutrient medium as described contained tryptophan in a concentration of approximately 10<sup>-4</sup> molar, but not 5-hydroxytryptophan. For certain experiments the medium was fortified with 10<sup>-3</sup> molar tryptophan or 10<sup>-5</sup>-10<sup>-3</sup> molar 5-hydroxytryptophan. At 2 day intervals the media were poured off and stored at -70° C for serotonin assay; new media were added, and the incubation was resumed. Each tube contained 25 U of penicillin, 25 µg of streptomycin, and 0.5 µg of amphotericin B ('Fungizone'). Each experimental group contained seven culture tubes.

All assays for serotonin were performed on the same day on 0.1 ml. aliquots of medium. The method of assay involved the formation of a fluorescent product after extracting the serotonin<sup>12</sup>. The identity of the serotonin was confirmed by thin-layer chromatography in an ethanol : dioxane : benzene : ammonia system (5 : 40 : 30 : 5), followed by staining with Ehrlich's reagent.

Cells in explanted pineal fragments had a healthy appearance throughout the 10 day period of observation. Examined in preparations stained with haematoxylin and eosin, the cell population after the first 2-3 days appeared to consist almost entirely of pinealocytes. There was little cellular outgrowth from the explanted fragments until the fourth day; however, by the eighth to tenth day an extensive growth appeared from all fragments. This outgrowth also consisted almost entirely of pinealocytes, with occasional scattered, more elongated cell forms. These morphological findings were similar to those described<sup>9,10</sup> in plasma clot cultures of rat pineal fragments, except that the outgrowth in the present investigation included fewer cells other than pinealocytes.

The net synthesis of serotonin by the pineals was determined by comparison of the quantity of amine released into the medium during 2 day periods of incubation with the amounts present in two kinds of control tubes: "zero-time" tubes, which were frozen immediately after the addition of the pineal, and tubes which were incubated without pineal tissue. When incubated with 0.5 ml. of medium containing 10<sup>-4</sup> molar tryptophan, pineals were capable of synthesizing as much as 1.5 µg of serotonin in a day. (This amount is several times greater than the highest reported concentrations of serotonin in the rat pineal, but is consistent with the rapid rate at which the serotonin content of the gland normally increases *in vivo* early in the day<sup>1</sup>). The medium did not contain 5-hydroxytryptophan, and so the synthesis of serotonin in these conditions indicated that the pineal gland contained both the 5-hydroxylating and the decarboxylating enzymes (that is, tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase). The rat pineal has previously been shown to contain relatively large amounts of this latter enzyme<sup>13</sup>. Tryptophan hydroxylase activity has already been found in brain<sup>14</sup>, but not so far in the pineal.

To determine which of these two enzymes controlled the rate of serotonin synthesis by pineals in organ culture, glands were incubated with media fortified by the addition of tryptophan or 5-hydroxytryptophan (Table 1). Pineals incubated with 10<sup>-4</sup> molar tryptophan generated 4.3 × 10<sup>-9</sup> moles of the amine during the first 2 days of culture. (This represented an 8.5 per cent conversion of the amino-acid.) Increasing the concentration of the tryptophan ten-fold (to 10<sup>-3</sup> molar) did not increase the absolute yield of serotonin. Pineals incubated with 10<sup>-5</sup> molar 5-hydroxytryptophan generated 3.8 × 10<sup>-9</sup> moles of the amine in 2 days. When a greater concentration of this amino-acid was used, there was a marked rise in the rate of serotonin

synthesis (Table 1). These data indicated that the activity of the decarboxylase enzyme was considerably greater than that of the hydroxylating enzyme. They further suggested that, in the conditions of this experiment, the hydroxylation step determined the rate at which serotonin was formed.

Table 1. SEROTONIN SYNTHESIS BY RAT PINEAL GLANDS IN ORGAN CULTURE

Contents of medium	Moles of serotonin formed ( $\times 10^{-6}$ )/tube	Zero time	0-2 days	2-4 days
Tryptophan				
10 <sup>-4</sup> molar	1.0 $\pm$ 0.1	4.3 $\pm$ 0.3*	1.6 $\pm$ 0.2†	
10 <sup>-3</sup> molar	0.9 $\pm$ 0.2	4.3 $\pm$ 0.4*	3.8 $\pm$ 0.9†	
10 <sup>-4</sup> molar, no pineal	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	
5-Hydroxytryptophan (also contained 10 <sup>-4</sup> molar tryptophan)				
10 <sup>-4</sup> molar	1.1 $\pm$ 0.2	3.8 $\pm$ 0.4*	2.8 $\pm$ 0.3*	
10 <sup>-3</sup> molar	1.6 $\pm$ 0.3	6.5 $\pm$ 0.6*	8.1 $\pm$ 0.6*	
10 <sup>-4</sup> molar	1.6 $\pm$ 0.3	9.9 $\pm$ 0.6*	10.9 $\pm$ 0.8*	
10 <sup>-3</sup> molar, no pineal	1.4 $\pm$ 0.1	1.5 $\pm$ 0.2	1.4 $\pm$ 0.1	

Groups of seven culture tubes, each containing one pineal gland, were incubated for 2 days. The medium was then poured off and frozen, fresh medium was added, and the tubes were incubated for 2 more days. Control tubes were frozen immediately after the addition of the pineal, or were incubated without a pineal. Aliquots of the culture medium (0.1 ml.) were assayed for serotonin content.

\* $P < 0.001$ , differs from zero time controls.

† $P < 0.01$ , differs from zero time controls.

In all experiments, the synthesis of serotonin from 5-hydroxytryptophan was greatest on days 2-4. Significant, but decreasing, amounts of the amine continued to be formed for 8-10 days after initiation of the culture. The increase in decarboxylase activity between days 0-2 and 2-4 suggests that new enzyme is being formed in organ culture. This could represent more enzyme in each pineal cell, or unchanged levels in a larger number of cells.

Previous work has shown that the activity of 5-hydroxytryptophan decarboxylase in the rat pinealocyte is regulated by the sympathetic nerves to this organ<sup>15</sup>. It is presumed that the sympathetic nerve ending exerts its effects by release of a neurotransmitter substance. The development of an *in vitro* system which continues to synthesize serotonin for several days could provide a useful experimental tool for investigation of the neural control of pineal enzymes.

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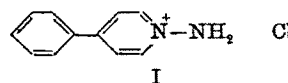
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## 1-Amino-4-phenyl Pyridinium Chloride: a Potential Antihypertensive Agent

DURING investigations of the synthesis, storage and release of peripheral catecholamines, 1-amino-4-phenyl pyridinium chloride (AH.2035) was found to cause a marked depletion of noradrenaline in mouse heart. The results reported here suggest that the compound (I) may be a useful antihypertensive agent.



Acute oral toxicity values for AH.2035A in different species are given in Table 1.

Table 1. ACUTE TOXICITY OF AH.2035A IN MOUSE, RAT, RABBIT, CAT AND DOG

Species	Mouse	Rat	Rabbit	Cat	Dog
Oral LD <sub>50</sub> (mg/kg)	161	713	150-300	> 75*	> 75*
(95 per cent fiducial limits)	(132-196)	(570-891)			

\* Oral doses of 150 mg/kg caused vomiting.

The noradrenaline contents of various organs in the mouse and rat were determined after acute and sub-acute oral administration of AH.2035A. Noradrenaline was extracted, isolated by ion exchange chromatography<sup>1</sup> and determined fluorometrically by a modification of the trihydroxy-indole reaction (Martin, L. E., and Harrison, C., to be published). In the mouse a single dose of AH.2035A (10 mg/kg) lowered the heart noradrenaline content by 66 per cent. In the rat repeated administration of AH.2035A (three doses of 5 mg/kg in 24 h) lowered the heart noradrenaline by 50 per cent. In neither experiment was the concentration of noradrenaline in the brain significantly affected. Thus, AH.2035A depletes peripheral but not central stores of noradrenaline in mouse and rat.

In dogs anaesthetized with pentobarbitone, AH.2035A injected intravenously in doses of 0.25-1 mg/kg produced pressor responses of 25-130 mm mercury which lasted for 10-45 min. After 1 mg/kg the rise in blood pressure was followed by a gradual fall to 50 mm below the pre-injection level during the next 2 h. The pressor responses to occlusion of the common carotid arteries or injection of tyramine were reduced throughout this period but that to noradrenaline was potentiated. The pressor effects of AH.2035A were prevented by previous treatment with reserpine, cocaine or phentolamine but not by hexamethonium. These effects are similar to those produced by guanethidine<sup>2</sup> in anaesthetized animals. AH.2035A differs, however, from guanethidine in that it does not cause acute adrenergic blockade. For example, AH.2035A did not block the inhibitory effects of sympathetic nerve stimulation in a Finkleman rabbit ileum preparation<sup>3</sup>.

Daily oral administration of AH.2035A in doses of 5 mg/kg to renal hypertensive dogs caused a pressor response on the first day of administration but not on subsequent days. On the second and third days of administration there was a gradual reduction in the mean arterial blood pressure of 30-40 mm mercury. When small doses of AH.2035A were given initially, and slowly increased to 5 mg/kg/day, only an antihypertensive effect was found. In this experiment as little as 1-2 mg/kg/day AH.2035A induced a significant reduction in systolic blood pressure. The antihypertensive action of AH.2035A in renal hypertensive dogs was not accompanied by diarrhoea or other noticeable side-effects.

The mechanism of the antihypertensive action of AH.2035A is not known for certain but is probably related to its action on stores of catecholamine in sympathetic nerve endings. The properties of AH.2035A and

its analogues are being investigated in greater detail and the results will be reported elsewhere.

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### Activation of Tyrosine Hydroxylation in Rat Brain *in vivo* by Chlorpromazine

PHENOTHIAZINES and related neuroleptic drugs, such as thioxanthenes, markedly increase the content of homovanillic acid (3-methoxy-4-hydroxyphenylacetic acid) in the brain, especially in the extrapyramidal centres of various animal species. This has raised the question of whether neuroleptic drugs accelerate the synthesis of catecholamines through a feed-back mechanism either as a result of blockade of dopaminergic and noradrenergic receptors or of alteration of the amine storage<sup>1-6,16</sup>. The present communication shows that *in vivo* chlorpromazine and related neuroleptics markedly enhance the hydroxylation of tyrosine which seems to be a rate-limiting step in the formation of catecholamines<sup>7</sup>.

In female rats weighing 60–80 g, injected subcutaneously with 0.2 mg/kg L-3,5-<sup>3</sup>H-tyrosine (11.1 mc./mg), the <sup>3</sup>H-catechol derivatives of brain (homogenized in three volumes of 4 normal perchloric acid) were isolated by chromatography on alumina and measured in a liquid scintillation counter<sup>8</sup>. The endogenous tyrosine in blood plasma and brain stem (including basal ganglia, but without medulla and pons) was determined by a spectrofluorimetric method<sup>9</sup> which showed a similar recovery of added tyrosine (78 ± 5 per cent) in normal rats and rats treated with chlorpromazine. The animals receiving chlorpromazine (10 mg/kg intraperitoneally) were kept at an environmental temperature of 31° C in order to prevent a decrease of the body temperature

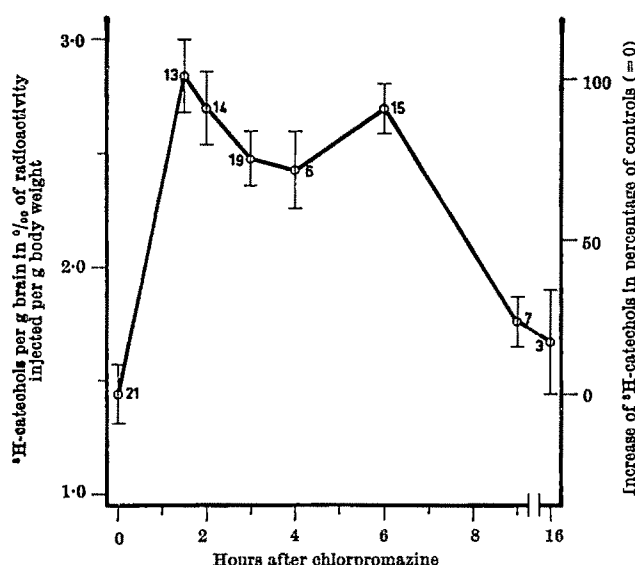


Fig. 1. Tyrosine hydroxylation in rat brain after a single intraperitoneal injection of 10 mg/kg chlorpromazine. <sup>3</sup>H-Tyrosine was administered subcutaneously to all animals 1 h before killing them. Controls (0 time) received <sup>3</sup>H-tyrosine only. Each point represents an average value with standard error; the number of experiments (each with a pool of two rats) is indicated beside the points.

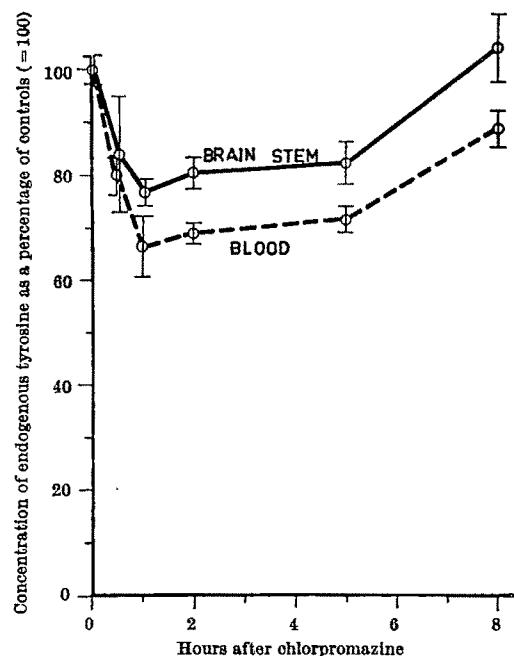


Fig. 2. Tyrosine content in the brain stem and blood plasma of rats after a single intraperitoneal injection of 10 mg/kg chlorpromazine. Each point represents a mean of between eight and fifteen determinations of a pool of six animals ± S.E. Absolute values of controls (thirty-two experiments): brain stem 5.0 ± 0.1 µg/g; blood plasma 4.0 ± 0.2 µg/ml.

(maximal deviation from the normal rectal temperature of 1°–2° C as measured by a thermocouple).

In rats receiving <sup>3</sup>H-tyrosine, chlorpromazine increased the formation of <sup>3</sup>H-catechol derivatives in the brain by 80–100 per cent for a period of 1.5–6 h (Fig. 1). The total radioactivity of the brain, however, did not change significantly, which indicates that the penetration of tyrosine into the brain was not altered by the drug. The concentration of endogenous tyrosine in brain stem and blood plasma was significantly diminished by 20 and 30 per cent, respectively, 1–5 h after the treatment with chlorpromazine (Fig. 2), thus showing an inverse time course as compared with that of <sup>3</sup>H-catechols. These alterations may be characteristic for chlorpromazine-like neuroleptics, because chlorprothixene also enhanced the transformation of <sup>3</sup>H-tyrosine into <sup>3</sup>H-catechols and decreased the concentration of endogenous tyrosine. Other psychotropic drugs, however, such as imipramine, phenobarbitone and chlordiazepoxide, were less effective.

The increased transformation of <sup>3</sup>H-tyrosine into <sup>3</sup>H-catechols in the brain of intact rats indicates that chlorpromazine and chlorprothixene enhance the hydroxylation of tyrosine. It remains to be determined whether these neuroleptics act by a direct activation of tyrosine hydroxylase or by an indirect mechanism, for example, facilitation of tyrosine transport, enhancement of the turnover of catecholamines as a result of interference with catecholaminergic receptors or storage sites<sup>1,3,4,6</sup>. The decrease of endogenous tyrosine in brain and blood might, in part, result from the increased formation of catechol derivatives, but an additional activation of other pathways of tyrosine metabolism, for example, transamination<sup>10–12</sup>, must also be considered. The enhanced formation of <sup>3</sup>H-catechol and the diminution of endogenous tyrosine do not, however, seem to result from an alteration of protein synthesis, because this was not found to be affected by chlorpromazine in the brain of normothermic rats<sup>13</sup>.

In conclusion, an acceleration of the hydroxylation of tyrosine, which is considered to be a limiting step in the biosynthesis of catecholamines *in vivo*, might be connected with the increase of cerebral homovanillic acid due



to neuroleptic drugs. Furthermore, an enhanced synthesis of dihydroxyphenylalanine (DOPA) is possibly related to an increased formation of melanin observed in patients under prolonged treatment with neuroleptics<sup>6,14,15</sup>.

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### Gel Filtration Radioimmunoassay to distinguish Human Chorionic Gonadotrophin from Luteinizing Hormone

EXISTING immuno- and bio-assays are incapable of readily distinguishing human luteinizing hormone (LH) from human chorionic gonadotrophin (HCG). A procedure capable of making this distinction should make it possible to solve a variety of problems, including whether the high levels of luteinizing activity associated with seminoma are due to HCG or LH, whether the blood of newborn infants contains HCG or LH, and whether or not moderately high levels of "LH" found by radioimmunoassay in patients thought to be cured of choriocarcinoma represent a recurrence of the neoplasm. The present communication describes a means for making this distinction. Advantage was taken of the different gel filtration properties of these two hormones to effect their separation, and the resulting elution peaks were then located by radioimmunoassay.

All the steps of the procedure were carried out at 4° C. The separation was performed in a 1.5 × 80 cm column of 'Sephadex G-100' which was equilibrated with 0.05 molar phosphate buffer at pH 7.6 and eluted in the same buffer at a rate of 5 ml./h using a peristaltic pump. Samples (1 ml.) were applied beneath the buffer on the surface of the gel, and sucrose was added as required to aid in the transfer. 1 ml. portions were collected in conical centrifuge tubes, and collection began when 0.5 ml. of sample had entered the gel. Blue dextran with a molecular weight of  $2 \times 10^6$  was used to determine the excluded or void volume ( $V_0$ ) and was measured by spectrophotometry at 630 mμ. HCG and LH were quantitated in the tubes by radioimmunoassay<sup>1</sup>. After gel filtration, 0.5 ml. of 1:80,000 anti-HCG in 1:400 normal rabbit serum containing 0.02 molar ethylenediamine tetraacetic acid (EDTA) in 0.14 molar saline buffered at pH 7.0 with 0.01 molar phosphate was added to each tube. After 24 h of continuous agitation, 0.5 ml. of HCG labelled with iodine-131 was added, followed 24 h later by 0.5 ml. of sheep anti-rabbit gamma globulin, the latter in a dilution which maximally precipitated the rabbit globulin in each tube. After incubation for 48 h each tube was centrifuged, decanted and counted. The elution volume ( $V_e$ ) for LH and HCG was determined by finding the tube which gave maximal inhibition of the reaction between the labelled HCG and its antibody. All results were expressed as the

ratio of  $V_e/V_0$  after subtracting the "dead" volume between the bottom of the gel column and the tip of the delivery tube in the fraction collector. In separate experiments, 1 ml. of serum containing HCG labelled with iodine-131 and LH labelled with iodine-125 was passed through the column and the hormones located by differential gamma-ray spectroscopy. The samples which contained HCG were made up from sera from pregnant women and serum from a woman with choriocarcinoma. The samples containing LH consisted of sera from women during the menstrual cycle<sup>2</sup>. Unknown samples consisted of sera from the umbilical vein of newborn infants.

The results are listed in Table 1. HCG could readily be distinguished from LH. The sensitivity of the radioimmunoassay procedure allowed the determination to be made on 1 ml. aliquots of serum from normal menstruating females at mid-cycle. The radioiodinated hormones gave  $V_e/V_0$  ratios indistinguishable from those of unlabelled hormones, but their elution peaks were sharper than those measured by radioimmunoassay for the endogenous hormones in sera. It is possible that some of the circulating hormones are bound to serum proteins.

Table 1. SEPARATION OF HCG AND LH BY GEL FILTRATION

	No. of samples	$V_e/V_0$
		Mean $\pm$ S.E.M.
Samples containing LH		
LH- <sup>125</sup> I	3	1.49 $\pm$ 0.02
Sera, women at midcycle	3	1.52 $\pm$ 0.04
Samples containing HCG		
HCG- <sup>131</sup> I	3	1.25 $\pm$ 0.07
Sera, pregnant women	2	1.32 $\pm$ 0.03
Sera, patient with choriocarcinoma	1	1.35
Unknown samples		
Sera, newborn infants	3	1.31 $\pm$ 0.01

Radioimmunoassay of three sera obtained from the umbilical vein of infants at the time of delivery by caesarean section showed that the hormone levels were comparable with those of non-pregnant adults. Analysis of these sera by gel filtration and radioimmunoassay showed that they contained a material with a  $V_e/V_0$  similar to that of HCG and not of LH (Table 1). Accordingly, it seems that a small amount of HCG normally enters the foetal circulation (concentrations of maternal HCG, 7–22 IU/ml., ranged from 570 to 800 times higher than those in the newborn babies).

We thank Dr. W. D. Peckham for the gift of purified human LH used for radioiodination (potency,  $5.3 \times$  NIH-LH-S1; lot No. WDP-VII p. 833).

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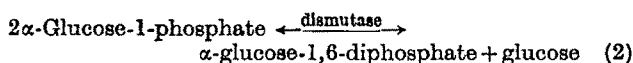
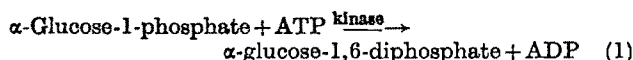
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### Hypothesis on the Mode of Conversion of Glucose into $\alpha$ -Glucose-1-phosphate

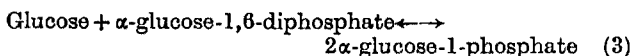
SOME 10 years ago much evidence had accumulated that phosphorylase does not synthesize glycogen from  $\alpha$ -glucose-1-phosphate *in vivo*. The confirmation of this belief came with the discovery of glycogen synthetase,

and its synthesis of glycogen from uridine diphosphate glucose (UDPG)<sup>1</sup>. There is now a parallel situation in another area of glycogen metabolism, namely, repeated evidence from tracer experiments that the conversion of glucose into glycogen in liver and muscle does not proceed by way of glucose-6-phosphate. Several reports, originating with Beloff-Chain *et al.*<sup>2</sup> and most recently by Threlfall<sup>3</sup>, combine to indicate that there is a pathway from glucose to  $\alpha$ -glucose-1-phosphate—and thence to UDPG and glycogen—that does not have glucose-6-phosphate formation as an intermediate step. In other words, hexokinase, glucokinase, glucose-6-phosphatase acting in its synthetic role, all forming glucose-6-phosphate, together with phosphoglucomutase, converting the 6-phosphate into  $\alpha$ -glucose-1-phosphate, may well have no direct role in the conversion of glucose into glycogen.

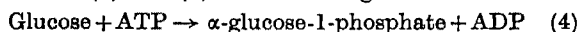
It occurred to us that an answer to the problem might be found by examining the actions of two enzymes already known in muscle, namely,  $\alpha$ -glucose-1-phosphate kinase<sup>4</sup> and  $\alpha$ -glucose-1-phosphate dismutase<sup>5</sup>. These enzymes catalyse the following reactions



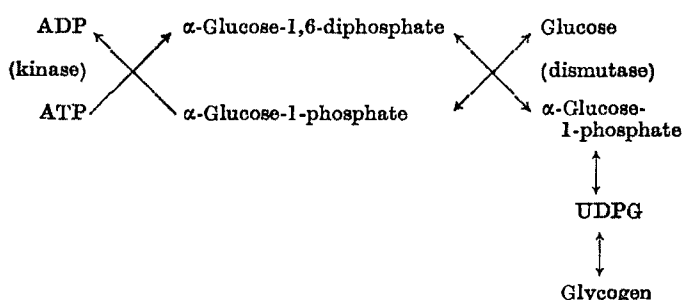
Both reactions have previously been studied in terms of their production of  $\alpha$ -glucose-1,6-diphosphate, the coenzyme of phosphoglucomutase. They may, however, serve a different purpose. Reaction (2) is reversible, and can be written as



If reactions (1) and (3) are added together we have



The whole system can be expressed as



Reaction (4) would be essentially irreversible because of the irreversibility of reaction (1). Experiments to test the hypothesis are in progress.

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## Redox Pump Theory for Active Secretion of Hydrogen Ions

PERMIT me to bring to the notice of Professor Britton Chance and Leena Mela an obvious omission from the references to their paper<sup>1</sup> on proton movement in mitochondrial membranes. Is it possible that they can discuss the release of hydrogen ions at interfaces, associated with redox processes, without mentioning Conway's redox pump theory<sup>2</sup> for the active secretion of hydrogen ions by the cells of the gastric mucosa? Conway's theory, which predates other redox theories mentioned, specifically suggests electron transfer from flavoproteins to cytochromes as being particularly favourable for the active release of hydrogen ions from the cells of the gastric mucosa. He has also presented evidence<sup>3</sup> for a similar mechanism in fermenting yeast.

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## BIOLOGY

### Pharmacodynamics of Dieldrin in Pigeons

CONSIDERABLE interest has been aroused in recent years by the presence of residues of organochlorine insecticides in the tissues of birds in Great Britain, and a comprehensive review of the situation up to 1965 has been made<sup>1</sup>. No information has been published yet on the pharmacodynamics of any of these compounds in birds. We are investigating this topic and some of our results are summarized in this report.

A group of pigeons was fed a diet containing 50 p.p.m. of the main constituent of technical dieldrin, that is, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4 $\alpha$ ,5,6,7,8,8 $\alpha$ -octahydro-1,4-endo, exo-5,8-dimethanonaphthalene (HEOD) for 6 months. The pigeons were then fed a normal diet and killed at intervals. The concentrations of HEOD in the adipose tissue, brain, liver and breast muscle of the birds were determined by gas-liquid chromatography in combination with an electron capture detector. The results of the determinations are shown graphically in Figs. 1 and 2. It is apparent that HEOD was being eliminated from the body of the pigeons, and that the concentration of HEOD in the four body tissues was decreasing in an approximately exponential manner. Further, the rates of decrease of HEOD in the four tissues were similar.

The regression lines for the relationship between the concentrations of HEOD in the tissues (transformed to their corresponding logarithms) and time of feeding on the normal diet have been calculated; these are given in Table 1 and are also given in Figs. 1 and 2. An analysis of covariance<sup>2</sup> of the transformed results shows that the four rectilinear regression lines fit the data satisfactorily (that is, the mean squares of the deviations from linearity are not significantly different from the within group mean squares) and that there is no significant departure from parallelism. Consequently the pooled slope of the regression lines can be calculated. This pooled slope is  $-0.0064$  (confidence limits,  $P=0.95$ ,  $0.0055-0.0073$ ). The concentration of HEOD in these four tissues has been decreasing in parallel and so it can be postulated that the total content of HEOD in the whole bodies of the pigeons has been declining at this rate. Consequently the change

of concentration of HEOD in the whole pigeon when exposure ceases can be represented by

$$C_t = C_0 e^{-bt}$$

where  $b$  is 0.0064 and  $C_0$  is concentration of HEOD when exposure ceased.

The biological half-life of HEOD in the pigeon can be calculated from the relationship  $t_{1/2} = (\log_{10} 2)/b$ , whence the estimated mean half-life is found to be 47.2 days (confidence limits,  $P = 0.95$ , 41.4–54.9 days).

The intercepts of the calculated regression lines give an estimate of the concentration of HEOD in the four tissues after feeding on a diet containing 50 p.p.m. of HEOD for 6 months. These estimates are given in the fourth column of Table 1. The intercepts for the four tissues are significantly different and are in the order of fat > liver > muscle > brain. Using the concentrations in the four tissues the storage ratio, concentration of HEOD in tissue/concentration of HEOD in diet, can be calculated, and these estimates of the tissue storage ratios are given in column 5 of Table 1. The concentrations of HEOD in these four tissues of living pigeons are considerable and it is noteworthy that they are much higher than those found in the tissues of the majority of dead birds which have been analysed.

Table 1. CONCENTRATIONS OF HEOD IN THE BODY TISSUES OF PIGEONS AS A FUNCTION OF TIME AFTER TERMINATION OF EXPOSURE

Tissue	Regression line $y = bt + a$	Bio- logical half-life (days)	Estimate of concentration in tissue when feeding with HEOD was termi- nated (p.p.m.)	Storage ratio
Fat	$y = -0.007359t + 3.9620$	40.9	448.6	9.0
Liver	$y = -0.008183t + 2.9354$	48.7	32.7	0.65
Muscle	$y = -0.008782t + 2.7559$	44.4	16.3	0.33
Brain	$y = -0.005269t + 2.3932$	57.1	7.7	0.15

Pigeons had been fed a diet containing 50 p.p.m. of HEOD for 6 months.  $y = \log_{10}$  (concentration in p.p.m. of HEOD in tissue  $\times 10^4$ );  $t$  = time in days after ingestion of HEOD diet ceased.

The parallel decline in the various tissues is noteworthy, because it indicates that a dynamic equilibrium exists between the concentrations of HEOD in these tissues, an equilibrium that arises, no doubt, from the circulation of HEOD in the blood. It is plausible, therefore, that

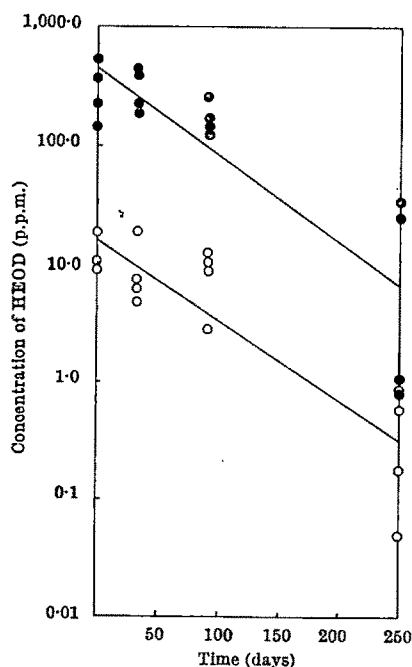


Fig. 1. The change of concentration of HEOD in the fat and muscle of pigeons, which have been fed 50 p.p.m. of HEOD for 6 months, after dietary intake of this compound had ceased. ●, Fat; ○, muscle.

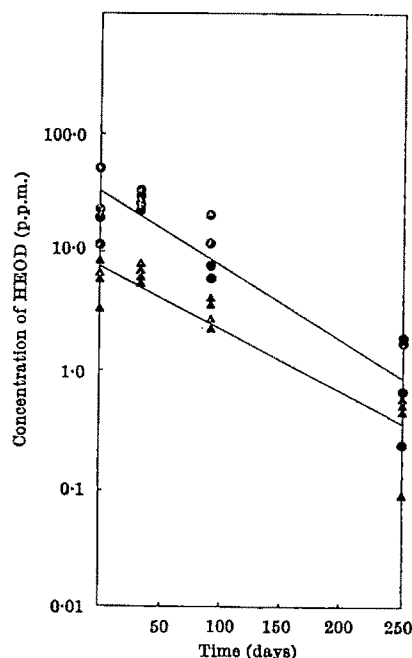


Fig. 2. The change of concentration of HEOD in the brain and liver of pigeons, which had been fed a diet containing 50 p.p.m. of HEOD for 6 months, after dietary intake of this compound had ceased. ●, Liver; ▲, brain.

the dynamics of uptake, distribution and elimination of HEOD in birds agree with the compartmental model that has been used to explain the pharmacodynamics of drugs in experimental animals and man<sup>3,4</sup>. Furthermore, an elimination mechanism of the exponential type, demonstrated for HEOD in our experimental pigeons, implies that continuous uniform exposure of pigeons (and, presumably, other bird species) will result in an equilibrium concentration in the tissues in accordance with an asymptotic relationship of the type  $C_t = C_{eq} (1 - e^{-bt})$ , where  $C_t$  is the concentration after exposure for time  $t$ ,  $C_{eq}$  is the asymptote ( $C_t \rightarrow C_{eq}$  as  $t \rightarrow \infty$ ), and  $b$  is the slope of the regression line for the elimination process when the concentrations are transformed to their corresponding logarithms. In the case of exposures that vary in a random manner over a period of time, the equilibrium concentration will vary within limits dependent on the variations of the exposure.

As in the case of mammals there will certainly be inter-specific variations in the half-life of HEOD in birds, in the ratios of concentrations of HEOD in the various tissues, and in the storage ratios for the tissues. All these factors, however, have implications of great importance in the assessment of the potential hazards to the general bird population arising from the use of dieldrin in agriculture. First, the concentrations of HEOD in any tissue may be used as an index of chronic exposure and consequent pharmacological effects. Second, the significant variations between tissues in the storage of HEOD indicate that the concept of transfer of HEOD along food chains with concomitant biological amplification requires more precise definition than has been given so far. Third, the ability to eliminate HEOD from the body is of importance in all birds, but particularly for wood-pigeons and other grain eating birds which may have seasonally varying exposures as a result of agricultural practice. Reports of increased mortality of wood-pigeons, for example, were made in the years 1956–61 and the deaths of these birds were attributed to the use of cereal seed dressed with, for example, dieldrin<sup>5</sup>. A detailed investigation of the dynamics of a wood-pigeon population in Cambridgeshire has been made by Murton and his co-workers<sup>6-8</sup> and this included an investigation of the effects of dressed cereal seed on the wood-pigeons

in 1961 (ref. 10). The breeding population in the area investigated was about 20 per cent smaller than normal in the spring of 1961 and an estimated mortality of at least 8 per cent was attributed to poisoning by toxic chemicals. The decline in the breeding population was not statistically significant, but there were other indications that the 1961 breeding population was abnormally low. The population numbers, however, had recovered in 1962 and so the effects of the apparently low breeding stock in 1961 were transitory. Samples of tissues from many of the dead birds collected in the spring of 1961 contained large amounts of HEOD and other organochlorine insecticides\* and it is highly probable that the surviving birds also had large, albeit non-fatal, body burdens of HEOD. The ability of the birds to eliminate HEOD between the breeding seasons was probably an important factor in the transitory nature of the effect of dieldrin on the population dynamics of the wood-pigeons in that area.

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## Oviposition Responses by Aphidophagous Syrphidae (Diptera)

CURRENT work on syrphid predators of aphids has demonstrated some behavioural responses of the ovipositing adult females which may be significant in relation to a general understanding of natural enemy action and also to the better use of natural enemies in the control of insect pests.

Females of aphidophagous Syrphidae oviposit close to aphids<sup>1-3</sup> and only very rarely on uninfested plants<sup>4-6</sup> when they lay single<sup>7,8</sup>, usually sterile<sup>9</sup>, eggs. I have found, however, that the age of the syrphid and the species may greatly affect oviposition behaviour. There is substantial variation in the rate of ageing, at least under caged conditions, but results for one experiment, showing a particularly marked decline in precision of response with age, are presented in Table 1, which demonstrates how the mean distance between the eggs laid by *Syrphus luniger* Mg. and the nearest aphid (*Brevicoryne brassicae* L. on brussels sprouts) increased as the syrphids aged.

This change in behaviour could be explained by a loss of discrimination by the female and/or by stimulus satiation. The loss of precision from stimulus satiation

should be reversible, whereas loss of discrimination through ageing would be irreversible.

When the stimulus (that is, the infested plants) was removed on the eighth day and replaced on the fourteenth day there was only a slight restoration of precision (Table 1). Furthermore, by the twentieth day, *S. luniger* females were laying eggs, still mostly fertile, on uninfested plants even in the presence of infested ones, and it is concluded that while there may have been some stimulus satiation, the chief cause of the reduction in precision was a loss of discrimination with age, which began immediately after the first oviposition.

A predator which is attracted to the host plant of the aphid and can oviposit in advance of infestation, or when the population of the prey is still very small, is likely to affect the number of the prey more and to be more useful in biological control than one which, however voracious, is attracted only by relatively large numbers of the prey. It may be that the failure of discrimination in ageing species of *Syrphus* directly increases their efficacy in natural control by providing increased predatory power on incipient aphid infestations on plants where the aphid stimulus would normally be inadequate to induce oviposition.

I have repeatedly observed the species *Platychirus peltatus* Mg. and *Melanostoma scalare* L. to lay fertile eggs in significant numbers on uninfested plants, apparently in response to the host plant of the aphid and not to the aphid itself. They would seem to be potentially more useful than *Syrphus* species for controlling aphids at an early stage of infestation.

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## Production of Dauer Pupae in *Bombyx mori* L.

THE average pupal period of silkworms is about 12-15 days at 25° C. When the brains of some strains are extirpated immediately after pupation, however, imaginal differentiation is not detectable for as long as 100-200 days, and these brainless pupae have been called "dauer pupae"<sup>1</sup>. The extent to which dauer pupae can be produced differs according to race and strain, but the reason for this is not known. With a cross between *J.122* and *C.115*<sup>1</sup> and the original race *C.R.*<sub>4</sub> (Morohoshi; unpublished results) the production of between 50 and 70 per cent dauer pupae was achieved by extirpation of the pupal brain.

Using silk thread, we ligatured hybrid larvae (*Bm* × *N.*<sub>24</sub>) 2 days before pupation in various ways, when the posterior parts of surviving silkworms developed into dauer pupae (Fig. 1), while the anterior parts died within 30 days of applying the ligature. Within the same race, some silkworms which had had either their brains extirpated immediately after pupation or their suboesophageal ganglion extirpated 2 days before pupation emerged as did untreated controls.

It would seem from these experiments that imaginal differentiation occurs when brain hormone is secreted in plenty, and that dauer pupae develop when the secretion is impaired. Accordingly it should be possible to produce dauer pupae in most strains by cutting off the secretion from the brain, provided the time of operation is correct.

Table 1. DECLINE IN PRECISION OF RESPONSE TO APHIDS IN OVIPOSITING FEMALES OF *Syrphus luniger* Mg.

Age of female from first oviposition (days)	Total No. of eggs laid	Percentage of eggs laid at stated distance from nearest aphid (mm)					
		0	1-5	6-10	11-15	16-30	30+
0-1	106	50.0	37.8	8.5	1.9	0.9	0.9
2-3	208	37.0	43.8	8.2	3.8	3.8	3.4
4-6	254	31.9	40.2	12.6	6.3	4.7	4.8
7-8	184	14.7	39.7	21.2	10.3	5.4	8.7
14-16	240	25.2	37.0	20.7	6.5	6.5	4.1

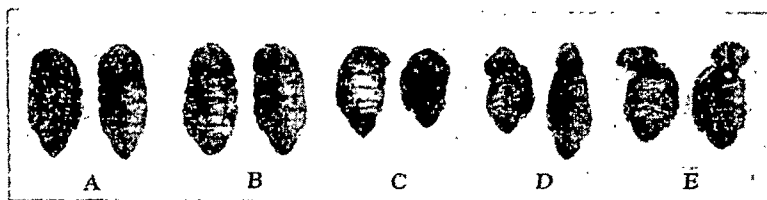


Fig. 1. "Dauer pupae" produced by ligaturing arvae of the second day before pupation in different regions: A, behind brain and before suboesophageal ganglion; B, behind suboesophageal ganglion and before prothorax; C, between pro- and mesothorax; D, between meso- and metathorax; E, between metathorax and abdomen. The photograph was taken after the "dauer pupae" had been kept at 25° C for 50 days after spinning their cocoons.

It is probable that in *Bombyx*<sup>1-3</sup> as well as in *Platy-samia*<sup>4-7</sup> the brain hormone stimulates the activation of the prothoracic gland which releases a hormone into the blood which initiates imaginal differentiation. It is possible that the strains in which dauer pupae occur by extirpation of the brain immediately after pupation, such as the hybrid between J.122 and C.115 or the original race C.R., resulted from a delay in the relative development of the pupae compared with other races.

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### Function of the Brain through Nerve Commissures on the Suboesophageal Ganglion in *Bombyx mori* L.

Fukuda and Hasegawa have verified<sup>1-3</sup> that voltinism in the silkworm is probably controlled by a hormone from the suboesophageal ganglion. Hasegawa has claimed that voltinism may be determined by the function of the suboesophageal ganglion alone, while Fukuda has assumed that it may be determined by that of the brain-suboesophageal ganglion complex at the pupal stage. It can be inferred from Fukuda's experiments that the brain controls the secretion of the suboesophageal ganglion. When the brain completely suppresses the secretion of the suboesophageal ganglion, the silkworm moths lay batches of non-diapausing eggs. When the brain accelerates the secretion of the suboesophageal ganglion, on the other hand, the moths lay batches of diapausing eggs. Fukuda concluded that the centre which controls voltinism lies in the pupal brain. Morohoshi<sup>4</sup> later recognized that inhibitory and accelerating functions of the brain are not completely absent but act only weakly.

Table 1. PRODUCTION OF DIAPAUSE EGGS BY TRANSPLANTING BOTH THE SUBOESOPHAGEAL GANGLION AND BRAIN-SUBOESOPHAGEAL GANGLION COMPLEX

No.	Recipient J.106 x Di	Donor	Race	Non-dia- pausing moths	Moths laid mixed eggs	Dia- pausing moths	Season of experi- ment
1	Control (Wounded)	J.106		26	0	0	
2	L ← H (SG)	x		25	2	1	Spring
3	L ← H (Br+SG)	Di		15	8	1	(1965)
4	Control (Wounded)		K	28	0	0	
5	L ← H (SG)	x		12	2	6	Summer
6	L ← H (Br+SG)	Di		8	1	13	(1965)
7	Control (Wounded)	J.106		33	0	0	
8	L ← L (SG)	x		24	9	3	Autumn
9	L ← L (Br+SG)	Di		13	14	3	(1965)

L, Low incubating temperature; H, high incubating temperature.

Investigations of a *Kp* strain have indicated that the brain of this silkworm displays only an accelerating function and only ever produces batches of diapausing eggs regardless of the temperature of incubation: however, it seems possible that there are ecologically and developmentally different types of bivoltine races. A cross between a bivoltine strain and the *Kp* strain produced a hybrid the *F*<sub>1</sub> offspring of which when incubated at a low temperature (17° C) always revealed batches of non-diapausing eggs but no diapausing ones. The diapausing gene of the *Kp* strain was thus recessive<sup>5</sup>.

From a genetical analysis of the *Kp* strain.

it is known that the function of the suboesophageal ganglion is controlled by a major gene which also controls bivoltinism and that the brain accelerates the function of the suboesophageal ganglion through nerve commissures. If, as a result of incubation at a low temperature, the brain of this strain were to inhibit the function of the suboesophageal ganglion, some non-diapausing eggs would be expected. No batches of non-diapausing eggs, in fact, appeared at the low temperature of incubation. This may mean that there is no inhibitory function in the brain of the *Kp* strain. The *Kp* diapausing strain produced non-diapausing eggs when its brain was extirpated immediately after pupation.

It can be seen from experiments Nos. 7-9 of Table 1 that the "non-diapausing" hosts transplanted with brain-suboesophageal ganglion complex (*Br+SG*) from "non-diapausing" donors produced more diapausing moths than did the "non-diapausing" hosts transplanted with the suboesophageal ganglion alone (*SG*). This may result because the brain from the "non-diapausing" donor does not inhibit the secretion of the suboesophageal ganglion through nerve commissures, but weakly accelerates that of the suboesophageal ganglion.

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### Oviposition of *Aedes aegypti* L. on a Dry Surface and Hygroreceptors

It is known that the mosquitoes *Culex* and *Anopheles* lay their eggs on water and that *Aedes* lays its eggs on wet surfaces. If a wet surface is absent, *Aedes* will not lay eggs<sup>1,2</sup> nor will *Culex*<sup>2</sup>. In *Anopheles*, however, some laying can be obtained on a dry surface<sup>2,3</sup>. When gravid females of *A. aegypti* were placed over a wet surface without being able to come into actual contact with it they laid a number of eggs on an interposed dry screen. An attempt was made to locate the hygroreceptors involved in oviposition.

In each of the experiments, twenty gravid *A. aegypti* were placed in a sieve of 80 mesh copper screen gauze. The sieve, with its top end covered with gauze, was placed over a Petri dish filled with wet filter paper to a thickness of about 1-1.25 cm. The filter papers were kept moist by the addition of water. The gravid mosquitoes were maintained at a temperature of 26° C and 70 per cent relative humidity for 3 days. At the end of this



period, no eggs were found on the top of the filter papers, but careful examination with a magnifying glass revealed white eggs irregularly scattered on that part of the screen which had been above the wet surface. These eggs did not darken but remained white and transparent because of the lack of contact with water. The number of eggs obtained in two such experiments was 120 and 150, that is, 6 and 7.5 eggs from each female, respectively.

Similar experiments were carried out with antennectomized (all flagellar segments removed) mosquitoes. The number of eggs obtained in six repeated tests was 102, 53, 71, 152, 71, 35—an average of four eggs from each female. Under normal conditions, the average number of eggs laid by each female on wet filter paper placed in a water container was found to be about a hundred for both intact or antennectomized mosquitoes. The only difference was that whereas intact mosquitoes laid most of their eggs (65 per cent) on the wet filter paper and the remainder on water, antennectomized mosquitoes laid most of their eggs (79 per cent) on the water and the remainder on the wet filter paper.

In two other tests, the proboscis was removed in addition to the antennae, in order to prevent any possible contact of the stylets (which are extremely fine) with the wet surface below. (It was thought that information concerning the existence of a wet surface might be obtained by means of the only organs that may come into contact with it.) Even under these conditions, thirty and ten white eggs were obtained in the two tests.

It has been shown that hygroreceptors in *A. aegypti* are located on the antennae<sup>4,5</sup>. The present experiments indicate that this mosquito possesses additional hygroreceptors on other parts of the body. They appear, however, to be far less sensitive than those located on the antennae, for Roth and Willis<sup>6</sup> have shown that when *A. aegypti* were given a choice of two relative humidities (75 per cent and 100 per cent) the lower humidity was preferred. This preference was progressively reduced when the antennal segments were removed, and was completely absent when all the flagellar segments were removed. Similarly, it has been shown<sup>6</sup> that intact female mosquitoes provided with water *ad libitum* avoided the air close to a wet surface, whereas similar antennectomized mosquitoes showed no reaction. It has also been found (unpublished results) that whereas intact mosquitoes easily locate inside their cage water contained in a jar with a 1 cm diameter hole in the centre of its cover, antennectomized mosquitoes cannot do so. When the first two flagellar segments of the antennae are retained, about 50 per cent of the mosquitoes are capable of finding the water; when the first three flagellar segments of the antennae are present the percentage rises to 90 per cent. It appears, therefore, that the hygroreceptors which are located on the body, other than on the antennae, are similar to what is known as "the common chemical sense" of insects. The receptors of the common chemical sense, the location of which is unknown, are far less sensitive to the odours than the olfactory receptors located on the antennae<sup>6</sup>.

The much smaller number of eggs laid by intact as well as antennectomized mosquitoes when prevented from coming into contact with the wet surface indicates that under normal conditions, that is, when the mosquitoes can freely contact water or wet surfaces, additional sense organs, probably moisture receptors, are involved in the process of oviposition.

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## Response of Fungi to Diurnal Temperature Extremes

REPEATED freezing and thawing of bacteria is generally considered to be lethal<sup>1,2</sup>, but *Aerobacter aerogenes* and a *Pseudomonas* species have survived and grown well in experiments simulating Martian temperature extremes of about +25° C to -75° C (ref. 3). The bacteria spent only about 4.5 h above 0° C with a maximum near 25° C in each 24 h cycle. Growth rates in liquid media were found to be comparable with the non-cycled controls held at 25° C. In similar work with desert algal-lichen soil crusts<sup>4</sup>, the algae survived repeated freezing and thawing but did not grow during the treatment. The algae were grown later on a modified Pochon solution incubated at 27° C-30° C. The diurnal temperature extremes were -79° C or -195° C for 15 h and 22° C for 9 h for periods of 1-7 days. As far as is known, no comparable work has been done on the response of fungi to diurnal temperature extremes.

This report presents the results of a preliminary investigation of the response of various fungi to diurnal temperature extremes of -94° C to 23±2° C. The fungi were grown on malt agar (BBL) and two or four agar disks 10 mm in diameter were transferred to Petri dishes containing a sterilized University of California type soil mix. Each dish contained 50 g of air dried soil mix, 5 ml. of 1 per cent sterile glucose solution, and sufficient sterile water to bring the soil moisture to about 50 per cent field capacity at the beginning of the experiment and 38 per cent at the end. The dishes were then cooled to -94° C for 19.5 h in darkness in a Revco ultra low temperature freezer, removed from the freezer, and warmed to 23±2° C for about 4.5 h. The treatment lasted for 35 days. During the warming phase the dishes were exposed to 80±10 ft. candles of cool-white fluorescent light. The control dishes were kept at room temperature and, depending on the growth rate of the fungus, hyphae traversed the entire soil surface in 1 or 2 weeks. The rates of sub-zero warming and cooling experienced by the fungus were determined by placing a 36-gauge copper-constantan calibrated thermocouple on the soil surface and recording the temperature change on a temperature recorder (Fig. 1).

The treated dishes were examined under a Bausch and Lomb 'StereoZoom' microscope (at ×30) for hyphal growth during and after the thirty-five cycle treatment. Because of the small amount of mycelium produced at the

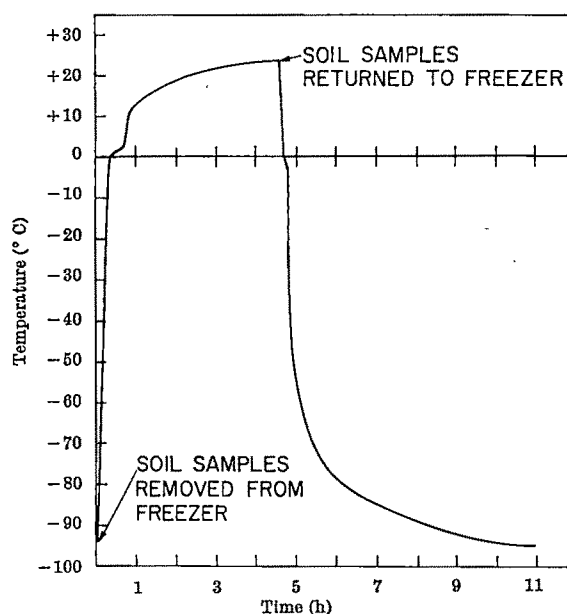


Fig. 1. Rate of temperature change of the soil mix surface during one 24 h cycle of warming and cooling. The minimum temperature is reached 11 h after the beginning of the warming and cooling phases.

end of the treatment, mycelial growth was arbitrarily measured from the edge of the agar plug to the farthest point of advance on the soil surface. It was recorded as no growth, growth less than 1 mm, or growth greater than 1 mm but less than 4 mm. Growth more than 4 mm was not observed. The results after the thirty-fifth cycle are shown in Table 1.

Table 1. RESPONSE OF VARIOUS FUNGI ON STERILE SOIL MIX TO DIURNAL TEMPERATURE EXTREMES

Organism	No growth	1 mm growth	1 mm-4 mm growth
<b>Phycomycetes</b>			
<i>Phytophthora</i> species	×		
<i>Rhizopus nigricans</i>	×		
<b>Ascomycetes</b>			
<i>Ceratocystis</i> species	*		
<i>Nectria cinnabarina</i>	*		
<i>Ophiobolus graminis</i>			×
<i>Pleospora herbarum</i>		×	
<b>Fungi imperfecti</b>			
<i>Alternaria dauci</i>			×
<i>Ascochyta pisi</i>		×	
<i>Curvularia</i> species		×	
<i>Dicoccum asperum</i>	×		
<i>Fusarium roseum</i>		×	
<i>Helminthosporium sativum</i>		×	
<i>Phoma</i> species			×
<i>Stachybotris atra</i>	×		
<i>Stemphylium ilicis</i>			×
<b>Myxomycetes†</b>			
<i>Physarum flavicomum</i>	×		
<i>Physarum gyrosum</i>	×		
<i>Physarum polycephalum</i>	×		

Growth was measured after the thirty-fifth cycle of  $-94^{\circ}\text{C}$  for 19.5 h and  $+23^{\circ}\text{C}$  for 4.5 h.

\* Organism grew after subsequent culture on potato dextrose agar incubated at  $25^{\circ}\text{C}$ .

† Subjected to one cycle only, death determined by lack of protoplasmic streaming, protoplasmic disorganization, and failure to recover after subsequent incubation at  $25^{\circ}\text{C}$ .

*Alternaria dauci* showed the greatest mycelial development of the fungi growing in excess of 1 mm but less than 4 mm (Fig. 2). Fungi that did not seem to grow during the treatment were tested for survival by incubating pieces of the agar plug at  $25^{\circ}\text{C}$  on potato-dextrose agar plates for 3 weeks. After 3 weeks incubation, the mycelia of *N. cinnabarina* and *Ceratocystis* species were well developed and did not differ morphologically from stock cultures. On the other hand, *Stachybotris atra*, *Rhizopus nigricans*, *Dicoccum asperum*, and *Phytophthora* species did not develop on potato-dextrose agar plates, and microscopic examination ( $\times 100$ ) failed to show germinating hyphae emanating from the agar plug pieces.

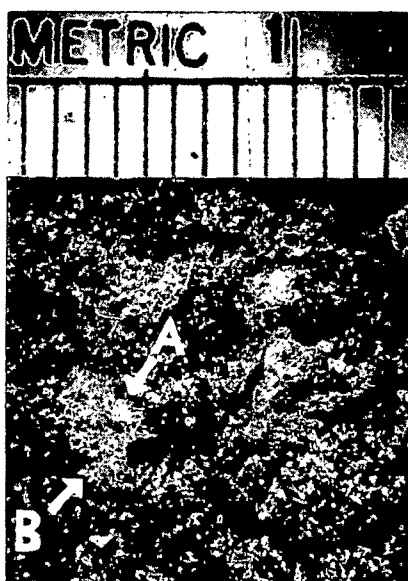


Fig. 2. Growth of *Alternaria dauci* on the soil mix surface after thirty-five diurnal warming and cooling cycles. Growth was limited but measurable. Arrow A points to the edge of the agar plug and arrow B indicates the furthest advance of the mycelium from the agar plug.

In separate experiments with slime moulds, the plasmodia of *Physarum polycephalum*, *P. gyrosum*, and *P. flavicomum* were cultured on 'Cellophane' strips and allowed to migrate over oatmeal agar overnight. The 'Cellophane' strips were then placed on the sterile soil mix surface and subjected to only one cycle. After the warming phase, the 'Cellophane' was removed from the soil mix surface and the plasmodium examined ( $\times 100$ ) for protoplasmic streaming. No movement was detected and the plasmodium appeared to be disorganized. The strips were then incubated on oatmeal agar for 1 week but the plasmodium failed to recover. These results are not surprising in view of the report<sup>5</sup> that the plasmodium of *P. polycephalum* is killed after a 5 sec exposure to only  $-1^{\circ}\text{C}$  or  $-2^{\circ}\text{C}$ .

The rates of cooling and warming experienced by the fungi (Fig. 1) were not constant and only an approximate value can be obtained. Between  $-94^{\circ}\text{C}$  and  $0^{\circ}\text{C}$  an approximate sub-zero warming rate of  $4^{\circ}\text{C}$  to  $5^{\circ}\text{C}/\text{min}$  can be calculated with a cooling rate of about  $1^{\circ}\text{C}$  to  $2^{\circ}\text{C}/\text{min}$  between  $0^{\circ}\text{C}$  and  $-40^{\circ}\text{C}$ . These rates compare favourably with the rates reported by many investigators (summarized by Mazur<sup>6</sup>) for the optimal survival of yeast cells suspended in distilled water and cooled to  $-30^{\circ}\text{C}$  or lower.

In those species of fungi that survived the exposure to diurnal temperature extremes but did not grow, growth on the agar plug may have occurred but was so limited that it was not detected. Data indicated, however, that certain fungi were able to survive and produce limited mycelial growth when exposed to simulated Martian diurnal temperature extremes. Although most of the hyphae and spores were probably killed after the first few cycles, resistant portions apparently succeeded in growing and adapting to this temperature regime.

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## Levels of Monoamine Oxidase in the Brain of C57BL/6J Mice after Exposure to Defeat

It has been widely demonstrated during the past several years that changes in brain levels of serotonin have a direct effect on changes in behaviour<sup>1-12</sup>. Norepinephrine has also been shown to be directly involved with behaviour<sup>9,13</sup>. In addition, norepinephrine has been demonstrated to be connected with the behavioural changes observed when an animal is attacking or being attacked<sup>14</sup>, and both serotonin and norepinephrine have been reported to be involved in the learning ability of mice<sup>15-17</sup>. These observations were all made after changing the brain amine levels by the administration of drugs or by electrically stimulating the brain. No work, however, has been reported to show the effect of purely behavioural manipulation on the brain amine levels.

Before the role of serotonin and norepinephrine in behaviour can be clarified, it is necessary to investigate the enzyme systems involved in their metabolism. With this in mind, an investigation of monoamine oxidase, the enzyme which oxidatively de-aminates serotonin and

norepinephrine, was undertaken to determine the effect on this enzyme of exposure to fighting aggression.

The animals used were mice of strain C57BL/6J. A colony of fighter mice was first trained according to the method of Scott<sup>18</sup> for use in fighting encounters with experimental mice.

The experimental mice were weaned and isolated at 21–25 days of age. After at least 40 days of isolation, these "naive" mice (not trained to fight) were exposed to trained fighters for 0 (unfought controls), 1, 4 or 8 five-minute periods in one day. Ten mice were each exposed at each of these periods. Twenty minutes after the last fight, the mice were killed by decapitation and the brains quickly removed; the hypothalamus, amygdala and frontal cortex were dissected out, frozen in acetone-dry ice and weighed to the nearest 0.1 mg. The three brain areas were kept frozen for future analysis.

Monoamine oxidase was assayed by a modification of the methods described by Wurtman and Axelrod<sup>19</sup> and McCaman *et al.*<sup>20</sup>. Twenty  $\mu$ l. of brain homogenate (1 g/25 ml. of water) was mixed at 0° C with 0.1 ml. of 0.8 mM 5-hydroxytryptamine-2-<sup>14</sup>C (in 0.1 M phosphate buffer of pH 7.2) and incubated at 38° C for 30 min. The reaction was stopped by the addition of 2.0 ml. of 2 N hydrochloric acid which also served as a wash to remove excess labelled substrate. The radioactive 5-hydroxy-indoleacetic acid formed in the reaction was extracted with 6 ml. ethyl acetate. Four ml. of the ethyl acetate was then transferred to a counting vial followed by 2 ml. ethyl alcohol and 10 ml. toluene scintillator solution. Each sample was run in triplicate and the enzyme units calculated by the following equation:

$$\frac{\mu\text{moles serotonin} = \text{converted/g tissue/h}}{(\text{N mmoles substrate}) (\text{counts of sample}) (2000)} \\ \frac{2/3 (\text{counts of N moles substrate}) (\text{g of sample})}$$

The results showed that monoamine oxidase exhibited different trends for each of the areas investigated (Table 1).

Table 1. EFFECT OF EXPOSURE OF NAIVE MICE TO TRAINED FIGHTERS FOR A DIFFERENT NUMBER OF 5-MIN PERIODS IN ONE DAY ON THE MONOAMINE OXIDASE ACTIVITY IN THE HYPOTHALAMUS, AMYGDALA AND FRONTAL CORTEX

Fights/day (No.)	Monoamine oxidase activity $\mu$ M serotonin/g/h		
	Hypothalamus	Amygdala	Frontal cortex
0	6.467	5.659	5.973
1	9.009	7.513	5.993
4	9.704	5.763	5.987
8	8.847	4.672	4.412

The normal value for the hypothalamus was found to be 6.467 enzyme units (one unit = the amount of enzyme contained in one g of tissue which converts one  $\mu$ mole of 5-hydroxytryptamine to one  $\mu$ mole of 5-hydroxy-indoleacetic acid in 1 h) which was higher, but not significantly, than the frontal cortex or the amygdala which had normal levels of 5.973 and 5.659 units, respectively.

After one fight, an insignificant increase was observed in the hypothalamus, to a value of 9.009 units. Four fights brought the monoamine oxidase activity of the hypothalamus to a value of 9.704 units, which was significantly ( $P < 0.05$ ) higher than normal, but not significantly different from the one-fight value. This was followed by a decrease after eight fights to a value of 8.847 units which was no longer significantly higher than normal.

The amygdala showed a significant ( $P < 0.005$ ) increase to a value of 7.513 units after one fight. The activity then returned to a normal value of 5.763 units after four fights, which was followed by a further decrease to 4.672 units after eight fights which was significantly ( $P < 0.05$ ) below normal.

No change was observed in the frontal cortex during the first four fights. After eight fights, however, a decrease below normal, to 4.412 units, was found which approached significance at the 0.05 level.

It is interesting that both the hypothalamus and amygdala show initial increases whereas the frontal

cortex is unaffected. This indicates a rapid use of the amines in the hypothalamus and amygdala. After eight fights, however, the monoamine oxidase activity of both the amygdala and frontal cortex decreased below normal while the activity in the hypothalamus remained slightly above normal. This could mean that an active mobilization of the enzyme and/or the amines was taking place from the amygdala and frontal cortex to other brain areas such as the hypothalamus. The high activity of monoamine oxidase in the hypothalamus also indicates that this is the area of greatest utilization of serotonin and norepinephrine.

It should be noted that several workers<sup>21–23</sup> have suggested that there may be different monoamine oxidases for different amines or that there may be more than one active site involved in the enzyme. If this is the case, since we used serotonin as the substrate in the enzyme analysis, the monoamine oxidase actually measured was that for serotonin.

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### Resistance to Water Transport in Plants— a Misconception Misconceived

WE agree with Professor Levitt<sup>1</sup> that there is a valid objection to "van den Honert's law" but believe that the argument he develops is fallacious.

We assume that transport of liquid water through the plant is linearly related to differences in water potential and that vapour transport across the air filled spaces of the leaf is linearly related to differences in vapour pressure. This is essentially the point Professor Levitt makes. In a steady state, the flow of water may be represented by

$$f = \frac{P_r - P_w}{R_L} = \frac{p_w - p_a}{R_V}$$

where  $P_r$  and  $P_w$  are the water potentials at the root surface and the mesophyll cells,  $p_w$  and  $p_a$  the partial pressures of water vapour above the evaporating surface in the cell wall and ambient air, and  $R_L$  and  $R_V$  the resistances to flow in the liquid and vapour phases, respectively. Professor Levitt has ensured, by expressing  $P$  and

$p$  in similar pressure units, that  $R_L$  and  $R_V$  are dimensionally similar; however, nothing is learned by comparing their magnitudes since they refer to physically dissimilar phenomena. What is required is a comparison of the effect on flow of a relative change in  $R_V$  with a similar relative change in  $R_L$ . From the relation  $V_0 dP_w = v_0 dp_w$ , where  $V_0$  and  $v_0$  are the specific volumes of liquid water and water vapour respectively,

$$\frac{R_V}{R_L} \cdot \frac{(\partial f / \partial R_V) R_L}{(\partial f / \partial R_L) R_V} = \frac{R_V}{R_L} \cdot \frac{v_0}{V_0}$$

Professor Levitt discusses an example in which  $R_V/R_L = 1/2,500$ .  $v_0/V_0 = 33,000$  (at  $30^\circ\text{C}$ ), however, and thus a change in resistance to vapour flow will alter the transpiration rate 13 times more than a similar relative change in the resistance to liquid flow. van den Honert's argument exaggerates the relative importance of  $R_V$ ; nevertheless, his conclusion remains qualitatively correct. Indeed, were it not for this and the large energy requirement for vaporization, few land plants could survive.

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### Resistance to Water Transport in Plants

DR. LEVITT<sup>1</sup> directly compares a resistance for flow of liquid water with one for gaseous diffusion of water vapour. A comparison of the resistances in the plant tissues with those in the gaseous phase is, however, only possible even as an approximation if both are expressed in terms of the same fluid. If this comparison is made in terms of water vapour we can specify, for each part of the system that is considered separately, an equivalent air path; this is a tube of air of unit cross-sectional area of such a length that, with the equivalent potential difference ( $\Delta P$ ) maintained between its two ends, it would allow the same net flow of water as that observed to pass through the system in the steady state.

For diffusion of water vapour in such a tube of air

$$\frac{P_T V}{t} = \frac{K.A.(P_1 - P_2)}{l}$$

where  $P_T$  is the total pressure (atm),  $\frac{P_T V}{t}$  is the volume ( $\text{cm}^3$ ) of water vapour calculated as at unit pressure passing through the tube in time  $t$  (sec),  $K$  is the diffusion coefficient for water vapour ( $\text{cm}^2 \text{sec}^{-1}$ ),  $A$  is the cross-sectional area ( $\text{cm}^2$ ) and  $l$  is the length (cm) of the tube and  $P_1 - P_2$  is the difference in partial pressure (atm) of aqueous vapour between the two ends. The rate of flow  $\frac{P_T V}{t}$  is thus in terms of  $\text{cm}^3 \text{sec}^{-1}$  of aqueous vapour calculated as at unit pressure and at the temperature of the experiment. The resistance is

$$\frac{(P_1 - P_2)t}{P_T V} = \frac{l}{KA} = R \text{ sec cm}^{-3} \quad (1)$$

The expression used by van den Honert<sup>2</sup> was

$$\frac{dm}{dt} = \frac{P_1 - P_0}{R_r} = \frac{P_2 - P_1}{R_x} = \frac{P_3 - P_2}{R_l} = \frac{P_4 - P_3}{R_g}$$

or for the whole system

$$\frac{dm}{dt} = \frac{P_4 - P_0}{R_r + R_x + R_l + R_g} \quad (2)$$

Here  $dm/dt$  (in unspecified units) was the rate of water transport in the steady state,  $P_1 - P_0$  to  $P_4 - P_3$  were potential differences in terms of diffusion pressure deficit (atm) and  $R_r$ ,  $R_x$ ,  $R_l$  and  $R_g$  were resistances (again in unspecified units) for the roots, xylem, leaves and gaseous phase respectively.  $dm/dt$  can be equated to  $\frac{P_T V}{t}$  and

the equivalent partial pressures of aqueous vapour (atm) can be substituted for diffusion pressure deficits, with a change of sign.

We can then use equation (1) to calculate the resistance in the plant ( $R_r + R_x + R_l$ ) with potential difference ( $P_0 - P_3$ ) and that in the gas phase ( $R_g$ ) with ( $P_3 - P_4$ ). The ratio of these resistances is obviously  $(P_3 - P_4) \div (P_0 - P_3)$ ; in the example given by Dr. Levitt this ratio is 13 : 1, whereas for the osmotic potential differences the ratio was 20 : 1. The arbitrary choice of water vapour or liquid water as the basis for comparison thus leads to different values, showing that the method is at best very approximate. The difference arises from the logarithmic relation between osmotic pressure and equivalent vapour pressure; there is, at least, no doubt as to the part of the system with the greatest resistance.

In making these calculations it must be assumed that the environment provides a heat flow to the leaves at a rate just sufficient to maintain the system isothermal, with the production of  $P_T V/t \text{ cm}^3 \text{sec}^{-1}$  of water vapour.

Although van den Honert<sup>2</sup> reached a correct qualitative conclusion as to the relative magnitude of the resistance in the plant and gaseous phase, respectively, he did not state at all clearly the implications for the survival of the plant. He suggested that control of rate of water transport in the plant could only be effective in the gaseous phase, that is, reduction of stomatal aperture, and wrote: "If, for instance, the filtration resistance of the roots is increased, it will have little effect on the rate of water transport; its only effect will be an increase of diffusion pressure deficit in the whole plant". That would, however, depend on the absolute increases in resistance that could be achieved in the two phases, and if equation (2) could be accepted it is obvious that the same absolute increase in any of the resistances would have the same effect on steady state flow. If the resistance in either phase became infinite, water transport would, of course, come to an end. The important difference is that the main effect of resistances in the liquid phase, with the exception of that for the final path from the mesophyll cell to the intercellular space system of the leaf, is to restrict the replacement of the water lost by transpiration; resistances in the gaseous phase restrict that loss. Thus, the former may be considered "harmful" resistances and the latter "protective". A large increase of resistances in the liquid phase would reduce transpiration, but the leaves could be killed and desiccated before a steady state was reached.

Whether water movement through plants obeys the Ohm's law analogy must be tested experimentally by finding whether rate of flow is directly proportional to potential difference. There is some evidence that it is not<sup>3,4</sup>, and for such a heterogeneous and non-rigid system as a plant perhaps this is not surprising.

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## PSYCHOLOGY

## Memory Enhancement by Anticholinesterase as a Function of Initial Learning

WHEN a well learned habit is clearly remembered its recall is blocked by an intracerebral injection of the anticholinesterase di-isopropyl fluorophosphate (DFP)<sup>1</sup>. When, however, the same habit is almost forgotten its memory is enhanced by treatment with the same drug<sup>2</sup>. Here we show that when a habit is only partially learned its recall is enhanced by injection of the anticholinesterase DFP, and further that the better the original learning of the habit the worse the recall of the habit after treatment with the drug.

Seventy-two male albino rats (Sprague Dawley, Holtzman strain, 400 g) were trained in a Y-maze to escape a shock by running to the lit arm of the maze as previously described<sup>1,2</sup>. The light in the correct arm was dim to make the task difficult. The rats were randomly assigned to one of six groups before initial training. The first two groups were given thirty trials of learning. The second two were given seventy trials, and the third two 110 trials. The first group of each pair was injected with the drug in the same manner and quantity as already described<sup>1,2</sup>, 5 days after initial learning. The second group of each pair was simply injected with the vehicle peanut oil, 5 days after training, again as previously described<sup>1,2</sup>. All groups were tested for recall of the habit 24 h after the injection. The difference between the number of correct choices during the last ten trials in original training and the number of correct choices during first ten trials of the post-injection test were used as an index of recall.

The three groups injected with peanut oil and trained to three different levels forgot approximately the same amount in terms of an increase in errors during the first trials of retraining (Table 1). On the other hand, the rats trained for only thirty trials and then injected with DFP showed a large and significant enhancement of recall when compared with their own performance in the last ten trials of initial training and the performance of the thirty trial control group in recall.

Table 1

Treatment of trials (DFP or peanut oil)	No. in initial learning	Learning score		Retention score		Difference score Retention - Learning	
		Mean	S.D.	Mean	S.D.	mean	S.D.
DFP	30	6.75	1.76	8.75	1.05	2.00	2.24
PO	30	8.00	2.08	6.75	1.48	-1.25	1.42
DFP	70	8.50	1.37	8.58	1.56	0.08	2.32
PO	70	8.25	1.48	7.92	1.18	-0.33	1.61
DFP	110	9.75	0.63	7.50	1.35	-2.25	0.90
PO	110	8.92	1.49	8.00	1.25	-0.92	2.02

Duncan's new multiple range test on difference scores

	30 DFP	70 DFP	110 DFP	30 C	70 C	110 C
30 DFP	—	*	**	**	**	**
70 DFP	*	—	ns	ns	ns	ns
110 DFP	**	*	—	ns	*	ns
30 C	**	ns	ns	—	—	ns
70 C	**	ns	ns	ns	—	ns
110 C	**	ns	ns	ns	ns	—

DFP, Di-isopropyl fluorophosphate; PO, peanut oil; C, control.

This enhancement of recall did not occur in the groups which had seventy or 110 trials when they were treated with DFP. Table 1 shows that the greater the number of initial trials of learning the greater the degree of forgetting after treatment with DFP and the smaller the amount of enhancement of recall with this drug. This trend is linear and statistically significant at the 0.01 level (analysis of variance). The difference in amount of forgetting displayed by the 110 trial DFP and control groups just misses significance.

An alternative test of the hypothesis in this investigation should show that there is more facilitation by DFP in animals which had learned less, and more impairment in those who had learned more within each group. Cor-

relations between learning and retention were computed within each group and the correlations were compared (Table 2). The difference in the thirty trial groups is significant and in the correct direction. In the seventy trial groups it is in the right direction. It is to be expected on purely mathematical grounds that the correlation between initial performance and retention should fall off as the initial performance approaches a perfect score for all the members of a group, because any difference in amount of initial learning cannot be mirrored by a difference in score. The present results show the same effect of facilitation as a pilot study using an easier discrimination task with 14-day-old memories.

Table 2. COMPARISON OF INTRAGROUP CORRELATIONS BETWEEN LEARNING AND RETENTION

	DFP	Control	Standard error	Z	P
30	-0.18	+0.70	0.47	2.23	<0.05
70	-0.23	+0.28	0.47	1.10	ns
110	+0.17	-0.05	0.47	0.60	ns

DFP as an anticholinesterase delays the destruction of acetylcholine. At low effective levels of acetylcholine synaptic transmission is facilitated, a fact used in the treatment of myasthenia gravis. With increasing quantities of acetylcholine facilitation turns into progressive synaptic block. The findings reported here are therefore interpreted<sup>3</sup> to show that during learning a set of synapses is stimulated to increase their capacity to emit transmitter, and that with increased learning of the same habit the capacity of these synapses is boosted further. Consequently, facilitation is seen with poorly learned habits, while progressive blocking of recall is observed with well learned habits, keeping dose of drugs and learning task constant.

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## Effects of Neonatal Amygdalectomy in the Maternally Reared and Maternally Deprived Macaque

IN the monkey, bilateral amygdalectomy results in a number of behavioural changes which have been well documented<sup>1-4</sup>. These changes consist of (1) reduction in aggressiveness and relative tameness toward man; (2) increased orality, coprophagia and the mouthing of inedible objects; (3) hypermetamorphosis; and (4) hypersexuality. While these changes may be enhanced by extending the lesion to include the temporal neocortex and hippocampus, it has been established that these behavioural alterations can be produced by ablation of the amygdaloid nuclei alone<sup>2</sup>.

This lesion produces a profound alteration in adult behaviour and so it would be of interest to examine the development of animals subjected to similar ablations at various ages. In the kitten<sup>5,6</sup>, one of us (A. J. K.) found that lesions of the amygdaloid nuclei from 2-50 days of age did not result in the behavioural changes seen after similar ablations in the adult. Growth for the first year of life was unaffected as was the time of appearance of play and aggressive behaviour. This report will deal with the effects of amygdala lesions in the monkey produced during the first week of life (Fig. 1).



Monkeys reared in social isolation display marked fear reactions, lack of socialization and bizarre stereotyped motor behaviour<sup>7</sup>. One of the prominent effects of amygdectomy is a reduction in fear responses, and so it was of major interest in this investigation to observe the effects of this lesion on the maternal deprivation syndrome.

All monkeys used in this investigation were from the genus *Macaca*—four *M. mulatta*, three *M. speciosa* and one *M. radiata*. All neonates were born in the laboratory from imported pregnant females or from laboratory matings. Of the newborns, two were returned to their mothers after operation and two were reared artificially to examine the effects of this lesion on the maternal deprivation syndrome. Similarly there were two groups of juvenile operates, two which were maternally reared and two which were artificially reared and which displayed the deprivation syndrome at the time of operation. Data were collected about the growth of the neonates, infant-maternal interaction, visual exploratory behaviour, and, in the juveniles, quantitative measures of affective changes. All lesions were made by aspiration under direct exposure. Anaesthesia was accomplished with intraperitoneal pentobarbital sodium, 12 mg/lb. body weight. In the neonates, surgery was carried out within the first five post-natal days.

Post-surgically, the monkeys were given penicillin and subcutaneous fluids. They were kept in an incubator until the following morning when two were returned to their respective mothers and two were kept in the incubator and nursed by bottle with a synthetic milk preparation. The neonates were alert and vocal from 6 to 12 h after operation. Those that were returned to their mothers were readily accepted and oriented to the nipple almost immediately. The infants displayed good grasping and sucking, and remained on the nipple in the manner of normal neonates. In all gross respects they appeared to be unaffected by the operation. In time, the infants were observed to nibble at solid food, separate themselves for progressively longer periods and at greater distances from the mother, and to respond to strangers by quickly returning to the mother's breast. This is behaviour typical of the normal infant. No lethargy or hyperactivity was noted.

Those neonates who were maternally deprived were also alert and responsive after operation. They readily nursed from the bottle and learned to climb a module and to feed on their own after a short training period. They were nibbling solids at 4–6 weeks and were totally weaned to solid food without difficulty. By 3–4 weeks of age, two maternally deprived amygdectomized monkeys showed cowering, rocking, excessive non-nutritive sucking of thumbs and feet and general withdrawal. As they grew older they would withdraw at the approach of observers, cower and rock in a corner or hide under cover. This is characteristic for normal monkeys raised under similar conditions as has been well documented<sup>7</sup>. From

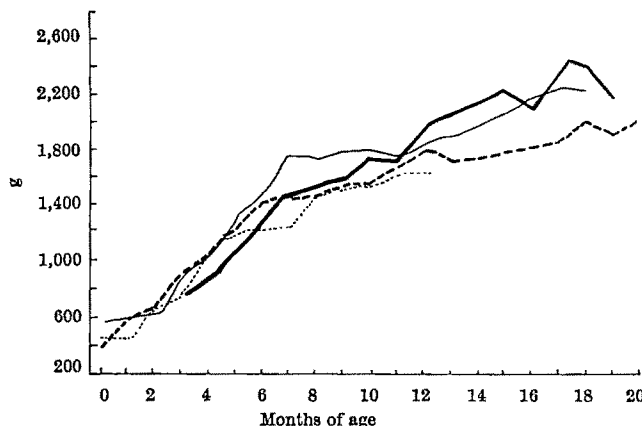


Fig. 2. Growth curves for eight infants from birth to 20 months. Each line represents two animals. ...., Amygdala lesioned, maternal deprivation; ----, amygdala lesioned, maternal reared; —, maternal deprivation; ———, maternal reared.

4 months to 1 yr the maternally reared amygdectomized monkeys remained active and alert and would nip when handled. In the rhesus, grimacing, withdrawal, piloerection, defecation, threatening postures and barking all occurred in response to the presence of observers as in the normal rhesus. There was no evidence of coprophagia, excessive mouthing, or sexual behaviour until the time of being killed at 1–2 years of age. While the *M. speciosa* juveniles were more docile than the rhesus, they likewise did not display evidence of the amygdala syndrome. Growth curves for eight females, four with amygdala lesions and four unoperated, are shown in Fig. 2. Two monkeys in each group were maternally deprived and two were mother-reared. No marked differences are seen for the first year of life, but there may be a tendency for the operates to show less weight gain after 1 yr. This group was killed before puberty, and so only the early growth phase can be demonstrated.

The marked reduction of fear responses in adult monkeys subjected to amygdectomy does not develop when the lesion is produced during the early post-natal period. Nor does it affect the marked fear and withdrawal which develop in the maternally deprived monkey. While some diminution of the deprivation syndrome occurred in a juvenile group, the subjects did not resemble the typical amygdectomized preparation (our work, in preparation). These results would suggest that (a) the effects of amygdectomy are age-dependent and may require some degree of sexual maturation before becoming manifest; (b) the lack of influence of amygdectomy on the maternal deprivation syndrome would indicate that this behaviour is mediated by sub-cortical structures, is fixed rather early in life, and is not grossly influenced by higher brain systems; and (c) growth for the first year of life and appropriate infant-maternal transactions are not grossly affected by neonatal amygdectomy.

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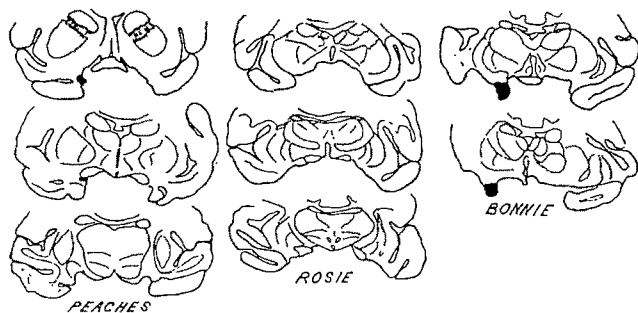


Fig. 1. Serial sections through the brains of three monkeys sustaining amygdala lesions during the first week of life. The black areas represent remaining amygdala tissue.

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## AGRICULTURE

### Influence of Algal Growth-promoting Substances on Growth, Yield and Protein Contents of Rice Plants

GERMINATION of paddy seeds is accelerated after treatments with algal hormones<sup>1,2,4</sup>. If rice seeds are treated with extracts of the *Phormidium* species before sowing, the algal hormone in the extract markedly promotes growth and development of both roots and shoots of rice seedlings<sup>3</sup>. The present investigation deals with the effect of algal hormones in extract of *Phormidium foveolarum* (Mont.) Gomont on vegetative growth, crop yield and protein content of rice seeds.

Seeds of paddy type 21 were pre-soaked for 24 h in 0.5, 1 and 5 per cent water and ether extract of *Phormidium foveolarum* suspended in water. Seeds soaked in distilled water served as a control. The method of hormone extraction and pre-soaking has been described in another communication (Gupta and Shukla, in the press). Immediately after soaking, seeds were sown in earthen pots filled with standardized garden soil. Four plants per pot were sown and raised to maturity. Observations were recorded fortnightly.

The beneficial effect was maintained throughout the duration of the crop.

Ether extract suspended in water also exercised a beneficial effect on vegetative growth of plants. The maximum increase in height of plants and length of leaves was observed in plants treated with 1 per cent algal extract, while number of leaves, breadth of leaves and numbers of tillers was markedly better in 5 per cent ether extract. The difference in 1 and 5 per cent treatment, however, was on the whole slight.

Observations on the dry weight of the plant (straw), total weight of all ears per plant, weight of a single ear and weight of 1,000 grains are recorded in Table 2. The data suggest that in the case of water extract the best results are obtained with 5 per cent while in ether extract suspended in water 1 per cent is most effective. The protein content of seeds was also increased in 1 per cent ether extract suspended in water and 5 per cent water extract.

The results indicate that the treatment with algal extract containing hormones exercises a marked beneficial effect on the growth and development of rice plants and the yield is increased significantly. Apart from the better yield the quality of the grains is also improved because they are richer in proteins (Table 3).

Table 1. EFFECT OF PRE-SOAKING SEEDS IN EXTRACTS OF *Phormidium foveolarum* ON VEGETATIVE GROWTH AND DEVELOPMENT OF RICE PLANTS\*  
(All figures quoted below give average of 14-22 plants)

Age of seedlings	Height (cm)		No. of tillers		No. of leaves		Total length of leaves (cm)		Total breadth of leaves (cm)	
	†C	5 per cent	C	5 per cent	C	5 per cent	C	5 per cent	C	5 per cent
15 days	31.34	41.5	0	2.78	4.25	9.5	90.22	181.52	2.44	6.14
30 days	61.62	77.57	1.93	9.5	8.00	28.78	219.75	848.7	5.95	34.6
45 days	84.6	101.5	5.25	12.14	14.12	43.9	403.5	1,438.6	9.05	53.6
60 days	85.87	131.5	5.6	12.14	14.12	43.9	403.5	1,438.6	9.05	53.6
	Critical difference = 13.90		Critical difference = 0.5792		Critical difference = 8.02		Critical difference = 553.08		Critical difference = 15.48	
	Difference		Difference		Difference		Difference		Difference	
	5 per cent - C = 22.16		5 per cent - C = 5.95		5 per cent - C = 20.88		5 per cent - C = 697.9		5 per cent - C = 30.36	
	Water extract		Ether extract suspended in water							
	C	1 per cent	C	5 per cent	C	5 per cent	C	1 per cent	C	5 per cent
15 days	31.34	43.4	0	0.88	4.25	6.25	90.22	150.4	2.44	3.4
30 days	61.62	76.2	1.93	5.72	8.0	17.55	219.75	506.5	5.95	15.94
45 days	84.60	95.5	5.25	7.1	14.12	21.4	403.5	674.9	9.04	19.43
60 days	85.87	126.7	5.6	7.2	14.2	21.4	403.5	674.9	9.04	19.43
	Critical difference = 13.234		Critical difference = 1.314		Critical difference = 3.787		Critical difference = 144.463		Critical difference = 1.6487	
	Difference		Difference		Difference		Difference		Difference	
	1 per cent - C = 19.60		5 per cent - C = 2.03		5 per cent - C = 5.53		1 per cent - C = 246.93		5 per cent - C = 7.94	

\* All figures quoted are averages of results from 14-22 plants.

† Control.

Table 2. EFFECT OF PRE-SOAKING SEEDS IN EXTRACTS OF *Phormidium foveolarum* ON VEGETATIVE GROWTH AND YIELD OF RICE PLANTS

Extract	Vegetative dry weight of plant (g)				Total weight of all ears (g)				Weight of single ear (g)				Weight of 1,000 seeds (g)			
	Con- trol	0.5 per cent	1 per cent	5 per cent	Con- trol	0.5 per cent	1 per cent	5 per cent	Con- trol	0.5 per cent	1 per cent	5 per cent	Con- trol	0.5 per cent	1 per cent	5 per cent
Ether extract sus- pended in water	4.08	5.9	5.9	8.0	3.6	7.4	7.7	6.4	1.1	2.1	2.2	1.8	1.7212	1.8716	2.5832	1.7280
Water extract	4.08	4.7	5.0	17.6	3.6	3.6	4.0	18.7	1.1	1.5	1.7	2.3	1.7272	2.0026	2.0026	2.5834

Table 3. EFFECT OF PRE-SOAKING SEEDS IN EXTRACTS OF *Phormidium foveolarum* ON PROTEIN CONTENT OF RICE PLANTS

Extract	Control		0.5 per cent		1 per cent		5 per cent	
	Total nitrogen (per cent)	Total protein (per cent)	Total nitrogen (per cent)	Total protein (per cent)	Total nitrogen (per cent)	Total protein (per cent)	Total nitrogen (per cent)	Total protein (per cent)
Ether suspended in water	1.75	10.93	1.61	10.05	1.81	11.31	1.5	9.75
Water extract	1.75	10.93	2.08	13.0	2.83	17.68	3.67	22.83

After harvesting the matured plants, the weight of straw, the weight of ears per plant, the average weight of a single ear and the weight of 1,000 seeds were recorded. Nitrogen contents of the seeds were estimated by Kjeldahl's standard micro method and their protein contents were calculated<sup>5</sup>.

Observations were made on all seeds which germinated within 96 h and the results from 14-22 replicates in various treatments were averaged. The results of the beneficial effect of concentrations of water extract and ether extract suspended in water are recorded in Table 1. The data show that the height of the plant, the number of tillers, the number of leaves, the length and the breadth of leaves increased considerably following treatment with 5 per cent water extract as compared with the control.

A comparison of treatments with 1 per cent ether extract suspended in water and 5 per cent water extract shows that both yield and protein content are very significantly increased in the treatment with 5 per cent water extract.

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<sup>1</sup> Gupta, A. B., *L. J. Sci. and Tech. Ind.*, 2 (1), 84 (1964).

<sup>2</sup> Gupta, A. B., and Lata, K., *Hydrobiologia*, 24, 430 (1964).

<sup>3</sup> Gupta, A. B., and Shukla, A. C., *L. J. Sci. and Tech. Ind.*, 2 (3), 404 (1964).  
Garrard, A., *New Phytol.*, 53, 165 (1954).

<sup>4</sup> Gupta, A. B., *Hydrobiologia*, 28, 213 (1966).

<sup>5</sup> Sondheimer, E., in *Org. Const. High. Pl.*, 205 (Trever Robinson, 1963).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 20

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 9.30 a.m.—Colloquium on "Message Switching".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 2 p.m.—Colloquium on "Precision Resistance Standards".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Dr. K. R. Sealey: "The Siting and Development of British Airports".

UNIVERSITY OF LONDON (in the Engineering Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. G. Kendall, F.R.S.: "Delphic Semi-Groups".\*

## Monday, February 20—Tuesday, February 21

INSTITUTION OF ELECTRICAL ENGINEERS (joint symposium with the I.C.E. and the I.Mech.E., at the Institution of Civil Engineers, Great George Street, London, S.W.1)—Symposium on "Area Control of Road Traffic".

## Tuesday, February 21

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.20 p.m.—Dr. D. J. B. White: "Blakeney Point (Norfolk) Nature Reserve".\*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. M. J. Batkinson, Mrs. S. Day, Prof. N. Mullineux, Mr. K. C. Parton and Mr. J. R. Reed: "Calculation of Switching Phenomena in Power Systems"; Mr. J. P. Blekford and Mr. P. S. Doepel: "Calculation of Switching Transients with particular reference to Line Energisation".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the I.Mech.E. and Society of Instrument Technology, at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. J. Redding and Mr. L. C. Towle: "Barrier Method of Ensuring the Safety of Electrical Circuits in Explosive Atmospheres".

PLASTICS INSTITUTE, LONDON SECTION (at the Kensington Close Hotel, Wright's Lane, London, W.8), at 5.30 p.m.—Mr. J. Morrish and Mr. J. E. Weaver: "Cost Accounting and the Fabricator".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. H. O. J. Collier: "Humoral Factors in Bronchial Constriction" (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. Paul Fraise (Paris): "The Speed of Perception". (Further lectures on February 22 and 24).\*

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (in Lecture Theatre C, Chemistry Department, Imperial College, London, S.W.7), at 7 p.m.—Dr. D. W. Turner: "Molecular Photoelectron Spectroscopy and the Investigation of Molecular Orbital Energy Levels".

## Wednesday, February 22

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (joint meeting with the Food Group, at the School of Pharmacy, Brunswick Square, London, W.C.1), at 2.30 p.m.—Meeting on "The Effects on Microbiological Condition of Minor Ingredients in Foods".

ROYAL STATISTICAL SOCIETY (in the Gustave Tuck Lecture Theatre, University College London, Gower Street, London, W.C.1), at 5 p.m.—Prof. E. J. Williams (University of Melbourne): "The Analysis of Association among Many Variates".

MENTAL HEALTH RESEARCH FUND (at the Edward Lewis Theatre, Middlesex Hospital Medical School, Cleveland Street, London, W.1), at 5.30 p.m.—Dr. Heinz F. R. Prechtl (University of Groningen): "Neurological and Behavioural Sequelae of Pre- and Perinatal Brain Damage".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Prof. N. M. Hancock: "The Cellular Basis of Bone Modelling". (First of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation).\*

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the I.E.R.E. Medical and Biological Electronics Group, at the I.E.R.E., 9 Bedford Square, London, W.C.1), at 6 p.m.—Meeting on "Electrodes for Long-Term Physiological Measurements".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. P. L. Mothersole, Mr. D. S. Hobbs and Mr. D. J. King: "A Dual Standard Colour Television Receiver".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Maurice Goldsmith: "The Science of Science".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Department of Mechanical Engineering, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Discussion on "Desiccation—Are We Too Generous?" led by Mr. D. C. Allen.

## Thursday, February 23

BRITISH COMPUTER SOCIETY (at the City University, St. John Street, London, E.C.1)—Symposium on "Reduction of Costs in Distribution".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. C. J. Dean, Mr. P. Feldschreiber, Mr. L. D. G. Hamilton, Mr. J. T. Lett, Mr. J. G. Little and Miss Kathleen Steele: "The Repair of X-ray Damage to the Deoxyribonucleic Acid in *Micrococcus radiodurans*".

ASSOCIATION FOR SCIENCE EDUCATION (at Goldsmith's College, New Cross, London, S.E.14), at 5.15 p.m.—Meeting on "Experimental Closed Circuit Television in Science Teaching" introduced by Mr. T. Gibson.

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Climatic Trends and their Hydrological Consequences" introduced by Mr. H. H. Lamb.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion meeting on "The Manley-Rowe Relations" opened by Prof. J. Brown.

UNIVERSITY OF LONDON (at Senate House, London, W.C.1), at 5.30 p.m.—Prof. Sir Hans Krebs, F.R.S.: "Biological Concepts in Biology" (The John Coffin Memorial Lecture).\*

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. A. E. R. Thomson: "Studies on Human Peripheral Blood Lymphocyte Populations". (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (in the Engineering Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. G. Kendall, F.R.S.: "Renewal Sequences and Their Arithmetic".\*

## Friday, February 24

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Mr. G. Shaw: "Photochemistry in Polymer Media".

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "Thermal Balance in the Ionosphere". Chairman: Prof. D. R. Bates, F.R.S.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. M. R. Harris and Mr. W. Z. Fam: "Analysis and Measurement of Radial Power Flow in Machine Airgaps".

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Charlotte Auerbach, F.R.S.: "Mutagenesis Research in Edinburgh. I, Delayed and Unstable Mutations".\*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. C. A. Coulson, F.R.S.: "Science and Religion".

## Friday, February 24—Saturday, February 25

UNIVERSITY OF BRADFORD, POSTGRADUATE SCHOOL OF STUDIES IN POLYMER SCIENCE (at the University)—Symposium on "Structural Analysis of Polymers".

## Saturday, February 25

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Adrian Digby: "Daily Life and History of the Aztecs".\*

## Monday, February 27

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Prof. G. A. Barnard: "The Bayesian Controversy in Statistical Inference".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. B. M. Weedy and Mr. J. P. Perkins: "Steady-State Thermal Analysis of a 400 kV-Cable Through Joint"; Dr. B. M. Weedy: "Thermal Aspects of Changes in the Environment of Underground Cables".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. R. Pollard: "Storage Systems for Telephone Switching".

UNIVERSITY OF LONDON (in the Engineering Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. G. Kendall, F.R.S.: "The Arithmetic of the Kingman Semi-Group".\*

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Charlotte Auerbach, F.R.S.: "Mutagenesis Research in Edinburgh. II, Mutagen Specificity".

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 6.15 p.m.—Dr. R. Gold and Mr. M. Paneth: "Management of Pulmonary Embolism".\*

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Meeting on "The Automotive Industry—Are Reinforced Plastics Making Progress?"

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with two years experience in general pathology, including morbid anatomy) in FORENSIC MEDICINE—The Secretary, The London Hospital Medical College (University of London), Turner Street, London, E.1 (February 21).

ASSISTANT LECTURER (with an honours degree and qualified in formal logic) in PHILOSOPHY—The Registrar, University of Warwick, Coventry, Warwickshire (February 23).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (below the age of 30 with a Ph.D. degree or equivalent research experience) in APPLIED MATHEMATICS, BIOLOGICAL SCIENCES, CHEMISTRY, ENGINEERING AND TECHNOLOGY, AND PHYSICS—The Academic Registrar, The City University, St. John Street, London, E.C.1 (February 24).

LECTURER in the DEPARTMENT OF INORGANIC CHEMISTRY—The Registrar, The University, Newcastle upon Tyne 2 (February 25).

LECTURER in the DEPARTMENT OF PHYSIOLOGY—The Secretary, The University, Aberdeen (February 25).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Hull (February 25).

POSTDOCTORAL FELLOW in PHYSICAL CHEMISTRY—The Registrar, The University, Leeds, 2 (February 25).

POSTDOCTORAL FELLOW (Theoretical Physicist) to work on the electronic properties associated with dislocations in crystals—Dr. D. Pugh, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, C.1 (February 25).

RESEARCH ASSISTANT in the SUB-DEPARTMENT OF COMPUTATION in the DEPARTMENT OF MATHEMATICS—The Registrar, The University, Hull (February 25).

ASSISTANT LECTURER in ORGANIC or INORGANIC CHEMISTRY—The Registrar, The University, Leicester (February 28).

LECTURER in LOGIC and METAPHYSICS in St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (February 28).

LECTURERS in both THEORETICAL AND EXPERIMENTAL PHYSICS—The Registrar, University of Warwick, Coventry, Warwickshire (February 28).

SENIOR LECTURER/LECTURER (preferably with postgraduate experience in the fields of entomology (other than taxonomy), mammalogy and wild life management and comparative physiology) in the DEPARTMENT OF ZOOLOGY, University of Ghana—The Assistant Registrar, Universities of Ghana Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (February 28).

POSTDOCTORAL RESEARCH FELLOW in the DEPARTMENT OF PHYSICS in the field of theoretical solid state physics, with particular reference to magnetooptical phenomena—The Deputy College Secretary, Westfield College (University of London), Kidderpore Avenue, Hampstead, London, N.W.3 (March 3).

SENIOR LECTURER IN VETERINARY PATHOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, March 3).

AGRICULTURIST (with a degree or diploma in agriculture) to organize field experiments using small plots—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/97 (March 4).

LECTURER/ASSISTANT LECTURER in BIOLOGICAL CHEMISTRY—The Secretary, The University, Aberdeen (March 4).

LECTURER in VETERINARY PATHOLOGY—The Secretary of the University Court, The University, Glasgow (March 4).

CHAIR OF PLANT PHYSIOLOGY—The Registrar, The University, Nottingham (March 6).

LIBRARIAN/MAP CURATOR (fully or partly qualified librarian with some experience or interest in map collections) in the DEPARTMENT OF GEOGRAPHY—The Secretary, The University, Southampton (March 6).

READER in MATHEMATICS at Royal Holloway College—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 7).

ASSISTANT LECTURER or LECTURER (with particular interests in economic geology or in fields related to engineering geology) in APPLIED GEOLOGY—The Registrar, The University, Sheffield (March 10).

LECTURER in HEAT TRANSFER and FLUID DYNAMICS in the DEPARTMENT OF NUCLEAR ENGINEERING (applicants need not necessarily have a nuclear engineering background)—The Registrar, Queen Mary's College (University of London), Mile End Road, London, E.1 (March 10).

OFFICIAL TUTORIAL FELLOW in PURE MATHEMATICS—The Secretary, Balliol College, Oxford (March 11).

ASSISTANT LECTURER (preferably with research experience in physiology, experimental parasitology or behaviour) in ZOOLOGY—The Registrar, The University, Nottingham (March 13).

CHAIR OF BIOCHEMISTRY—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11 (March 17).

PROFESSOR OF APPLIED OPTICS in the DEPARTMENT OF APPLIED PHYSICAL SCIENCES—The Registrar (Room 39, O.R.B.), The University, Reading (March 18).

LECTURER (man or woman graduate) in BOTANY—The Registrar, University Senate House, Bristol, 2 (March 20).

LECTURER or ASSISTANT LECTURER (with qualifications in comparative physiology) in ZOOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (March 20).

LECTURER/SENIOR LECTURER in PARASITOLOGY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 24).

READER (with research interests in any branch of chemical engineering, high academic qualifications and substantial research accomplishments) in CHEMICAL ENGINEERING—The Secretary, Royal College of Advanced Technology, Salford 5, Lancs, quoting Ref. CE/1 (March 27).

KEEPER (with an appropriate university degree and preferably museum experience) of ZOOLOGY—The Secretary, National Museum of Wales, Cardiff (March 29).

LECTURER or ASSISTANT LECTURER in INORGANIC CHEMISTRY; and a LECTURER or ASSISTANT LECTURER in PHYSICS at the University of Botswana, Lesotho and Swaziland—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 31).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT OF ANIMAL GENETICS—The Secretary, University College London, Gower Street, London, W.C.1 (March 31).

PROFESSOR and HEAD of the DEPARTMENT OF BOTANY, Makerere University College, University of East Africa—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 31).

WAYNEFLETE PROFESSOR OF METAPHYSICAL PHILOSOPHY—The Registrar, University Registry, Oxford (April 8).

SEDLERIAN PROFESSOR OF NATURAL PHILOSOPHY—The Registrar, University Registry, Oxford (April 22).

ENTOMOLOGIST (national of the United Kingdom or Republic of Ireland, with a Bachelor's degree in biological sciences and some tropical experience, preferably on malaria) in the British Solomon Islands, to assist with the Malaria Eradication Project—The Ministry of Overseas Development, Room 403, Eland House, Stag Place, London, S.W.1.

LECTURER (with research interests in topics such as numerical analysis, mathematical statistics and probability, continuum mechanics) in APPLIED MATHEMATICS—The Registrar (Room 39, O.R.B.), The University, Reading, quoting Ref. AM.2.

MASTER to teach CHEMISTRY to Open Award standard—The Master, Dulwich College, London, S.E.21.

Beaches in Britain. By Prof. C. Kidson. (An Inaugural Lecture delivered at the University College of Wales, Aberystwyth.) Pp. 19. (Cardiff: University of Wales Press, 1966.) [2411]

Office of Health Economics. Disorders Which Shorten Life: A Review of Mortality Trends for those between the Ages of 15 and 44. Pp. 33. (London: Office of Health Economics, 1966.) 2s. 6d. [2411]

Television for Children. By Doreen Stephens. (B.B.C. Lunch-time Lectures, Fifth Series—1. Pp. 18. (London: British Broadcasting Corporation, 1966.) [2411]

Causes of the Slow Rate of Economic Growth of the United Kingdom. By Prof. Nicholas Kaldor. (Inaugural Lecture.) Pp. 40. (London: Cambridge University Press, 1966.) 5s. net. [2411]

### Other Countries

Astro-Archaeology. By Gerald S. Hawkins. (Research in Space Science. Special Report, No. 226.) Pp. vii + 65. (Cambridge, Mass.: Smithsonian Institution, Astrophysical Observatory, 1966.) [2411]

Tulane University Computer Science Series. Monograph No. 3: Tulane Information Processing System, Version 2 including Meditran—System Manual. By Ronald H. Dreyfus, Wilson J. Nettleton, Jr., and James W. Sweeney. Pp. vi + 45. (New Orleans Louisiana: Tulane University, Division of Medical Computing Sciences, 1966.) [2511]

Metropolitan Life Insurance Company. *Statistical Bulletin*, Vol. 47 (September 1966): Fatal Firearm Accidents in the United States. Hearing Impairment in the United States. Survival and the Life Cycle. Leukemia Mortality Leveling Off. Pp. 12. (New York: Metropolitan Life Insurance Company, 1966.) [2511]

American Geographical Society. Serial Atlas of the Marine Environment. Folio 12: Distribution of Decapod Crustacea in the Northwest Atlantic. Pp. 4 + 4 plates. (New York: American Geographical Society, 1966.) [2511]

Control Procedures in Drug Production. (Proceedings of a Seminar, July 17-22, 1966, sponsored by The University of Wisconsin Extension Services in Pharmacy and the School of Pharmacy, in co-operation with Pharmaceutical Manufacturers Association and the Food and Drug Administration, U.S. Department of Health, Education and Welfare.) Edited by William L. Blockstein. Pp. 207. (Madison, Wisconsin: University of Wisconsin, 1966.) 32. [2811]

Suomen Geodeettinen Laitoksen Julkaisuja—Veröffentlichungen des Finnischen Geodätischen Instituts. No. 62: Horizontal Angles in the First Order Triangulation of Finland in 1920-1962. By Jorma Korhonen. Pp. 115. (Helsinki: Geodeettinen Laitos, 1966.) [2811]

Instituto Antártico Argentino. Contribucion No. 95: Algunos Aspectos de la Investigacion Espacial en las Regiones Polares. Por Otto Schneider. Pp. 21. (Buenos Aires: Instituto Antártico Argentino, 1965.) [2811]

Pubblicazioni della Università degli Studi di Firenze—Facoltà di Scienze Matematiche, Fisiche e Naturali. Fascicolo No. 82: Mappa del Focculi Solari in Radiazione K del Ca 11 per Gli Anni Internazionali di Quietudine Solare (IQSY), 1964-1965. 1—Anno 1964. By Giovanni Godoli. Pp. 187. Fascicolo 83: Catalogo delle Surges Solari al Bordo (Bright Surges on Limb, BSL), Osservate ad Arcetri Durante il Periodo 1959-1964. By Giovanni Godoli, Fabrizio Mazzucconi e Giancarlo Noel. Pp. 83. Fascicolo 84: Osservazioni Esecutive all'Osservatorio Astrofisico di Arcetri Durante l'Anno 1965. Pp. 87. (Firenze: Università degli Studi di Firenze, Facoltà di Scienze Matematiche, Fisiche e Naturali, 1966.) [2911]

National Research Council of Canada. NRC No. 9017: Review 1966—Fifty Years of Science. Pp. xxii + 310. (Ottawa: National Research Council of Canada, 1966.) 32. [2911]

Svenska Linné-Sällskapet Årsskrift, Årgång 48, 1965. Pp. 101. (Uppsala: Svenska Linné-Sällskapet, 1966.) 20 Sw.kr. [2911]

Australian Atomic Energy Commission. Fourteenth Annual Report, 1965-66. Pp. 104. (Coogee, N.S.W.: Australian Atomic Energy Commission, 1966.) [2911]

New South Wales: Department of Agriculture. Contributions from the New South Wales National Herbarium. Flora Series, Nos. 30-31: Flora of New South Wales by Various Botanists. Produced under the direction of K. Mair. No. 30: Pontederiaceae. By O. D. Evans. No. 31: Phyllodraceae. By O. D. Evans and L. A. S. Johnson. Pp. 5. (Sydney: Government Printer, 1966.) [2911]

Australia: Department of National Development. Atlas of Australian Resources—Second Series. Mineral Deposits. Second edition. (Canberra: Resources Information and Development Branch, Department of National Development, 1965.) [2911]

De Beers Industrial Diamond Division: Diamond Research Laboratory. The Grinding Centre—Descriptive Brochure. Pp. 8. (Johannesburg: Diamond Research Laboratory, 1966. Available from the Industrial Diamond Information Bureau, London.) [112]

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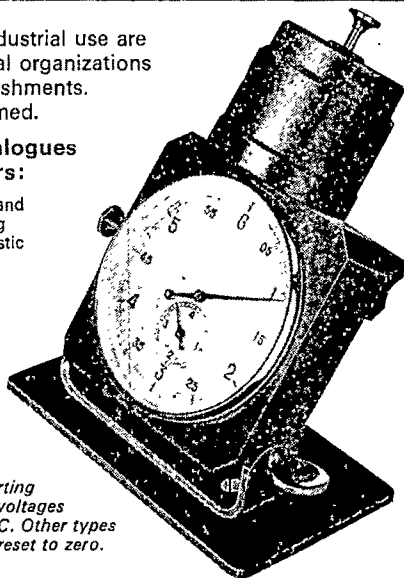
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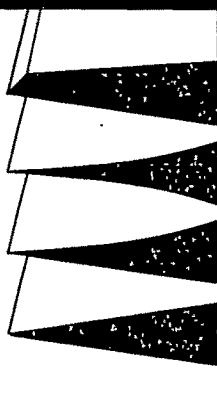
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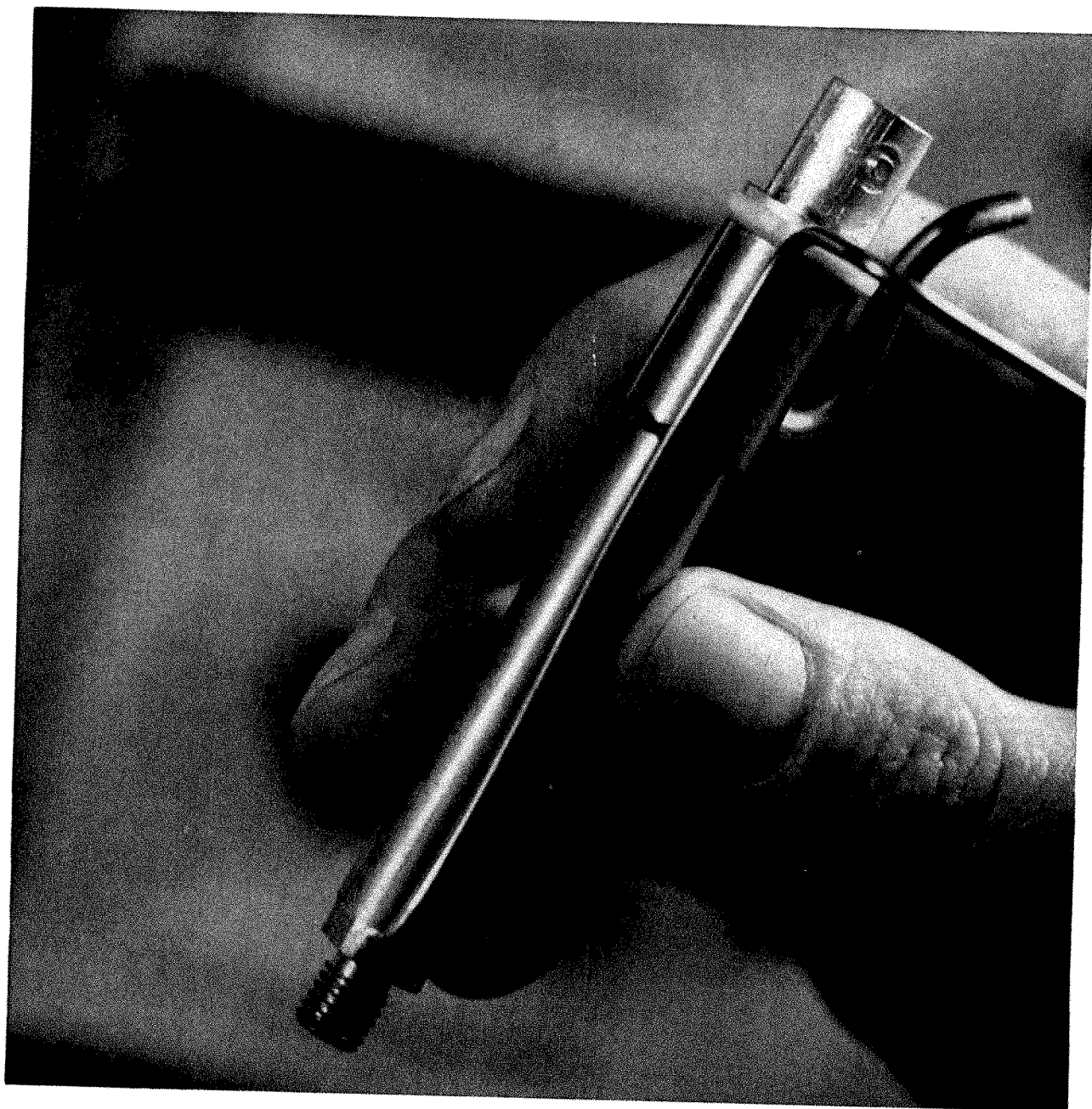
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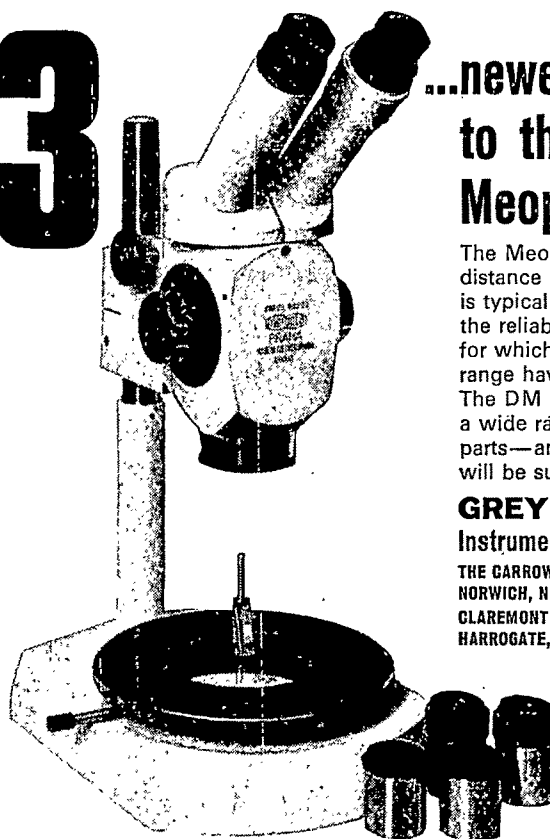
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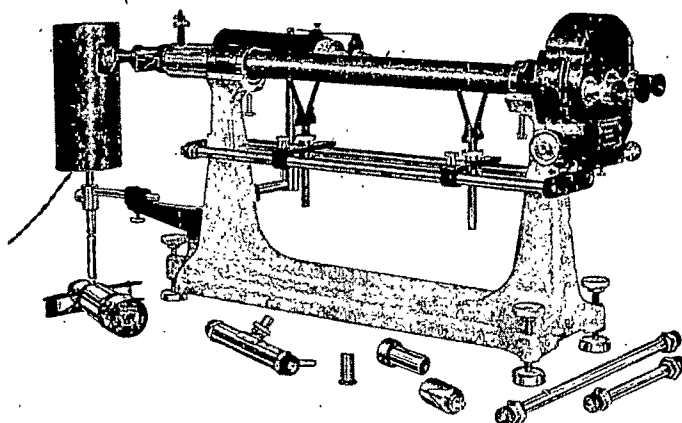
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## NEW BROOM, SWEEP CLEAN

THE address by Professor P. M. S. Blackett to the Parliamentary and Scientific Committee was a courageous exercise. It is not every day that a distinguished public servant says in public what he really thinks. It is true that Professor Blackett is President of the Royal Society as well as the chief adviser at the Ministry of Technology, but even holders of that powerful office have usually chosen to "work behind the scenes" as the saying goes. If Professor Blackett goes on like this, he may easily persuade others who give advice to governments without becoming civil servants that they do not ever afterwards have to bite off their tongues, which would help enormously to improve the quality of public life. And he will find that for every Christmas card list from which he disappears, there are a dozen people willing to listen to old arguments for the first time. One of the delicate ironies of his address last week is that it will have won him (and the minister to whom he gives advice) friends and admirers in circles ordinarily moved by the deepest suspicion of what he is about.

What Professor Blackett had to say about the mergers of industrial enterprises is of course a central part of the new doctrine of the Ministry of Technology. The chief interest in what he had to say last week, however, is the seemingly innocent confession that the British Government may have "taken the wrong road" immediately after the war by putting too high a proportion of its research and development work with government establishments specifically set up for the purpose. It would have been better, the argument goes, if British industry had been more intimately involved. For one thing, manufacturing industry would have been given more direct experience—much of it undoubtedly painful—of creative innovation. For another, there would now have been a better distribution of the technically skilled labour force, with more people in industrial positions where they can contribute directly to the health of the British economy. Certainly, the skilled labour force would now have been more flexible. But if there have been mistakes, it may not be too late to put them right. The inference from what Professor Blackett has been saying is that the Ministry of Technology will now be doing everything it can to rectify an unbalanced situation, and to transfer more people and more work to industry. To begin with, of course, the ministry may find that it is itself its own worst enemy, for there are still many who think that it is possible to run an industry from an office. But, in the long run, it will find that the policy towards which it is now moving is not merely sensible but successful.

With industrial research, in any case, diagnosis may well turn out to be easier than cure. The trouble is

not that the government establishments have grown too big, but that they have grown into institutions. According to the most recent triennial survey of the employment of scientists and technologists, government agencies employed 32,000 qualified scientists and engineers in 1965, or just about one-seventh of those actively at work in Britain. The skilled labour force in government employ is more than a quarter of that at work in industry, although the proportion is declining slowly. The proportion is very much the same in Britain as in the United States, where 14 per cent of all qualified people were working for governmental organizations in 1964. In the circumstances, it would obviously be wrong if the British Government now promptly set out to sack a substantial number of the scientists and engineers on its payroll. It is true that there should be a much more easy flow of talent from the government establishments to industry and the universities—and in the other direction as well—but what really needs to be accomplished is a change in the conception of how a government should seek to stimulate industrial innovation. In the past two decades, there has been a tendency in Britain to believe that it is at once cheaper and more effective to carry out research and development centrally, for this tends to eliminate duplication and to concentrate ability in one team. The difficulty, of course, is that of making sure that research and development of this kind is sufficiently in touch with economic reality. In any case, research and development is not an end in itself, but often a means by which industrial companies learn to make full use of new techniques. In the circumstances, it will be entirely welcome if the British Government now resolves to carry out more research and development by letting contracts to laboratories outside its own organization. It is easy enough to imagine that by holding out some promise that this may happen, Professor Blackett will have made himself some unexpected friends.

How, then, should the British Government decide to place its money? Obviously the Civil Service must continue to shoulder a great part of the responsibility of deciding what kinds of developments should be encouraged. The government has done something to help in this direction by setting up planning groups at Harwell and Malvern, but there is obviously much more that can be done. It is also worth asking whether some government laboratories which at present exist to provide services to industry might not now be happier if they were autonomous organizations able to act more quickly than is at present easy. There remains the problem of how the Government can best stimulate industrial improvement. Professor Blackett is strong on the importance of the structure of industry, and

right to point to the anomalies which spring from the great fragmentation of many industries. But simply to arrange for mergers will not guarantee a cure. In exactly the same way, research and development by itself is not a sufficient condition for industrial prosperity. It is also necessary to arrange that industry has some attainable goal towards which to work. In fostering the development of computers, for example, the British Government would be well advised to spend at least as much energy (and possibly more money) on the encouragement of use as on the development of innovation. And the best way of bringing about mergers is to arrange that the economy should become thoroughly competitive. In other words, the economists have as much to say about technological improvement as have the technologists themselves. If this is how the Ministry of Technology is thinking, everybody will be delighted.

## MONEY FOR RESEARCH COUNCILS

THE Council for Scientific Policy seems to have won a fair settlement from the British Government over the budgets for the research councils in the financial year immediately ahead (see page 749). Less than a year ago, when the council produced its first annual report, there were some grounds for fearing that the spending of the councils would be kept on a much tighter rein. The possibility was real that the research councils might be kept on such short commons that they would be unable to take on new projects. Since then the economic situation has not improved, to say the best of it. In the circumstances an increase of 9 per cent in the aggregate budget of the four research councils operating in the natural sciences is as much as could reasonably have been expected. It is true that much of the extra money will be eaten up by the steadily increasing cost of keeping skilled men efficiently at work, and the Science Research Council will be particularly hard pressed by the welcome growth of the postgraduate population at the universities. But there should be something left over with which to break new ground. That is something to be grateful for.

There remains the problem of next year—and the one after. Will the Government be willing to underwrite the steady growth of the research councils which circumstances appear to make necessary? And are the research councils properly organized to do what is now expected of them? There is certainly a case for asking whether the medical and agricultural councils are right in spending a substantial part of their income on the direct support of research units and groups. More grant giving might give them more flexibility. And what should be the relationship between the research councils and the University Grants Committee? There is much to be said for Lord Bowden's argument that the research councils should be thought of as permanent supporters of university research, not merely as sponsors of new projects. But that

implies still faster growth. In other words, there are plenty of questions to occupy the Council for Scientific Policy in the year ahead.

## DR. ROBERT OPPENHEIMER

It is sad but also inevitable that Dr. Robert Oppenheimer should have acquired the public reputation of the first designer of nuclear weapons. What went on at Los Alamos during the last war was too important to be forgotten. One of Oppenheimer's most remarkable achievements is that he was able to make bombs and still seem to remain on the side of the angels. In the years immediately after the war, he was well served by the way in which the respect and admiration of his colleagues, and indeed of the whole profession of physics, were somehow translated into public esteem. It is also remarkable that he survived so well the inquiry into the granting of his security clearance to work as a consultant for the U.S. Atomic Energy Commission. The inquiry may have been a public scandal, and a malign attempt on the reputation of a distinguished man, but it was also damaging. In the eyes of all but a handful of fanatics, Oppenheimer was acquitted of the serious but implicit charge of disloyalty, which is only right and proper. But he emerged from the inquiry a little more like ordinary mortals, with feet of clay. What did he really tell the security men about Chevallier? And was he right to accept security restrictions for others but not himself? These remain absorbing questions. It is a tribute to him that they never seriously diminished the regard in which he was held.

With Oppenheimer dead, however, it is a pity that these cloudy issues must for many people conceal what must in retrospect be his greatest achievement. His reputation among his colleagues rests not on weapons nor on quasi-judicial inquiries, but on the way in which, almost single-handed, he carried the quantum theory to the United States. In this esoteric field he was a kind of St. Augustine. In the early thirties, he gave his contemporaries and their students an opportunity to swim quickly with the new tide then sweeping through Europe. By his art as a teacher, he made them see how important the consequences would be. In doing so, he helped to lay the foundations for a community of physics in the United States which is one of the intellectual attainments of this past few decades. Oppenheimer himself may not have done the most spectacular work, but his collaborators and students were scattered everywhere. If he had been less modest, he could have claimed to have had a hand in most of what was done in the thirties to make nuclear physics intelligible. But Oppenheimer was more than just a physicist. He had a flair for seeing wider issues. He was a great talker, with a gift for putting things well. After the hearings on his security clearance, he sometimes seemed to be a remote and even lonely figure, yet he somehow retained a flair for seeming young. That is yet another reason why he will be acutely missed.

# NEWS AND VIEWS

## British Science Spending

THE threatened squeeze on the research councils, which seemed all too imminent only a few months ago, has been postponed for at least another year. According to the preliminary estimates for expenditure by the British Government in the financial year 1967-68 (*Vote on Account 1967-68*, Cmnd. 337, H.M.S.O., 2s. 3d.), the allocation to the four principal research councils will increase by 9.6 per cent to a total of £68.3 million. Understandably, there is a still sharper increase of the budget of the newly formed Social Science Research Council from £709,000 to £1,162,000. On the face of things, it may therefore seem that the research councils are doing as well as can be expected at a time of financial stringency, and they seem to be well pleased with the way that things have turned out, even though they have recently been used to an even faster rate of growth—an increase of 20 per cent between 1965-66 and 1966-67, for example. The budgets of some of the councils may be more elastic than those of others, so that some councils may be less well placed to undertake projects in the year ahead. The allocations to the four research councils dealing with the natural sciences in recent years, in millions of pounds, are as follows:

	1965-66	1966-67	1967-68
Science Research Council	28.6	33.9	36.6
National Engineering Research Council	3.5	6.2	7.7
Medical Research Council	10.3	11.9	12.0
Agricultural Research Council	9.3	10.3	12.0
Total	51.7	62.3	68.3

The cost of research and development carried out within the budget of the Ministry of Defence will be marginally less during the coming financial year than in 1966-67. According to the *Defence Estimates* published last week (Cmnd. 312, H.M.S.O., 19s.), the total cost of defence research and development in 1967-68 will amount to £260 million, compared with £275 million in the previous financial year. Much of the decrease is accounted for by reduced expenditure on aircraft research and development.

## The Size of Technology

THE merging of the Ministry of Aviation with the Ministry of Technology on February 15 will have seemed much like a gnat swallowing an elephant. The entire cost of running the research and development establishments under the old Ministry of Technology was about £11 million, but those it has now acquired cost roughly £45 million a year to run (*Civil Estimates 1966-67*). But the Ministry of Technology is now also responsible for the expenditure of the Ministry of Aviation on industrial research and development, which cost £194 million last year, and support for the European Launcher Development Organization (ELDO), which cost £11.3 million. To round off Mr. Wedgwood Benn's new empire, there are the United Kingdom Atomic Energy Authority, whose civil activities cost £28.7 million in 1966-67, the National Research and Development Council, and the 8 research associations and the National Computing

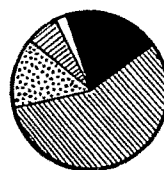
### RESEARCH EXPENDITURE

	Expenditure (£)	
	Staff (salaries, etc.)	Capital and re-current expenses
Ministry of Technology		
Building Research Station	983,000	277,000
Fire Research Station	199,000	63,000
Forest Products Research Station	247,000	43,200
Hydraulics Research Station	281,000	95,200
Government Chemist Laboratory	591,000	86,200
National Engineering Laboratory	1,038,000	1,465,000
National Physical Laboratory	2,341,000	1,339,000
Torry Research Station	241,000	121,800
Warren Spring Laboratory	539,000	272,000
Water Pollution Research Laboratory	246,000	72,600
National Research Development Corp.		£7,400,000
U.K.A.E.A.		£28,723,000
Research Association grants		£3,570,000
National Computing Centre		£300,000
Ministry of Aviation		
Aeroplane and Armament Exptl. Est.	}	£44,830,000
Explosives R and D Establishment		
National Gas Turbine Establishment		
Rocket Propulsion Establishment		
Royal Aircraft Establishment		
Royal Radar Establishment		
Signals Research and D Est.		
U.K.A.E.A.		£54,900,000

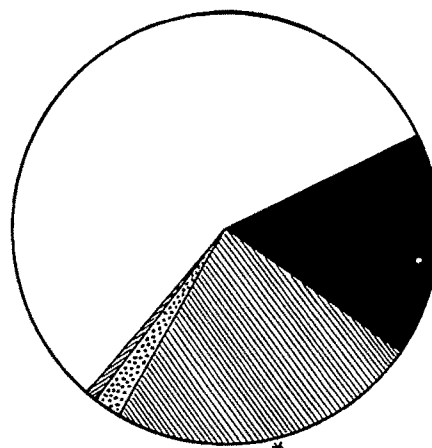
Centre which cost £3.6 million and £0.3 million a year respectively.

Not all the new establishments seem to fit easily into the Ministry of Technology. It is difficult to see, for example, how it will use the Explosives Research and Development Establishment, or the Aeroplane and Armament Experimental Establishment. It might have been more consistent with the Ministry's pre-occupation if these establishments had been hived off to the Ministry of Defence. They will, in fact, be controlled from the building of the Ministry of Defence, since Mr. John Stonehouse, looking after the new acquisitions as

Old Ministry of Technology,  
total £51.35 million



New Ministry of Technology,  
total £344.5 million



- Research Associations
- N.R.D.C.
- Research Establishments
- U.K.A.E.A.
- Industry

\*This allocation includes the payment at present made by the Ministry of Aviation for special materials.

Minister of State at the Ministry of Technology, will have his office there.

The Ministry of Technology is to be organized in three main groups—Engineering, Research, and Aviation. The new controllers will be Dr. G. G. Macfarlane (Research) and Mr. Ieuan Maddock (Industrial Technology) (*Nature*, **213**, 645; 1967), while the Aviation section—the largest of the three—will be controlled by Air Marshal Sir Christopher Hartley.

## Profession of Physics

AN even more detailed analysis of the structure of professional physics has now been published by the American Institute of Physics, based in part on the information obtained from the National Register (*Physics Manpower 1966*, American Institute of Physics, \$2.50). The survey is particularly informative on the recruitment of physicists at different parts of the education system, and on the immigration into the United States of physicists from elsewhere.

The survey suggests that it is as yet too soon to know what the effect will be of the new physics courses produced by the Physical Science Study Committee on education in physics in the United States. Of those graduating in physics in 1966, only 16 per cent will have followed the PSSC course at high school. In future years, however, the crop should be larger, for it is estimated that in the academic year 1964–65, roughly 50 per cent of high school physics students in the United States were enrolled in a PSSC course. The survey points out that the numbers of physics teachers in high schools have been declining as a fraction of science teachers as a whole, which seems to imply an increase in the numbers of mathematicians and others on high school faculties. In the universities, the Institute of Physics has found that from year to year a smaller fraction of those who enrol for a degree elect to become physics majors at the end of their course. In fact, just over 1 per cent of male entrants to American universities in September 1962 chose to specialize in physics in September 1965, with universities on the north-eastern seaboard producing more physics students than their share.

Allowing for the relatively high rate of drop-out between one year and the next, it is estimated that the number of bachelor degrees in physics will decline from 5,500 or so at present to 4,500 or so by the end of the decade. But, because of the steadily increasing tendency for graduates to stay on for postgraduate work, it is expected that the output of higher graduates will gradually increase at least until the 1970s. In practice, just about a half of those graduating in physics seem to stay on for further study. The number of Ph.D. degrees awarded each year in the United States has increased from roughly 490 in 1955–56 to 983 in 1964–65. By the early seventies there are expected to be about 1,200 Ph.D. graduates each year.

On immigration into the United States, the report points out that roughly 12 per cent (1,800) of physics graduate students in the United States come from abroad and that 69 per cent (1,280) of these foreign students are from Asia. In 1964–65 Europe contributed 211 graduate students in physics to the total. On the employment in the United States of physicists with degrees from foreign universities, it appears that Europe contributes 72 per cent of the total of 1,315 at

work in 1964. The United Kingdom contributed 362 of these, West Germany 258, and Canada 137.

## R & D at the R.I.

“THE richest of all Lords is use” according to Emerson, and Lord Rothschild might have taken this as his text for his Friday evening discourse at the Royal Institution on February 17. His theme, “Research in a Very Big Industry”, gave him an opportunity to emphasize the virtues of the operations of monopoly corporations at the frontiers of knowledge, and to comment on the differences between academic and industrial research. Industrial research is more concerned with getting exploitable results than is the academic variety, and its results should ideally have some value and be available when they are wanted. Lord Rothschild felt that these questions are still too far in the background in much academic research. He had, he said, come across cases where work had been going on for years and years without anyone bothering to ask “Is it useful?”. The excuse given for this type of work, that it adds to the sum total of human knowledge, was “trite and inadequate”. So far as industry was concerned, it was as well to be wary in assessing the value of research to a company. In general, research does help growth, but the difficulty is to know whether companies with high growth rates favour research because they have a high growth rate or whether it is the research that produces the high growth rate.

## False Teeth in Space

THE view that “Mankind takes his dental problems with him wherever he goes” was the theme of the paper read by B. Lawrence Shalit to the conference on “Planetology and Space Mission Planning” held in New York at the end of 1965, the proceedings of which have now been published by the New York Academy of Sciences (*Annals*, **140**, Art. 1, 1; 1966). Although most of the contributions to the symposium dealt with the potential accomplishments of various rocket systems and the desirability of space missions such as landing on asteroids, Mr. Shalit put forward his plan for dental care in space “to prevent the astronauts from being incapacitated by dental problems and, secondarily, to protect the oral cavity of the individual astronaut from permanent damage of any type during space flight”.

At least for short flights, Mr. Shalit believes that it will be possible to correct most dental defects before launching, so that none of the common dental troubles need disqualify people from becoming astronauts. Moreover, Mr. Shalit rejects the notion that it would be simplest “to equip all astronauts with full dentures”; not merely would this make mastication less efficient but “the loss would be psychologically upsetting or even unacceptable”. He does, however, recommend that before sending men into space, dental surgeons should eliminate non-vital and suspicious teeth, third molars “if, in the opinion of the examiners, this is indicated”, the instruction of astronauts in the use of temporary fillings and analgesics, and the careful planning of meals so that starchy items are eaten first. On longer space flights, Mr. Shalit suggests that the potential hazards of dental caries should be minimized by “reduction in frequency of eating”, and that astro

nauts should be trained in scaling teeth. Mr. Shalit does not, however, consider that they should be trained to extract teeth because that "requires considerable clinical experience".

## Sponsored Research

by our Special Correspondent, Newcastle upon Tyne

IN the first flush of enthusiasm for nuclear power about ten years ago, C. A. Parsons and Co. set up a large research centre here to handle the development of nuclear engineering and materials. When hopes of a rapid expansion in nuclear power receded, the laboratory began to diversify and in 1962 it became International Research and Development Co., Ltd.—the largest establishment carrying out sponsored research in the United Kingdom. The company gives the impression of having had to struggle to maintain the heady optimism of the early nuclear days, but now seems to have overcome the danger of redundancy among staff and under-use of its ample facilities.

Starting IRD almost looks accidental; running it does not. The managing director is Dr. H. M. Finniston, and the research manager is Dr. H. Rose. Both have strong nerves, and need them. As Dr. Rose explains, "The company has no budget. We need more than £1 million a year to keep operating, but never know where it will come from". In fact about 75 per cent comes from industry, the rest mainly from defence contracts; only about 10 per cent of the work now has a nuclear slant. The company employs about 500, of whom 200 are professional staff, mostly graduates.

The activities of IRD are wide ranging. A great deal of effort is going into a long-term project of magneto-plasma-dynamic (MPD) power generation, which involves passing a stream of hot helium seeded with caesium through a magnetic field, thus generating electric power directly. IRD is working with a closed cycle system and the design calls for a nuclear reactor to heat the gas to about 2,000° C. An important objective is the study of the phenomenon of magnetically induced ionization which could have the effect of raising the electron temperature in the plasma without requiring such a high gas temperature. One of the long-term attractions of the closed cycle MPD system is its potential independence of fossil fuels. Elsewhere in Britain, the Central Electricity Generating Board is at present spending £2 million over three years on an open cycle MPD system at Marchwood. IRD has yet to find a sponsor for the future of its project, which will cost between £50,000 and £100,000 a year.

IRD is quite open about projects such as MPD, which are far from being commercial propositions. About some others, the company is more guarded, either for commercial reasons or because the sponsors are security conscious. In the engineering department work has been carried out on cryogenics, superconducting magnets and motors, and a new kind of homopolar machine. Here there is little direct information, but a barely suppressed sense of excitement. The applied sciences department uses lasers and low temperature engineering in a number of applications. There is a large chemistry department, working on studies of corrosion and on chemical processes. In the materials technology section, explosive treatment of metals has been studied; in addition to explosive forming, now

well known, welding treatments have been developed which may have important commercial applications.

IRD is interesting not only for what it does, but also for what it represents. If the Ministry of Technology contrives to diversify its laboratories, they may well be much like this. The idea is attractive, but Dr. Rose sees dangers if it is carried too far. He fears that in a laboratory controlled by the Civil Service, industry may be brought in too late on important projects, and the effect will be to double the amount of money spent. Diversified government laboratories, of course, may attract funds which would otherwise find their way to IRD, but this is not a field in which Britain is embarrassed by riches; there should be enough at least for the best projects in each laboratory.

## Weather Prediction

BRITISH meteorologists are delighted at the success with which Mr. F. H. Bushby and Miss Margaret Timpson of the Meteorological Office at Bracknell have been able to forecast rainfall by computer. The essence of what they have been doing was embodied in a paper read to the Royal Meteorological Society on February 15 and soon to be printed in the society's *Quarterly Journal*. Briefly, they have used a three-dimensional model of the atmosphere in which the possibility of precipitation is adequately taken account of. Numerical integration of the equations representing the model atmosphere has been carried out over a three-dimensional grid of points, with those at ground level separated by 40 km. Thus the physical scale of the weather forecasts obtained is comparable with that of the frontal systems with which precipitation is usually associated. Most of the numerical forecasting systems already in use depend on an integration network whose dimensions are more nearly comparable with the scale of large-scale phenomena such as anticyclones and the like. This system of equations has been used to produce a number of 24 hour forecasts for the British Isles and north-west Europe. A full integration takes 8 hours on the Atlas computer at the Chilton Computer Laboratory of the Science Research Council, which means that the meteorologists will now join the queue of those who would like to see the early coming of still faster computers.

The success of the few 24 hour forecasts so far produced has been impressive. In the prediction of the movement of a small wave depression across the south of Britain on December 1, 1961, for example, the progressive deepening of the depression was forecast, although the numerical value of the lowest pressure was 3 mbar too high after 12 hours and 3 mbar too low after 24 hours. At least a part of these discrepancies seems to be accounted for by large scale oscillations in the atmosphere with a time period of several hours, but fortunately their amplitude does not increase with time. One of the particularly cheerful features of the work is that an initially smooth network of observations can yield a sharp frontal system with the passage of time. Forecasts of actual rainfall agreed well with reality, although the axis of the predicted belt of rainfall was farther north than it should have been. Because the model does not, as yet, include the effects of convection, some thunderstorms were missed. It is acknowledged that the consequences of friction and topography are not included in the atmo-



spheric model, which means that rainfall may not be fully accounted for in the work so far carried out. None of this, however, has prevented meteorologists long trained to keep a quizzical eye on proposals for numerical forecasting from saying that the Bushby and Timpson article is a milestone in numerical forecasting.

## Traffic Delay Banished

SCHEMES for controlling the movement of traffic within large parts of urban communities were discussed at a joint symposium of the Institutions of Mechanical, Civil and Electrical Engineers on February 21 and 22. There seems to be great and widespread enthusiasm for area traffic control systems, but not yet enough experience for engineers to know precisely what the benefits will be. This, however, does not prevent people making forward estimates. Those in charge of the computer-controlled traffic light system now being installed in west London expect, for example, that the social benefit of their scheme will amount to more than £4.5 million in the coming decade. This estimate is based on calculation that the average annual value of delay at a traffic signal stop line is £10,500. The capital cost of the equipment now installed is £550,000. The calculation is that reduction in delay at stop lines of a mere 2 per cent will provide an 8 per cent return on the investment in control equipment, and that a 5 per cent reduction in delay will be equivalent to an annual interest payment of 19.1 per cent.

But how is it possible to be sure that computer control will reduce delay and not aggravate it? Various traffic control experiments, in Glasgow and Toronto as well as in several other cities, seem to differ considerably in the principles on which control is carried out. In the west London experiment, the state of traffic throughout the experimental area will be recorded by counts of vehicles on all the arms of signalled intersections, records of the development of queues at intersections, and the measurement of the speed of vehicles at several points within the system. The object of the control system will be to minimize the integrated delay experienced by vehicles. The computer system will be provided with a library of control patterns theoretically designed to increase what is called the progression of traffic within the area, and appropriate items from these will be selected at intervals of ten minutes or so by the control computer. No doubt it will be some time yet before there is enough experience of these different systems to know what criteria are most important in deciding between the many possibilities.

## Polymorphism in Transfer RNA

by a correspondent in Molecular Biology

THE determination of the base sequences of a number of transfer RNAs, and the apparent presence in them of extensive common features, poses the problem of how these analogies are reflected in the tertiary structure of the molecule, and indeed whether it can be said to possess a tertiary structure in the same sense as an enzyme. Results which bear on this problem have recently been reported from the laboratories of Fresco and of Sueoka. This month Fresco and his associates

(Lindahl *et al.*, *Proc. U.S. Nat. Acad. Sci.*, **57**, 178; 1967) describe further attempts to define the active and inactive states of transfer RNA. These are forms differing somewhat in their physical properties—in respect of partition coefficients in countercurrent distribution systems, for example, and in hydrodynamic characteristics, which can be rapidly interconverted by heating with magnesium ions (inactive to active) or with a chelating agent (active to inactive).

Many of the experiments which are described have been performed on three specific transfer RNAs, but the most extensive studies are on leucine-accepting tRNA from yeast. In the first place it is shown conclusively that only the active or "native" form will accept its amino-acid on incubation with the relevant synthetase. Secondly, the leucyl-tRNA charged with its amino-acid can likewise exist in the same two conditions, and it is shown that only the native form is a substrate for enzymatic removal of the leucine. The question then arises of whether the transfer enzyme recognition site on the RNA has been destroyed in the "denatured" form, or whether the enzyme recognizes the RNA but cannot operate on it as a substrate. This question has been partly answered by the use of an oxidized tRNA, in which the terminal sugar has been eliminated, and which is therefore incapable of binding an amino-acid. Such a molecule evidently retains its normal conformation, for it is capable of interacting with the enzyme, and is thus a powerful specific inhibitor. It is found that whereas both the native and denatured forms of this oxidized RNA inhibit considerably, the former has a greater effect. It thus appears that the enzyme-recognition site remains partly intact in the denatured form, but that the enzyme can nevertheless not operate on such a conformation.

A quite different enzyme for which transfer RNA (irrespective in this case of its amino-acid specificity) is a specific substrate, is adenylyl transferase, which is responsible for the incorporation of the terminal adenylic acid residue into the molecule. It is shown that here again the native conformation is strongly preferred. Finally, it has been demonstrated that only the native leucyl-tRNA will function to any significant extent in a cell free protein synthesizing system from *E. coli*. It appears, moreover, to have been established that this effect does not arise from the formation in the denatured form of a new functional anticodon pertaining to some other coding triplet than that for leucine.

This work appears to leave little doubt that transfer RNA is not a "shapeless" molecule which will take up its correct conformation only in the presence of a relevant enzyme. It points rather to the conclusion that the molecule, like a globular enzyme, can assume several conformations, one of which is preferable by a relatively small margin of free energy. This raises in turn the interesting question whether the conformational polymorphism of tRNA is related to the multiple requirements of its function. It is interesting to note in this connexion the report by Kuriki and Kaji (*Biochem. Biophys. Res. Commun.*, **26**, 95; 1967) that discharged phenylalanine-tRNA, which has been prepared by recovery from polyphenylalanyl-tRNA under mild conditions, will not accept phenylalanine. Therefore the polypeptidyl-tRNA is either chemically more labile than the free RNA or, more probably, the latter

has been recovered in a conformationally inactive state, which it would be satisfactory to identify with the "denatured" form of Fresco and co-workers.

## Parliament in Britain

THE House of Commons caused something of a stir on February 13 by putting the words "brain drain" on to an order paper which has usually been free from such popular excesses. In the event, Mr. Quintin Hogg set out to reprove the government for pursuing policies which have aggravated the emigration of skilled people from Britain. In his diagnosis of the causes of emigration, Mr. Hogg said that the sheer excellence of the British education system "makes us more vulnerable to the brain drain", but this was not a part of his complaint against the government. Rather, he was cross that "the society of the richest country in the world has been plundering the educational systems of Western Europe". But, that said, Mr. Hogg was against the restriction of movement among scientists, and opposed to the opting out of areas of research in which people are particularly vulnerable to emigration. Instead, he wanted to see a stronger European base for scientific work in Britain, more collaboration on European projects such as the Concord aircraft, a counter-recruitment drive and less fierce taxes.

The case for the government was put by the Minister of Technology, Mr. Anthony Wedgwood Benn, and by the Secretary of State for Education and Science, Mr. Anthony Crosland. Both of them were quick to point out that public expenditure on research and development has been increasing steadily. Mr. Benn said that steps are being taken to collect better statistics, that the group under Dr. F. E. Jones would be reporting on the brain drain in May this year, that there is a possibility of keeping a register of British scientists at home and abroad, that recruitment in the United States is being pushed forward vigorously but that, in any case, the "central problem" is to strengthen British industry and help it to make proper use of graduates. "It is not really the government who are alone in the dock today." But Mr. Benn also wanted the universities to change their outlook about the value of careers in business. He agreed with Mr. Hogg about the need for European projects, but was hopeful about the plans being laid by the British Government. Mr. Crosland argued that the brain drain worked in two directions, with people moving from other countries to the United Kingdom and with emigrants returning after a spell abroad. Of the fellows of the Royal Society, 10 per cent were born abroad. But the problem is "concentrated in the last analysis on the policy, attitude and demands of the United States", and it could become more serious if the demand for skilled people in North America continued to increase as quickly as it had been doing. According to Mr. Crosland, a continuing failure on the part of British industry to attract bright graduates from the universities is also a possible cause of aggravation of the problem. He was all for more vigorous attempts by industry to make better use of scientists, as well as for attempts to induce more emigrants to return to Britain. He did not share the Opposition's view on taxes, however, and argued that the people who go abroad to work are not to be found among those on whom the marginal rate of tax is very great.

## University News:

### London

PROFESSOR L. COOPER, at present professor of mathematics in the University of Toronto, has been appointed to the chair of mathematics tenable at Chelsea College of Science and Technology. The title of professor of pure mathematics has been conferred on Dr. K. W. Gruenberg, in respect of his post at Queen Mary College, and the title of professor of cell chemistry has been conferred on Professor K. S. Kirby, in respect of his post at the Institute of Cancer Research: Royal Cancer Hospital.

### Manchester

DR. J. A. DAVIS, at present reader in paediatrics in the Institute of Child Health, University of London, has been appointed professor of child health and paediatrics and director of the Department of Child Health, on the retirement of Professor W. F. Gaisford.

### Mount Allison, Canada

MOUNT ALLISON UNIVERSITY in New Brunswick, Canada, announces the establishment of a Ruggles Gates Chair in Biology. The chair was endowed by the late Professor Reginald Ruggles Gates, who obtained his B.A. and M.A. degrees at Mount Allison. The university has inaugurated a Gates Memorial Collection, to consist of his books, papers, and other items of personal property. The first Ruggles Gates professor of biology is Professor W. B. Stallworthy, who has been professor and head of the Department of Biology since 1956.

### Sheffield

DR. B. RAWLINGS, at present reader in civil engineering in the University of Sydney, has been appointed to the second chair of civil and structural engineering, and Dr. D. E. Newland, at present senior lecturer in mechanical engineering at Imperial College of Science and Technology, London, has been appointed to the second chair of mechanical engineering.

## Appointments

MR. D. H. HILL, of the United Kingdom Atomic Energy Authority, has been appointed first secretary (atomic energy) to the U.K. delegation to the European Economic Communities in Brussels.

## Announcements

At a general meeting, held at Hyderabad on January 3, the Academy of Zoology elected the following officers and members of the executive council for the triennium 1967-69: *President*, Prof. B. C. Mahendra (India); *Vice-Presidents*, Prof. E. H. Rapoport (Argentina), Dr. G. Schreiber (Brazil), Dr. F. Turček (Czechoslovakia), Dr. F. H. Glenny (U.S.A.), Prof. M. Ghilarao (U.S.S.R.); *Secretary*, Miss S. Sharma (India); *Joint Secretary*, Prof. S. Sharma; *Treasurer*, Mr. D. Charan; *Members of the Council*, Dr. R. L. Peterson (Canada), Prof. P. Heegaard (Denmark), Dr. T. A. Moussa (Egypt), Prof. J. Z. Wylezynski (France), Prof. W. Ullrich (Germany), Dr. A. Keve (Hungary), Prof. G. P. Sharma (India), Marquis N. Kuroda (Japan), Prof. J. ten Cate (Netherlands), Prof. W. E. Adams (New Zealand), Prof. G. Paspalev (Bulgaria), Prof. L. Fah-Hsuen (Taiwan), Dr. A. d'A. Bellairs (United Kingdom), Dr. A. Grobman (U.S.A.), Prof. H. M. Smith (U.S.A.).

DEAN N. J. PALLADINO of the Pennsylvania State University has been elected chairman and Dr. C. W. Zabel of the University of Houston has been elected vice-chairman of the U.S. Atomic Energy Commission's Advisory Committee on Reactor Safeguards for 1967.

THE John Murray Travelling Studentships for 1967 have been awarded by the Royal Society to Dr. A. E. Gill,

assistant director in research in dynamical oceanography, Department of Applied Mathematics and Theoretical Physics, University of Cambridge, and Mr. P. D. V. Savage, research student in the Department of Oceanography, University of Southampton.

DR. N. BEKKEDAHL, deputy chief of the Polymers Division, Institute for Materials Research, National Bureau of Standards, has been awarded the 1967 Charles Goodyear Medal by the Division of Rubber Chemistry of the American Chemical Society.

THE Ministry of Technology has produced a new booklet, *Register of Research in Machine Tools 1966-67*, which records briefly research work on machine tools and associated topics being carried out by the Ministry's research stations, and by research associations, universities and similar organizations. Copies can be obtained free from Publications Section, Ministry of Technology, Abell House, John Islip Street, London, S.W.1.

A NEW publication entitled *Journal of Molecular Structure* is to be published by Elsevier Publishing Company. Further information can be obtained from Elsevier Publishing Company, P.O. Box 211, Jan van Galenstraat 335, Amsterdam.

THE first issue of a new journal entitled *Journal of Experimental Marine Biology and Ecology* is scheduled to be published by the North-Holland Publishing Company in June 1967. The publication will be devoted to all aspects of the biology of living organisms in the marine environment.

MR. ROBERT FROMAN, P.O. Box 322, Garnerville, N.Y. 10923, is compiling a list of ways in which amateurs can make meaningful contributions to scientific work. He would welcome suggestions, which should be sent to him at his address.

THE Endeavour Essay Competition has once again been arranged by Imperial Chemical Industries Ltd. in conjunction with the British Association for the Advancement of Science. Prizes totalling over £200 are to be awarded by Imperial Chemical Industries, Ltd., and the prizewinners will also receive invitations to attend the whole of the British Association meeting at Leeds in 1967. Further information about this competition can be obtained from the Deputy Secretary, British Association for the Advancement of Science, 3 Sanctuary Buildings, Great Smith Street, London, S.W.1, and the envelope should be marked Endeavour Prize Essay.

A SHORT course on "The Safe Use of Electricity in Industry" will be held in the University of Bradford during March 9-11. Further information can be obtained from the Registrar, University of Bradford, Bradford 7.

AN Anglo-German welding conference is to be held in Düsseldorf during April 4-7. Further information can be obtained from Mr. L. W. Booth, The Institute of Welding, 54 Princes Gate, Exhibition Road, London, S.W.7.

A SYMPOSIUM on "Mechanical Design of Diesel Engines" is to be held at the Institution of Mechanical Engineers during March 1-2. Further information can be obtained from the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

A SHORT course on "Modern Developments in Bridge Design" will be held in the University of Bradford on Tuesday evenings between March 7-May 9. It is intended for practising engineers interested in the analysis and design of bridges. Further information can be obtained from the Registrar, University of Bradford, Bradford 7.

AN international symposium on "Modern Optics", organized by the Polytechnic Institute of Brooklyn, will be held in New York City during March 22-24. Further information can be obtained from Mr. Jerome Fox, MRI Symposium Committee, Polytechnic Institute of Brooklyn, 333 Jay Street, Brooklyn, N.Y. 11201.

A CONFERENCE on "Underwater Archaeology", sponsored by the University of Miami Division of Continuing Education and the Council of Underwater Archaeology, will be held in Miami during March 23-25. Further information can be obtained from Mr. Sanford Schnier, University of Miami News Bureau, Coral Gables, Florida.

A CONFERENCE designed to stimulate research and development of electrical power systems for automobiles, trucks and buses as a major step in reducing air pollution will be held in the University of Columbia during April 6-8. Further information can be obtained from Professor H. P. Gregor, Polytechnic Institute of Brooklyn, 333 Jay Street, Brooklyn 1, New York.

CORRIGENDUM. In the article entitled "Structure of Ribonuclease" by Dr. C. H. Carlisle *et al.* (*Nature*, 213, 557; 1967), the table of approximate cystine positions on p. 561 should be deleted and the following substituted:

I-VI	$x = 0.83, y = 0.85, z = 0.1$
II-VII	$x = 0.02, y = 0.25, z = 0.17$
III-VIII	$x = 0.28, y = 0.08, z = 0.43$
IV-V	$x = 0.90, y = 0.95, z = 0.42$

ERRATUM. In the article "Safeguards on Plutonium", by Leonard Beaton (*Nature*, 212, 1517; 1966), in line 14 of the paragraph headed "Options on Bombs" on page 1519 there appears the phrase "the private activity of the United States Atomic Energy Commission and certain politicians in the know". Mr. Beaton had written "the private activity of the A.E.C." and was not intending to refer to any particular public authority.

## THE NIGHT SKY IN MARCH

All times are in Universal Time

PLANETS	MOON				CONJUNCTIONS WITH THE MOON			
	New Moon		Full Moon					
					Venus	18d 21h, 1° N.		
					Mars	27d 22h, 2° N.		
					Jupiter	21d 07h, 5° S.		
					Saturn	—		
Times of rising (R) and setting (S) during the month								
Name	R/S	Beginning	Middle	End	Mag.	$D_9$ (10 <sup>6</sup> miles)	Zodiacal position	
Mercury	S	18h 15m	Unfavourable for observation			+1.3	62	Aquarius
Venus	S	20h 05m	20h 55m	21h 45m	-3.4	129		Pisces
Mars	R	22h 20m	21h 20m	20h 05m	-0.6	69		Virgo
Jupiter	S	5h 20m	4h 20m	3h 20m	-2.0	437		Gemini
Saturn	S	19h 20m	Unfavourable for observation			+1.2	975	Pisces

$D_9$  is the distance of planet from the Earth on the 15th of the month.

### OTHER PHENOMENA

13d 21h	Venus occulted by the Moon, visible in S. America.
21d 08h	Equinox
25d 05h	Uranus 3° S. of Moon

# Blackett on British Technology

Professor P. M. S. Blackett, President of the Royal Society and Special Adviser to the Minister of Technology, addressed the Parliamentary and Scientific Committee after lunch on February 16. What follows is an extract from his address.

My first comment is that I am an optimist about the future. Not, however, because I think most things are right—on the contrary, I think very many things are wrong—but, and here is an essential point, they are wrong in a way which can be understood and which can be put right reasonably quickly. As an experimental physicist, I know that the real moment of pessimism is when one cannot find out why one's apparatus will not work. As soon as one has found out what was wrong, then optimism returns, even if one has to scrap and rebuild a lot of the apparatus.

On higher education and pure scientific research, I will have little to say—mainly because I think our present system on the whole is good. Of course, there are defects. But many of the essential changes are in process of being carried out: and indeed have been under way for the past five years or so. The main changes needed are more university places, more universities trying out new methods, more emphasis in schools and universities on technology, more scientists taking up careers in industry (especially physicists), more emphasis on postgraduate studies (including management studies), closer contact of universities and industry.

As I have said, I am optimistic about the state of our universities and of fundamental science. I also, of course, recognize the great increase of government money which has made these results possible. I must confess, however, that I am concerned at the recent increase of fees for overseas students. Many of my scientific colleagues have eloquently criticized this action. In general, I agree with their main criticisms. In particular, I am worried at the possible damage to the postgraduate schools of our universities just at the time when it has become national policy to build them up. It is in the postgraduate schools of universities where the internationalism of learning bears most fruit.

Perhaps the most urgent task for many industries, in my view, is to rationalize their structure, preferably on their own initiative and without waiting for the Government, into fewer, larger and more specialized firms. These changes would, of course, bring longer manufacturing runs and so should achieve marked economies of scale. Most important of all, perhaps, the reduction in the number of firms and the increase of their size would allow a decisive increase in the available number of qualified scientists and engineers in each firm for all the stages of my chain, in particular for the building up of R & D groups of adequate size to be viable. It would also allow the best managers to be better utilized.

Of course, merging is only one way of getting larger firms quickly. The more usual method, and perhaps the more important, is by rapid growth of a single firm—perhaps initially quite a small one—under first-class management. I am convinced that in many cases the Government should assist such go-ahead firms in a deliberately selective way. Though I can understand how unpopular selective measures are in many parts of industry, I am convinced that only by some form of selective concentration of our technological, manufacturing and industrial resources in strong firms can Britain compete internationally in many advanced products. Selective financial assistance to selected firms from the Government

is especially needed when the main competition comes from foreign and international firms, like many in the U.S.A., which have been heavily financed by fall-out from space or defence contracts. Obvious examples are computers, instruments, microcircuits and aircraft.

I now want to discuss some aspects of government procurement. Recently it has been realized that some of the traditional methods of procurement by central government or by state corporations and authorities could be improved if the objective were accepted of bringing into being a few really first-rate and internationally competitive firms.

My second criticism is a very familiar one and relates to the practice of some Government agencies of spreading their orders for some products—for instance, heavy electrical equipment—round too many firms, so perpetuating fragmentation of R & D teams or even increasing it, as well as denying the advantages of scale in manufacture.

My third criticism relates to a fairly common failure of a Government agency, when placing a development contract with a firm for some product which the Government will itself purchase for its own needs, to so modify the specification as to make the product more saleable at home and abroad.

My fourth and last criticism of the Government today—I have plenty more ready for other occasions!—relates to some of the R & D programmes carried out in government research establishments and the use made of the results. For one thing, there is often a great lack of cost-consciousness. This has provoked some industrialists to accuse some government establishments of designing gold bricks. Then, when an establishment has completed some R & D programme it is quite usual to make the results available to all the relevant firms—in the sacred pursuit of fairness. In fact, it often happens that no British firm can profitably take it up without some degree of monopoly right in it, so no firm takes it up. The R & D is thus wasted. If and when the results are actually published, as they often are, then it is quite likely that they will be exploited first by quick-off-the-mark foreign firms.

The more general problem of the role of R & D effort in government stations and in industry raises very delicate questions. I have slowly come to the conclusion that Britain after the war inadvertently took a wrong turning when it continued to rely so much for defence and atomic energy R & D on its own government stations, rather than on industry. I believe that in the U.S.A. a bigger fraction of government funds for defence and atomic energy went to industry and less to government stations. Few would now doubt that the United States has gained greatly from the resulting strengthening of industry and the building up of very strong firms, and that Britain has lost relatively.

On the other hand, for long pay-off time projects, say 10 to 20 years, the less commercial atmosphere of some government stations may be an advantage. A major national problem today is how to divide our total technological resources between long and short pay-off investments and between government stations and industry. A high level of technological, economic and industrial statesmanship will be needed to solve this problem.

# Coding in the Auditory Nervous System

by  
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University of Birmingham

Experiments on how hearing works suggest that it is the connectivity of the central pathways, and not the existence of elaborate code patterns in individual structures, which mediates discrimination of such a wide range of sounds by the ear:

THE idea of auditory analysis by resonant structures is one of some antiquity. The earliest theories were framed in terms of cavity resonances, for the inner ear was then thought to be filled with air. Quite explicit theories of this type are to be found at the beginning of the seventeenth century. From the time of du Verney<sup>1</sup> onwards, however, the solid structures to which the auditory nerve is distributed came to be regarded as the resonators, and the only changes between 1700 and 1850 resulted from a continuing search for the precise location of the resonators, the field of search being progressively opened up by improvements in microscopic and histological technique. The postulated resonance seems to have been regarded mainly as a means of magnifying the weak sound stimulus rather than as an analytic mechanism, although du Verney did consider that as a result of differential vibration the auditory nerve would "receive different impressions which represent in the brain the various characteristics of tones". Haller<sup>2</sup> appears to have been the first to speculate on the possibility of distinguishing sound frequencies by the frequency of the 'tremors' in the auditory nerve fibres and thus to have foreshadowed the other great group of auditory coding theories—the frequency or "telephone" theories first formalized by Rutherford in 1886.

In 1857 Helmholtz<sup>3</sup> produced his famous theory, which can probably be regarded as the first attempt to formulate a detailed system of auditory coding. He did this by combining three previously enunciated principles. The first of these was Ohm's law of auditory analysis. It is a matter of common experience that, in notes sounded by most musical instruments, it is possible with practice to distinguish both the fundamental and one or two overtones. Ohm extended this observation in his hypothesis, and stated that any complex periodic sound wave behaved—as far as the ear was concerned—as if it consisted of a series of suitably related sine waves corresponding to its Fourier components.

Helmholtz was quick to recognize that a system of tuned resonators is precisely the kind of structure to realize Ohm's proposal physically, and he therefore emphasized the analytic rather than the magnifying properties of the resonance hypothesis of cochlear function already established.

The third principle was that which is now commonly known under the name of Müller's doctrine of specific nerve energies. Müller proposed, in effect, that there are five different kinds of sensory nerves—one for each of the five senses—and that stimulation of one particular kind of nerve, by whatever means, evokes specifically the sensory quality to which it is related. Other people carried the subdivision further and the idea reached its fullest extension with Helmholtz's proposal of a specific fibre or group of fibres for each distinguishable pitch. The Helmholtz theory, in its original form, was a pure place theory. Each subjectively distinguishable tone

had a corresponding resonator in the cochlea and each resonator was supplied by an individual nerve fibre or group of fibres distinct from all others. Frequency was thus coded in terms of activity or inactivity in particular fibres, and intensity in terms of the degree of that activity.

There are a great many problems raised by the Helmholtz hypothesis, of most of which Helmholtz was himself aware. It is not possible to discuss them in detail here; they have been well treated by Wever<sup>4</sup>. It is sufficient to point out the two general headings: the identity of the resonators, and their selectivity. In spite of the greatly increased detail which the compound microscope had revealed by the mid-nineteenth century—in particular the structure of the organ of Corti—it never proved possible to identify any component which would account in number and range of physical properties for the number and range of the known discriminable frequencies. Likewise, it proved impossible to reconcile the high degree of selectivity called for in the resonators with the experimentally determined decay time of the system.

These two difficulties caused Helmholtz later to abandon the idea of highly selective resonators and to suggest only that one particular resonator was most highly excited by a tone while those for some distance on each side would be excited to lesser extents. He thus implicitly abandoned the specific nerve energy "one tone, one fibre" code in favour of a code in which there was a non-unique array of fibres subjected to a unique pattern of activation.

Although both the general principle and the details of resonance in the cochlea continued to be matters of argument, the theory was quite widely accepted and taught in elementary textbooks for some seventy years. Perhaps its very elegance and simplicity made people feel it ought to be true and encouraged its acceptance.

## Behaviour of the Cochlear System

The essence of the Helmholtz theory was a series of transverse mechanical resonators, distributed along the length of a tapering basilar membrane. Tones of different pitch would set into vibration different but highly localized regions along the length of this membrane. Until the early 1940s no one had actually looked at the basilar membrane during activation of the ear by a sound stimulus. Then Békésy<sup>5</sup>, who had been working for some time on cochlear models, published several papers on the mechanics of real ears. The essence of his method was to examine the vibrating membrane through an artificial window in the cochlea with the aid of stroboscopic illumination. He found indeed that there was a region of maximum vibration of the membrane for a given frequency of stimulus, and that this maximum moved from the apex towards the base of the cochlea as the sound frequency was increased. The maxima were not very sharp, however, and considerable overlap of the vibration envelopes was evident between frequencies an octave or more apart. The method also made it possible to determine the



relative phase of the vibration, and the result proved crucial against a resonance theory. The phase lag of displacement of the basilar membrane relative to the driving force increases progressively from the base to the apex of the cochlea, reaching values as large as  $5\pi$  at the apical end; the lag at the position of maximum displacement may be  $2\pi$  or more. These values are not compatible with a true resonant system. The disturbance can best be thought of as a travelling wave originating at the basal end of the membrane and travelling towards the apex of the cochlea. As it travels it gradually increases in amplitude and then dies away rather abruptly. With high frequency driving forces the peak amplitude is reached close to the basal end, and the wave does not travel very far before dying out. With lower driving frequencies the wave travels farther and farther along the cochlea, and the maximum is progressively displaced towards the apical end (Fig. 1).

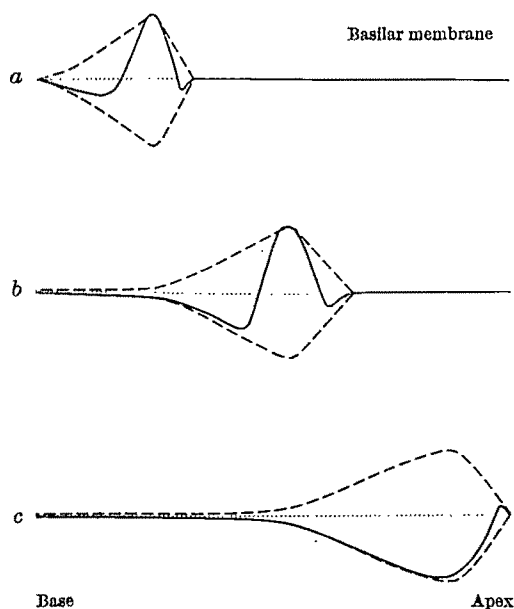


Fig. 1. "Instantaneous" pictures of the travelling wave on the basilar membrane for a high frequency, *a*; a middle frequency, *b*; and a low frequency stimulus, *c*. The dashed envelopes show how the disturbance travels farther along the membrane from base to apex before reaching a peak and dying out.

The basilar membrane carries on its surface a rather complex structure known collectively as the organ of Corti. This structure contains the hair bearing cells which form the mechano-neural transducers. Vibration of the basilar membrane results in deflexion of the hairs attached to these cells and this, in turn, by a process not fully understood, results in a discharge of impulses in the sensory nerve fibres associated with these cells. The greater the amplitude of vibration of the membrane, the faster are impulses discharged by the nerve.

Although the innervation of the cochlea is quite complex, in general terms we can say that the 30,000 to 40,000 fibres of the auditory nerve are evenly distributed to hair cells along the whole length of the basilar membrane. Because the vibration envelope of one tone can overlap that of other tones an octave or more away, and because the extent of the envelope varies with intensity, we might expect that a single auditory nerve fibre could be activated by many different tones, and this proves to be the case. Near its threshold a fibre responds to only a small range of frequency, but as the sound intensity is increased it responds over a greater and greater range until at high intensities it may be responding to any frequency within a band several octaves wide<sup>6</sup>. Each

fibre thus has a triangular frequency/intensity response area which roughly mirrors the shape of the vibration envelope.

Because any one fibre responds to a wide band of frequencies, it would be expected, conversely, that a large number of auditory nerve fibres will be discharging at any given stimulus frequency. Schuknecht<sup>7</sup> has shown that at the quite moderate sound pressure level of 40 dB, between 15 and 25 per cent of the whole system is activated by any one single frequency. But 25 per cent of 40,000 fibres is 10,000 fibres. It follows that if the frequency is changed by 1 per cent—an easily detectable change—something like 200 fibres will be added to one end and a similar number subtracted from the other. In other words, 98 per cent of the active fibres are activated by both tones. When we remember that the total number of fibres activated depends on intensity as well as frequency along the array, it is clear that there is no question of there being specific groups of fibres for specific discriminable frequencies. How then does the system function?

### Possible Pulse Interval Codes

An alternative to the resonance hypothesis was put forward by Rutherford<sup>8</sup> in the 1890s and has become known as the "telephone theory". Rutherford suggested that, rather than peripheral analysis taking place, the auditory nerve transmitted a frequency replica of the sound. Little was known in those days about the properties of the nerve impulse, but subsequent work has shown that it is not possible for a nerve to carry trains of impulses at frequencies greater than at most 1,000 per sec, and it usually cannot sustain rates greater than 500/sec for more than a very short time. This leaves a large proportion of the audible spectrum without representation. To deal with the frequencies above 500 c/s, it has been suggested that some form of alternation takes place<sup>4</sup>. According to this idea, a frequency of 10,000 c/s would be dealt with by twenty fibres each firing 500 pulses/sec and firing in rotation. Of course, this would only signal the frequency element of the sound, and considerably more fibres would be required to deal with the intensity parameter, but there are some 40,000 fibres available so that the hypothesis does not immediately fail on this score.

The primary question, however, is the experimental one: do the pulses in auditory nerve fibres bear any relation in time to the stimulus frequency? At low stimulus frequencies (say, 200 c/s) there is undoubtedly a very strong relationship. However, there is a good deal of "jitter" in the system which causes this relationship to become progressively less and less obvious as the stimulus frequency is increased. In the auditory nerve this relationship fails somewhere between 2,000 and 4,000 c/s, according to the time and space over which one chooses to integrate the measurements. The situation gets progressively worse as one ascends the neural pathway, owing to the variable nature of synaptic delays (the variation may be as much as 300  $\mu$ sec). This means, in effect, that for anything but the very lowest frequencies, a frequency code would have to be converted to some other form at a very early stage in the neural path. There is no evidence that any such conversion takes place. We do not, for example, find that single neurones at the higher levels of the nervous pathway respond only to narrow frequency bands; the band of frequencies to which a given fibre responds at, say, the collicular level is just as great as that of an auditory nerve fibre. We must conclude that although frequency representation is a possible mechanism for low frequencies, it is not a valid one for high frequencies.

Even at low frequencies there is a further problem to be considered. It is very easy when recording nerve impulses on an oscilloscope, or using them to prepare a pulse interval histogram, to distinguish a frequency of 990 from a frequency of 1,000. However, for the nervous system to do this, it too must have a suitable clock and there is no

evidence that a neural clock of this degree of accuracy exists.

We have considered two types of coding—individual channel activity and mean pulse frequency. Let us now consider another. The pulse trains in a nerve fibre are not, in detail, evenly spaced in time. This is especially true at higher levels in the system. It is clearly possible that a pulse interval code could exist, and such a code has a potentially high information capacity. In the past ten years or so, the possibility of such a code in the nervous system has attracted a lot of attention and a great deal of work has been done on analyses. So far, little encouragement has been forthcoming. Examination of such "patterns" in the auditory system has served only to support the conclusion that the distributions of pulse intervals are random.

In the early stages of the system, if we discount the relative deficiency of short intervals imposed by the refractory properties of the excitable elements, and any residual first order patterning of the type referred to here, there remains only an interval distribution which could adequately be accounted for by a random process. At higher neural levels the distributions are complicated by multiple delay paths, but there still appears nothing obviously specific to the stimulus. The objection raised here would also seem to apply even more cogently to the use of a pulse interval code: is there in the nervous system any mechanism for decoding such a pattern? Again we know of none.

### Coding of Stimulus Frequency and Intensity

Before going further, it may be worth while to consider what the auditory system actually has to do in hearing, say, a steady tone or a vowel sound. If we make measurements of the frequency and intensity difference limina (DLs) over the whole range of hearing, we can construct a two-dimensional diagram consisting of a number of cells or compartments which show at a glance the size of the DLs in any particular region<sup>8</sup>. If we count up the total number of these cells we find there are about 340,000. However, although many people have greater or lesser degrees of absolute pitch it would be absurd to suggest that anyone when presented with a tone at random can say "That is 6,130 c/s at 30 dB above threshold", and the auditory system is not called on to transmit data for any

such choice. Intensity difference limina are normally determined by comparing adjacent stimuli. Again, although speech has a highly complex waveform of potentially very high information content, little of this is used at any one time. Even highly degraded waveforms such as differentiated clipped speech yield 92 per cent intelligibility scores<sup>10</sup>. It seems that a very few frequencies, usually called the formant frequencies, or perhaps even the second formant alone, are sufficient to characterize the speech sounds as far as intelligibility is concerned<sup>11</sup>.

Let us see, therefore, how far we can get with a rather simple code. We have already seen that the pattern of activity in the array of auditory nerve fibres for a tonal stimulus is like that of Fig. 2 with fibres firing at various rates according to the degree of excitation of the part of the membrane from which they arise. In this array we could, for example, assess intensity in terms of the discharge rate of the most highly excited fibre, or by the total number of fibres carrying any activity. Likewise, we could assess the stimulus frequency by the position of the most active fibre (the position of the "peak") or by the relative position of the two ends of the array of active fibres. Neither of these two latter criteria is very good, however, as a small percentage of 'noise' pulses added at successive synaptic junctions could easily blur their locations.

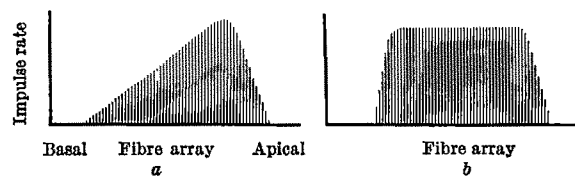


Fig. 3. Transformation of pulse rate distribution in the fibre array effected by the cochlear nucleus, *a*, input pattern; *b*, output pattern. Thick lines represent a low stimulus intensity, thinner lines a high intensity. Other conventions as in Fig. 2 (after Whitfield<sup>12</sup>).

If we examine the behaviour of a single auditory fibre at a higher level beyond the first cell station (cochlear nucleus) we observe two differences from an auditory nerve fibre<sup>12,13</sup>. First, the discharge rate is no longer a monotonic function of stimulus intensity, so that intensity cannot be signalled purely in terms of discharge rate. Secondly, over a rather wide range of stimulus intensity there is little change in firing rate. The curve tends to increase rather rapidly near threshold as the intensity is raised, and then to flatten off, falling again somewhat at very high intensities. Allanson and Whitfield<sup>14</sup> have pointed out that this effect leads to a transformation in which the noise-sensitive auditory pattern is transformed into one in which all the fibres are substantially either "hard on" or "hard off" (Fig. 3). In other words, if we consider only whether there is activity or no-activity in a given fibre, then the frequency is signalled by the relative position of the two edges, and the intensity by the number of active fibres between those edges. Such an arrangement is not only very resistant to degradation, but presents the information in a form which should be eminently detectable by the nervous system which is well adapted to locating regions of abrupt change in activity.

An immediate difficulty arises. Suppose we have not one single tone as stimulus, but two or more simultaneously as will certainly occur very often in real life. Because of the considerable overlap of activity, the auditory nerve pattern might look something like Fig. 4*a*, and this, after transformation, might be expected to give rise to a single block of active output fibres indistinguishable from that produced by a single, much louder tone, the frequency of which is located somewhere between the two tones we are considering. If we actually try the experiment, we find that although the fibres in the 'overlap' region are activated by either of the two tones sounded alone, if we sound them simultaneously these fibres are actually

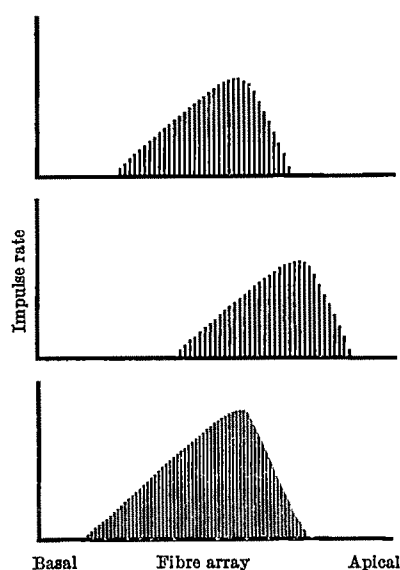


Fig. 2. Diagram to show the distribution of activity in the array of fibres originating from the basilar membrane. The height of each line represents the mean rate of nerve impulses in the corresponding fibre. The upper and middle diagrams represent two different stimulus frequencies, while the lowest diagram represents an increased stimulus intensity. In actuality there would be several hundred times as many fibres activated as are shown here.

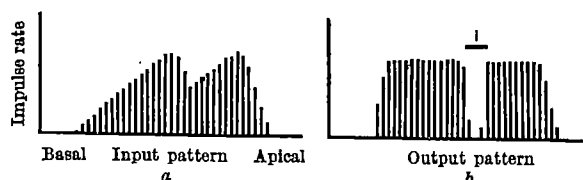


Fig. 4. Input/output relations for a stimulus involving two frequencies. Note the inhibition ( $i$ ) in the "overlap" region. Conventions as in Fig. 2 (after Whitfield<sup>19</sup>).

inhibited and fail to respond (Fig. 4b). This type of inhibition is a common feature of sensory pathways<sup>15,16</sup>. The effect of its existence is clearly to preserve the identity of separate stimuli—in this case the blocks of active fibres corresponding to the two tones.

It is also found experimentally that the fibre array preserves an orderly anatomical arrangement at least as far up as the inferior colliculus<sup>12</sup>. This means that channels which are adjacent frequency-wise are also spatially adjacent. The activity in the total array in response to a complex sound signal will therefore appear rather like that shown diagrammatically in Fig. 5a.

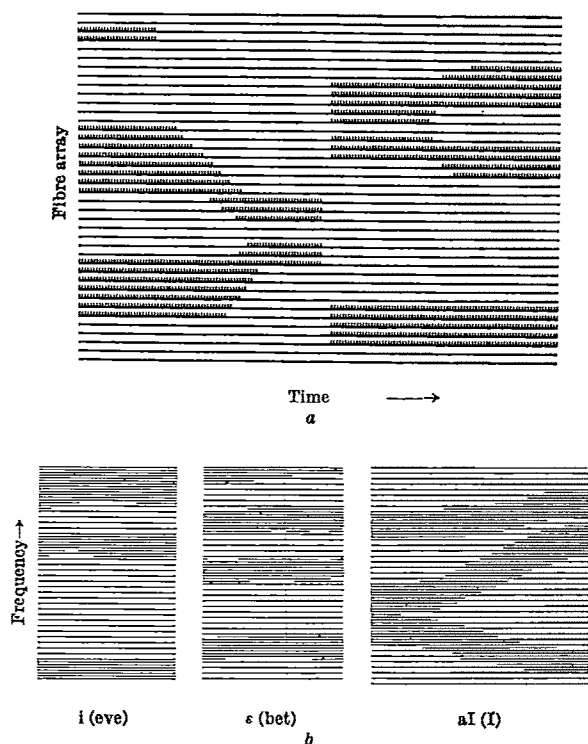


Fig. 5. *a*, Distribution of pulse activity in the array of fibres in the central auditory pathway, and the way this may vary with time as the components of the stimulus vary in frequency and intensity. *b*, Distribution of speech energy in different frequency bands for two vowels and a diphthong (after Potter, Kopp and Green<sup>20</sup>).

It is instructive to compare this with the time course of the formant frequencies of speech sounds (Fig. 5b). The evidence of Fig. 5a also suggests why discrimination between two successively presented tones, which would only require detection of the fact that the edge has moved, is so much more readily carried out than the absolute identification of frequency which would require memory identification of specific channels.

So far, we have considered only steady states and the relations between them. Identifications of frequency changes, however, are also important. For example, the feature which appears to distinguish between the consonants *b*, *d*, and *g* is whether the second formant frequency rises towards its final value, remains constant, or falls towards the final value respectively<sup>17</sup>. If we examine

neurons in the auditory part of the cerebral cortex, we find that there are such units which are specifically responsive to frequency changes<sup>18</sup>. A given neurone will fire only when presented with a falling frequency, while another will fire only when the frequency is rising (Fig. 6). The precise value of the frequency is largely unimportant provided there is a sufficient excursion in the correct direction. Provided the change is not too slow, the rate of change is not very critical.



Fig. 6. *a*, Response of a neurone in the auditory cortex to a rising frequency. The signal bar represents a steady tone of 12 kc/s which then rose linearly at the point shown to a new steady value of 13.2 kc/s. The rise time was 50 msec. In *b* the frequency returned from 13.2 kc/s to 12 kc/s in the same way; this failed to stimulate the neurone (after Whitfield and Evans<sup>18</sup>).

It seems likely that the qualities of pitch and intensity are distinguished by levels below the cortex, as discriminations of this type can be made, for example, by cats in the absence of the relevant cortical areas. Temporal patterns of sound, in which the only differences are those of the sequence of the tones and not their identity, cannot, however, be distinguished in these circumstances. There is some evidence of a similar situation in man.

#### Transient Phenomena and Sound Localization

So far we have been considering either steady state phenomena, or signals involving only fairly slow and limited changes of a single frequency. However, the ear has also to operate on a wide range of transient signals. One of the defects of the resonance theory was its failure to deal with this aspect of cochlear behaviour and its concentration on steady state frequency analysis. It is possible theoretically to produce for any waveform a pure frequency analysis, a pure time analysis, or any intermediate compromise. The mechanics of the cochlea do indeed represent such a compromise between the needs of frequency identification and the necessity for the temporal location of wave fronts. The latter are, of course, of particular importance to the organism in the location of sound sources.

If a short impulse is applied to the ear, a 'travelling bulge' is produced on the basilar membrane which propagates from the base to the apex of the cochlea. This wave has a velocity of the order of hundreds of metres per second near the base and slows to only three or four metres per second near the apex. Experiment shows that as a result of the passage of this bulge a given auditory nerve fibre discharges one or more impulses with a pulse interval corresponding to the steady-state frequency for which that fibre would be maximally excited<sup>19</sup>. There is thus a close link between the temporal pulse pattern produced by an impulse and that produced by a low frequency tone, and it would appear that the same mechanism could well process both. The 'click' has a marked superiority over the tone, however, in that it excites temporarily identifiable pulses in fibres at the basal end of the cochlea, and the steep wave front means that these initial pulses will be especially accurately timed.

For localization of a sound source, the relative time of arrival of the sound at the two ears and the relative timing of the corresponding nerve impulses could provide some of the necessary data. In criticizing the telephone and related theories of frequency analysis, we noted the way in which the relation between nerve impulse position and stimulus envelope, fairly good in the auditory nerve, became progressively degraded as more and more synapses were introduced along the pathway. We argued that this potential source of information could only be used if it

were transformed at an early stage into some other code. In the process of sound localization such a transformation does take place. There appears to be a special structure for this purpose—the medial (or accessory) olivary nucleus, which is situated in the brainstem at about the level at which the auditory nerves enter it. The nucleus is a plate like structure which has a sheet of nerve cells each innervated on one face by fibres of the auditory nerve of its own side, and on the opposite face by corresponding fibres of the auditory nerve of the opposite side. Hall<sup>20</sup>, using click stimuli, has shown that the probability that one of these cells will fire depends on the relative time of arrival of the stimulus at the two ears. Thus, if the stimulus to the ipsilateral ear precedes that to the contralateral ear by 500  $\mu$ sec the probability of the neurone firing might be, say, 0.75. If the ipsilateral stimulus lags by 500  $\mu$ sec the corresponding probability would be only 0.45. Relative intensity of the stimuli has a similar effect; if the ipsilateral click is more intense than the contralateral one, then the probability of firing is increased and vice versa. Artificial manipulation of the timing and intensity of dichotic clicks enables us to demonstrate the phenomenon of time intensity trading<sup>21</sup> in the apparent position of a source, but for a real single source the two effects reinforce one another, as the signal will be both earlier and louder at the nearer ear. Because this situation involves a higher probability of a particular cell firing, it will mean that more fibres leaving this nucleus will be activated; the code has been transformed from one in which the information is contained in the relative timing of impulses to one in terms of the number of active fibres. The end result of the transformation thus has the same form, and the same resistance to degradation, as the frequency code discussed earlier. Of course, the number of active fibres on a given side will be a function of the absolute intensity of the signal, as well as of its relative intensity, and to this extent there is an ambiguity. The relative number of active fibres, however, on the two sides is to a first approximation independent of the absolute intensity and thus could give a measure of lateralization. Although such a model has been proposed<sup>22</sup>, the anatomical site of a comparator has not yet been located. Nevertheless, at the cortex there are cells which, while they are readily activated by quite weak sounds anywhere in the quadrant from dead ahead to a position opposite one ear, are quite unaffected by much stronger signals originating in the corresponding quadrant on the opposite side of the head.

### Centrifugal Control

Finally, let me turn to the question of the control of the auditory input. Everyone is familiar with the phenomenon of "not hearing the sitting room clock tick until it stops". Continuously present or irrelevant signals tend to be suppressed at the expense of more immediately important ones. This control takes place quite early in the system. Thus the electrical response in the cochlear nucleus to a sound signal has been shown to disappear if the signal is repeatedly presented over a long period<sup>23</sup>. Distraction of attention by the presentation of an "interesting" visual stimulus may have a similar effect<sup>24</sup>.

These 'gating' operations, which selectively control the input, appear to be mediated by centrifugal pathways which parallel the ascending auditory system all the way from the cortex to the periphery. Even right out at the cochlea it is possible to inhibit activity in individual groups of auditory nerve fibres by stimulation of the centrifugal olivo-cochlear bundle<sup>25</sup>. Farther up the system, within the brainstem, the possibilities become more varied. Stimulation of one set of descending fibres will shut off the response to sound of a particular neurone in the cochlear nucleus<sup>26</sup>; on the other hand, stimulation of a different set of these fibres increases the sensitivity of cochlear nucleus cells, and may lower the sound threshold of a particular cell by as much as 15 dB<sup>27</sup>. It is clear that

these fibres control the sensory throughput at all stages. It is less clear if they simply control whether or not information gets through at all, or if they actually determine the route it takes.

Until quite recently, views on auditory localization tended towards the view that somewhere in the system there should be an area or set of structures uniquely activated by a discriminable stimulus. From this locus activity would presumably 'fan out' to produce a complex widespread response on the motor side. A diagrammatic representation might be made from two pyramids with a common apex. As we have seen, experiment simply does not support this concept of sensory organization. Just why such an arrangement should have been thought necessary is unclear. Among spinal reflexes, the classical 'scratch reflex' involves interconnection of a complex sensory pattern with a complex motor pattern, yet no one has ever postulated a 'scratch' unit or claimed that there were unique pathways leading to it. The 'pyramid' theory, if it were true, would of course have simplified our concept of the discrimination mechanism, as we should only require a gate to operate at a single point. However, the existence of widespread centrifugal connexions means that not only is diffuse gating a practical means of controlling a 'learned' discrimination, but that this gating could take place at almost any neural level. It is well established, for instance, that direct connexions exist between auditory nuclei and motor pathways at the upper medullary level<sup>28</sup>. These are generally described as 'auditory reflex' pathways, but there seems no reason why they should not be 'conditional' pathways whose activity or quiescence is controlled by the centrifugal system.

In summary, then, let me reiterate two points. There is no evidence anywhere in the auditory system for groups of neurones responding uniquely to particular discriminable stimuli, and multi-channel correspondences probably exist between the sensory system and effector systems, equivalent to those at the spinal level. The elaborateness of the sensory pathway seems to lie in its connectivity; there is no evidence to date for the existence of elaborate code patterns in individual fibres.

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# Rhinoviruses: a Numbering System

Several laboratories have collaborated on a project to identify distinct serotypes of rhinoviruses, and to organize them into a convenient numbering system. They have prepared the following statement\*.

RHINOVIRUSES have emerged as the most important of the known aetiological agents of adult upper respiratory illnesses<sup>1-7</sup>. Taxonomically, they are classified as a sub-group of the picornaviruses because of certain biophysical and biochemical properties which include (1) small size (15-30 m $\mu$ ); (2) ribonucleic acid (RNA) core; (3) ether resistance; and (4) complete or almost complete inactivation at pH 3.0 (ref. 8). This last property distinguishes the rhinovirus from the enterovirus sub-group of picornaviruses.

Almost ninety rhinovirus serotypes have been described as potential new candidate viruses<sup>8</sup>. This large number of described rhinoviruses and the knowledge that many of them had not been tested by neutralization tests against all previously reported serotypes prompted the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Development Branch (VDB) and the World Health Organization (WHO) to institute a collaborative rhinovirus programme to compare the antigenic relationships of rhinoviruses in order to arrive at an acceptable rhinovirus numbering system. It was considered essential to assemble the numerous rhinovirus serotypes into a suitable numbering scheme, because the rapidly increasing number of serotypes made interpretation of epidemiological data from various laboratories difficult and also prevented many laboratories from making epidemiologic investigations of rhinovirus infection. The Vaccine Development Branch, therefore, awarded a contract to the Children's Hospital Research Foundation, Children's Hospital, Columbus, Ohio, to act as a reference laboratory with the immediate task of performing reciprocal neutralization tests with the candidate rhinoviruses and sera submitted to the programme.

At a rhinovirus workshop held on January 25, 1965, and attended by active investigators in the field, each laboratory which was represented submitted a list of candidate rhinoviruses which had been tested against all available rhinovirus antisera and which appeared to be distinct serotypes. After this meeting a few additional rhinoviruses were added to the list by various laboratories; a total of sixty-eight viruses were included in this initial phase of the programme. Participants at this workshop agreed that the following requirements were to be fulfilled before a virus was submitted to the programme as a candidate prototype. These were (1) each candidate rhinovirus was to be "purified" either by three terminal dilution passages in tube cultures or by three single plaque passages; (2) data concerning the biophysical and biochemical properties of each candidate rhinovirus were to include evidence that the virus was ether resistant, acid labile, less than 50 m $\mu$  in diameter and possessed an RNA core; (3) antigenic distinctness of the candidate rhinovirus was to be established by neutralization tests with all other known candidate antisera which were available at the time the virus was submitted; (4) evidence of human origin was to be demonstrated by a fourfold or greater rise in neutralizing antibody in paired sera from at least one person from whom the virus was obtained or recovery of the virus from two or more individuals.

Each laboratory was asked to submit to the Reference Laboratory 25 ml. (in 1 ml. portions) of each candidate virus and 100 ml. (in 2 ml. portions) of specific hyper-immune antiserum. In the Reference Laboratory each of the sixty-eight viruses was tested by the neutralization technique (using approximately 32-320 T.C.D.<sub>50</sub>) against each of the sixty-eight antisera (at dilutions of from 1:2 to 1:20) while each submitting laboratory tested its candidate virus or viruses against all available sera in a similar manner. In this way each candidate virus was tested independently against each specific antiserum in at least two laboratories. When a virus was neutralized by the screening dilution of serum, a reciprocal neutralization endpoint test was performed to determine the extent of that relationship. All the viruses included in the programme were "purified" by tube terminal dilution or plaque methods by the laboratory describing the virus except for the "coryzaviruses" 11, 12, 13, 14, 15, 16, 17, 18, 28 which were "purified" by the Laboratory of Infectious Diseases (LID), NIAID, NIH, Bethesda, Maryland, and "coryzaviruses" 19, 21, 22, 24, 25, 26, 27, 29, 30 which were "purified" by the Common Cold Research Unit (C.C.R.U.), Salisbury, England, so that these "coryzaviruses" could be included in the programme. All sera were prepared by the laboratory describing the virus, or by Abbott Laboratories, Inc., under contract to the VDB, except for sera to "coryzaviruses" 11, 12, 13, 14, 15, 16, 17, 18, 28 which were prepared by the LID, NIAID, NIH, and sera for "coryzaviruses" (CV) 24 which was prepared by the C.C.R.U. and sera for "coryzaviruses" 19, 21, 22, 25, 26, 27, 29, 30 which were prepared by the California State Department of Public Health Laboratory (Dr. Lennette). All sera used in the programme were prepared from "purified" virus except for the aforementioned sera to "coryzaviruses" 19, 21, 22, 25, 26, 27, 29, 30 which were prepared as working reagents before the collaborative programme had begun and which were only available in small quantities but were used so that these "coryzaviruses" could be included in the programme. In addition, all neutralization tests for each virus were performed by the laboratory describing the virus with the exception of the "coryzaviruses" for which most of the laboratory tests were performed by the University of Chicago (Dr. Hamre), and Echo-28 for which most laboratories performed the required reciprocal neutralization tests because reagents for this virus were available to most laboratories before this collaborative programme was initiated.

In June 1966, when all the neutralization tests had been completed, a meeting on rhinovirus nomenclature was held, which was attended by all the collaborating laboratories with the exception of the Common Cold Research Unit of Salisbury, England. Data from the Salisbury laboratory were presented to the meeting.

At this meeting each laboratory presented the cross-neutralization data on the viruses which they had submitted and these results were compared with those of the Reference Laboratory. There was complete agreement among the collaborators and the Reference Laboratory with regard to major cross-reactions. Various collaborating laboratories reported minor cross-reactions. In the Reference Laboratory, however, it was observed that treatment of the sera with human liver powder eliminated these minor cross-reactions in almost all instances. A

\* The signatories are: A. Z. Kapikian (Chairman), R. M. Conant, V. V. Hamparian, R. M. Chanock, P. J. Chapple, E. C. Dick, J. D. Fenters, J. M. Gwaltney, jun., D. Hamre, J. C. Holper, W. S. Jordan, jun., E. H. Lennette, J. L. Melnick, W. J. Mogabgab, M. A. Mufson, C. A. Phillips, J. H. Schieble and D. A. J. Tyrrell.



complete description of the results of the neutralization tests carried out by the Reference Laboratory will be presented in a later communication from that laboratory<sup>10</sup>.

A candidate rhinovirus was considered to be distinct if at least twenty times the limiting concentration of specific antisera which neutralized 32–320 *T.C.D.*<sub>50</sub> of the other serotypes (that is, 20 antibody units) failed to neutralize 3–320 *T.C.D.*<sub>50</sub> of the candidate virus and if at least 20 antibody units of serum to the candidate virus failed to neutralize 32–320 *T.C.D.*<sub>50</sub> of each of the other serotypes. Of the sixty-eight viruses submitted to the programme six pairs of viruses and three groups of three viruses were found to be identical by the aforementioned criteria; this reduced the number of candidate rhinoviruses by twelve. In addition the only viruses which were found to be significantly related, but not identical, were Echo-28 and B632. After reviewing all available data, it was agreed that B632 be designated a sub-type of Echo-28. It was the unanimous agreement of the participants that the prototype strain among identical viruses should be that virus which was first described in a scientific publication or, if not yet described, then that

virus which was first submitted to the Reference Laboratory.

It was also unanimously agreed that a rhinovirus numbering system should be based on a strict chronological system, that is, precedence in number should be given to the virus which had been described earlier in the literature, or, if not yet described, precedence in number should be given according to the date the virus was submitted to the Reference Laboratory. According to these agreements a system of numbering rhinoviruses from 1 to 55 with one sub-type was unanimously adopted.

After this meeting, the data and numbering system were presented to the collaborators at the Common Cold Research Unit, Salisbury, England; they endorsed the system. The nomenclature system was also presented at the meeting of the Directors of the World Health Organization Respiratory and Enterovirus Reference Centres in Moscow, U.S.S.R., who unanimously approved it and suggested its publication as soon as possible. They also recommended that the numbering system be presented to the International Sub-committee on Virus Nomenclature for their information. The numbering system approved by the collaborating laboratories is shown in Table 1.

A second phase of the programme is now in progress, and additional viruses will be tested against the fifty-five numbered rhinoviruses, to determine whether they represent new serotypes. Some viruses were received at the Reference Laboratory too late to be included in the first phase and others were excluded for other reasons. For example, one large group of viruses, the "coryzaviruses" 20 and 34–53, could not be included because neither terminally diluted virus nor adequate amounts of antiserum were available. CH 82 (CHV/3/59) virus which was submitted to the first phase could not be included because untreated antiserum against this virus showed high level non-specific neutralization to many rhinoviruses<sup>1</sup>. The D.C. virus, first described in 1953, was not submitted to the first phase of the programme<sup>2a</sup>. These and other candidate rhinoviruses will be investigated in the second phase of the collaborative programme.

Investigators who wish to submit rhinoviruses for investigation in the programme should contact either the Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, Maryland (Dr. A. Z. Kapikian), or the Common Cold Research Unit, Salisbury, England (Dr. D. A. J. Tyrrell)—both laboratories are designated as WHO International Reference Centres for Respiratory Diseases Other Than Influenza.

Table 1. PROPOSED RHINOVIRUS NUMBERING SYSTEM

Rhinovirus No.	Prototype strain*	References describing indicated strains
1A	Echo-28	11, 12
1B	B632 [K779]	13, [14]
2	HGP	13
3	FEB	13
4	16/60	13
5	Norman	13
6	Thompson	13
7	68-CV 11*	15
8	MRH-CV 12	15
9	211-CV 13	15
10	204-CV 14	15
11	1-CV 15	15
12	181-CV 16	15
13	353 [5,007-CV 23]	16, 17, [18]
14	1,059	16, 17
15	1,734	16, 17
16	11,757	16, 17
17	33,342	16, 17
18	5,986-CV 17	18
19	6,072-CV 18	18
20	15-CV 19	18
21	47-CV 21	18
22	127-CV 22 [203F]	18, [19, 6]
23	5,124-CV 24 (100,319)*	18, (9)
24	5,146-CV 25 [147H]	18, [19, 6]
25	5,426-CV 26 (K2,218)* (55,216)*	18, (20), (9)
26	5,660-CV 27 (127-1)*	18, (19, 6)
27	5,870-CV 28	18
28	6,101-CV 29 (113E)*	18, (19, 6)
29	5,582-CV 30 (179E)*	18, (21, 6)
30	108F	21, 6
31	140F	21, 6
32	363	22
33	1,200	22
34	137-3	19, 6
35	164A	19, 6
36	342H	19
37	151-1	19, 6
38	CH 79† [201-3C]	1, 7, [19, 6]
39	209 [00052]	23, (6)
40	1,794 [134E]	23, [19, 6]
41	58,110 [137F]	23, [19, 6]
42	58,822 [248A]	23, (6)
43	58,750 (E2 No. 133)* (WIS 258E)* [04874]	23, (20), (24), [6]
44	71,560	23
45	Baylor 1 (037211)* (E2 No. 46)*	25, (6), (20)
46	Baylor 2 [477-CV 50] [CH 202†]	25, [26], [1]
47	Baylor 3 [1,979M-CV 46] [CH 310†]	25, [26], [1]
48	1,505	9
49	8,213	9
50	A2 No. 58	20
51	F01-4,081 (19,143)* [005-CV 45] [313G]	27, (9), [26], [19, 6]
52	F01-3,772 (16,413)* [515-CV 34]	27, (9), [26]
53	F01-3,928 [252B]	27, [6]
54	F01-3,774 [2,253-CV 49]	27, [26]
55	WIS 315E [Baylor 4]	24, [28]

\* Virus in parentheses represents a virus submitted to programme by a collaborating laboratory and found to be identical to the prototype strain; virus in brackets represents a virus not included in first phase or not submitted to programme and found to be identical with the prototype strain by a collaborating laboratory. Reference numbers are shown in a similar manner to above. Thus, reference number not in parentheses refers to prototype strain, number in parentheses refers to virus strain in parentheses. Number in brackets refers to virus strain in brackets.

† CH 79 was formerly designated as CHV/2/59; CH 202 formerly designated as CHV/7/59; CH 310 formerly designated CHV/1/60. The number immediately following any CV ("coryzavirus") designation in this table represents a type number assigned to the indicated strain by the investigators originally describing that strain (18, 26). "Coryzavirus" was the term originally used to describe these strains (15, 18, 26).

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(continued on next page)

## BOOK REVIEWS

## THE BIRTH OF AVIATION

## The Invention of the Aeroplane (1799-1909)

By Charles H. Gibbs-Smith. Pp. xxiii + 360. (London: Faber and Faber, Ltd., 1966.) 84s. net.

CHARLES GIBBS-SMITH's latest book recalls so vividly the birth of aviation that one is left with a strange sense of disbelief that the modern world of supersonic and space craft is barely half a century away from the epoch making events which he portrays. The story of man's struggle to fly is told in this book with a good balance between detail and narrative and with the support of over 250 contemporary photographs and many diagrams. The book surveys methodically the evolution of the aeroplane from Sir George Cayley's first design in 1799 to the successful meeting at Rheims in 1909 when man's conquest of the air was proved beyond all doubt and the world was left wondering, with good cause, whether this new invention would be for good or evil.

The years from 1799 to 1899 take up only the first seven short chapters under the heading of "The Fore-runners", of whom perhaps Sir George Cayley is the best known. Although brief, this section makes most interesting reading and the many excellent photographs are of considerable historical interest. The author is, however, primarily concerned with the critical decade between 1899 and 1909 to which some 200 pages are devoted. The progress in this period is dealt with in great detail in some sixty short chapters profusely illustrated and clearly written, with shrewd comment on men and machines which in total makes an interesting story as well as a historical survey.

The courage and determination of the men who made (one hesitates to say "designed") and flew these strange aircraft are now part of history, and are clearly shown in the many chapters which cover this important decade. So, too, are their squabbles without which, unfortunately, no history would be complete, but it is hard to believe that the Europeans, in their rivalry with the Wright brothers, were as stupid as the author suggests and continues to suggest to the point when the repetition becomes irritating and tends to spoil an otherwise excellent narrative.

The rest of the volume is concerned with special chapters and appendixes (of which there are eight). Balloons and airships are dismissed briefly in one chapter and engines and propellers in two, while Bleriot's historic Channel crossing in 1909 is also dealt with in one short chapter. The illustrations in all these chapters are, however, up to the high standard maintained throughout

the book. The important meeting at Rheims in August 1909 is covered in one chapter and one appendix which latter, incidentally, consists of contemporary reports from *Flight* and extends for some twenty pages of most interesting reading. Chapter 67 describes in some detail the six key aeroplanes flying at the Rheims meeting: Wright, Voisin, Henry Farman, Curtiss, Bleriot and Antoinette. Specifications are given together with full page plates of the general arrangement drawings which, although rather crude, give considerable general information of the type when examined in conjunction with the text. It is, however, unfortunate from the engineer's point of view that it has been found necessary to omit from the text the detailed illustrations which are necessary for a better technical understanding. The control systems of these same aircraft are illustrated and described in Appendix VI.

The remaining appendixes include a chronological list of the first powered aeroplanes in order of their first take-offs and a very detailed table of the first powered flights and take-offs, while another appendix lists the first aero-engines.

Throughout the book there is a wealth of data mostly of a general or semi-technical nature—including the main dimensions of the aircraft, the power of the engine, comparative performances—but there is a lack of detailed aerodynamic and structural data. Because of this, the book cannot be classed as a technical work, as the title might imply to anyone unfamiliar with the works of Mr. Gibbs-Smith. This will, however, in no way detract from the value of the book to the type of reader for whom it is intended, and while an aeronautical engineer may not find the detailed information which he may seek either in the text or through the bibliography he will find much to interest him.

The book is beautifully produced throughout, but particular mention should be made of the excellent standard of reproduction of the many illustrations. On a critical note, however, the short chapters or sections tend to give a disjointedness in reading and have resulted in some duplication of subject matter, a fault which is exacerbated by the use of a final section of notes relating to various chapters.

These are small criticisms, however, which detract only slightly from an excellent publication, obviously compiled with meticulous care and which, for such a production, is offered at a very reasonable price. A well written history of any science or technology is of interest and value to both student and expert, and this is particularly true of Charles Gibbs-Smith's latest book. It is a worthy addition to the bookshelf of anyone interested in aeronautics.

J. T. HENSHAW

## CHLOROPHYLL COMPENDIUM

## The Chlorophylls

Edited by Leo P. Vernon and Gilbert R. Seely. Pp. xv + 679. (New York: Academic Press, Inc.; London: Academic Press Inc. (London), Ltd., 1966.) \$25.

THE older literature on chlorophyll has been comprehensively and critically reviewed in a number of books, such as the third volume of Fischer and Orth, and *Photosynthesis* by E. Rabinowitch; but the progress in this field has been so rapid, especially in the past ten years, that an increasing need has been felt for some time for a new consideration of the facts.

A recent conference organized by NATO has summarized the present situation and its deliberations are being published in two volumes. Apart, however, from progress reports, which necessarily deal primarily with individual contributions, there is a need for authoritative reviews covering objectively all the most important aspects of chlorophyll research. This has been done successfully in

(continued from previous page)

<sup>20</sup> Submitted by Dr. William J. Mogabgab, Tulane University, New Orleans.

<sup>21</sup> Connelly, jun., A. P., and Hamre, D., *J. Lab. Clin. Med.*, 63, 30 (1964).

<sup>22</sup> Webb, P. A., Johnson, K. M., and Mufson, M. A., *Proc. Soc. Exp. Biol. and Med.*, 116, 845 (1964).

<sup>23</sup> Mufson, M. A., Kawana, R., James, jun., H. D., Gauld, L. W., Bloom, H. H., and Chanock, R. M., *Amer. J. Epid.*, 81, 32 (1965).

<sup>24</sup> Dick, E. C., and Blumer, C. R. (in preparation).

<sup>25</sup> Phillips, C. A., Melnick, J. L., and Grim, C. A., *Proc. Soc. Exp. Biol. and Med.*, 119, 798 (1965).

<sup>26</sup> Hamparian, V. V., Leagus, M. B., and Hilleman, M. R., *Proc. Soc. Exp. Biol. and Med.*, 116, 976 (1964).

<sup>27</sup> Submitted by Dr. E. H. Lennette and Dr. J. H. Schieble, California State Department of Public Health, Berkeley, California.

<sup>28</sup> Melnick, J. L. (personal communication).

<sup>29</sup> Andrewes, C. H., Chaproniere, D. M., Gompels, A. E. H., Pereira, H. G., and Roden, A. T., *Lancet*, i, 546 (1953).

*The Chlorophylls* under the editorship of Dr. L. P. Vernon and Dr. G. R. Seely. It is somewhat invidious to single out individual chapters. H. H. Strain and W. A. Svec have reviewed the methods used in preparation, estimation and isolation of the chlorophylls, a field to which they themselves have made important contributions. Apart, perhaps, from avoidable repetition they give a critical and helpful account of the methods available, and in particular emphasize the difficulties, which have not all been completely resolved.

Holt gives a valuable account of recently discovered chlorophylls, and he deals in particular with the chemistry of chlorobium chlorophylls 650 and 660. This chapter, however, might have been improved by quoting findings and interpretations which are at variance with Dr. Holt's own work. The biosynthesis of these compounds raises a number of interesting questions which have not yet been adequately answered. The chemical synthesis of chlorophyll *a* is discussed by Dr. Lwowski; the contrast between the approach of Fischer and that of Woodward is an interesting demonstration of on the one hand supreme competence based on the classical organic chemistry of the German school, and on the other of the magnificent achievement which rests largely on the use of modern concepts to predict the reactivity of certain groups in complex molecules. Katz, Dougherty and Boucher give a valuable account of the use of infra-red and nuclear magnetic resonance spectroscopy in chlorophyll chemistry.

The state of chlorophyll in plants or micro-organisms is not yet completely understood. The nature of the linkage between chlorophyll and its associated protein is still somewhat uncertain and so are the chemical or structural factors which endow certain chlorophyll molecules with special reactivity. Our knowledge in this field and the uncertainties are clearly indicated in the chapters dealing with the optical properties of chlorophylls and chloroplast structure, and the description of the photosynthetic apparatus in micro-organisms.

Protochlorophyll is ably discussed by N. K. Boardman and the biosynthesis of the chlorophylls is reviewed by L. Borograd, both of whom give a fairly complete and up to date account of their respective topics. In some ways, however, the most interesting chapters are those written by Seely and by Vernon and Bacon Ke. There is a certain amount of overlap between these two chapters and there is also unavoidably a certain bias in the treatment of what are still regarded as controversial subjects. The reader cannot but be impressed, however, with the sophistication of the techniques used and the difficulties inherent in obtaining information about events which of necessity proceed extremely rapidly. Both these chapters give a useful, informative and helpful account of results obtained, and also of problems presented by the interpretation of the results. Altogether, the book will be most valuable to the many scientists interested in botany, microbiology, biochemistry and also to those engaged in research on chlorophyll.

A. NEUBERGER

## MORE ABOUT SPORES

### Spores

*Their Dormancy and Germination.* By Alfred S. Sussman and Harlyn O. Halvorson. Pp. xi + 354. (New York and London: Harper and Row, Publishers, Inc., 1966.) \$14; 112s.

In this book two American workers, one a bacteriologist who has been concerned with the physiology of bacterial spores, and the other a mycologist who has concentrated on the behaviour of *Neurospora* ascospores, attempt to "highlight the common denominators in biological dormancy". They extend their treatment to germination.

A brief account of the structure of dormant spores is followed by a consideration of their powers of survival.

The American interest in space leads to a review of the possibility of spores remaining viable there, and the panspermia idea of Arrhenius is given a new airing. The structural changes involved in germination and its kinetics are discussed. Two chapters deal with the environmental influences on germination and the ways in which the process may be "triggered". There is a useful discussion of the physiological and biochemical changes during germination. The controlled synchronous development of biochemical activity in a population of germinating spores offers a beautiful system for the analysis of awakening metabolism. This is a matter of present day interest in a number of laboratories in various parts of the world.

In attempting to bring together information on bacterial and fungal spores, the authors are doing a service to microbiology. It is, however, clear that they have found it difficult to compose an opera with two stars, bacterium and fungus. Rarely do these appear on the stage together; there are few duets.

The book is well produced and is a credit to the publishers, but there are numerous misprints and slips—for example, I noted nine wrongly spelt generic names. Again in relation to mucoraceous moulds reference is made at times to zygosporangia where sporangiospores are meant, and in *Phycomyces* there is a mention of conidiospores, which do not exist in that genus. In spite of these minor blemishes, however, the book is a useful and valuable one.

C. T. INGOLD

## SYMPOSIUM ON DRUGS

### CNS Drugs

(A Symposium held at the Regional Research Laboratory, Hyderabad, India, January 24–30, 1966.) Pp. xv + 367. (New Delhi: Council of Scientific and Industrial Research, 1966.) Rs. 33.00; 66s.; \$10.

THIS publication is a compilation of the thirty-two papers presented at the meetings held in Hyderabad. Approximately half the contributions are from Indian scientists, and the others are from European or North American scientists. As the editorial committee points out in the preface, research in the field of CNS drugs has grown impressively in recent years, and has been from the outset obviously multidisciplinary. This makes it very difficult to achieve a nice balance between the various aspects of research in a meeting such as this; unhappily, the present publication fails to achieve this balance. Almost half the contributions describe structure-activity relations in various classes of centrally active drugs, both old and new (including Rauwolfia alkaloids; diazacycloalkanes; 1,4-benzodiazepines; azetidines; 6,7-benzomorphans; 6-aminoquinazolinones; 2-alkyl-3-aryl-4(3H)quinazolinones; 4 substituted 2,3-polymethylenequinolines; *N*-aryl-aryl anthranilates; tropanes; *N*- $\beta$ -phenylpropionamides and substituted diaminopyridines). These articles undoubtedly provide much valuable information to those interested in these groups of drugs. The emphasis on structure activity relations, however, inevitably means that the remaining papers are stretched too thinly over a very wide field. Half a dozen articles describe the neuropharmacology of new compounds or of substances extracted from natural products (the latter not to be scorned in a country from whose herbal medicines one of the first important tranquilizers, reserpine, was "rediscovered" only fifteen years ago). The topics of addiction, neuroendocrine relationships, sites of drug action in the CNS, and drug access to the CNS are covered well, but of necessity too briefly to be of lasting value. A lamentably small number of articles is devoted to biochemical aspects of drug action.

The symposium was published with reasonable expedition, although the quality of some of the illustrations is

poor and the publication could have been improved greatly by arrangement of the articles into sections under headings. In summary, a useful review of the chemistry and structure-activity relations of several groups of CNS drugs, but not a cohesive or representative account of research in this area.

L. L. IVERSEN

## BOOK OF BREWING

### Principles of Industrial Microbiology

By Alan Rhodes and Derek L. Fletcher. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies; Microbiology Division.) Pp. xviii+320. (London and New York: Pergamon Press, Ltd., 1966.) 35s. net.

THIS book is an account of the knowledge of everyday importance to the microbiologist working in the field of industrial fermentation, which is being rapidly transformed from a mysterious art to a science-based industry—beer will soon be produced according to a mathematical equation instead of the reaction of the chief brewer to a glance and a sniff at the liquor in the vat. Rather than treat each fermentation as a separate entity, which has been the usual approach to the subject, the authors have attempted to distinguish and discuss the common underlying principles. Such a unified approach to fermentation technology is clearly necessary.

This study appropriately covers the physical or engineering, biological and chemical principles. The account of the design of a fermentation unit includes the culture vessels for fermentations and equipment for recovery of products. The design of culture vessels, considering its basic importance to the whole of the technology, is dealt with superficially, but this could be a true reflexion of the industry's approach at the moment to this important subject. We learn that fermenters are designed by rule of thumb for the most part. Sterilization is also dealt with in a most elementary manner, for example, the theory of sterilization by filtration is not discussed or referred to. In the chapter on aeration and agitation, the discussion of agitation is full and stimulating. In contrast, aeration, despite the mass of information about it, is cursorily dismissed.

The technical importance of genetics and screening of organisms for useful products are effectively reviewed. The chapter on nutrition is an interesting account of media, but largely avoids the principles. The chapters on the biochemistry of fermentations provide a valuable advanced account of the latest concepts in a wide range of biosyntheses of industrial importance and their relation to basic metabolic pathways. Antibiotics, steroids, vitamins, vaccine production, food fermentation and effluent disposal are all mentioned. Important details of modern fermentation processes for the production of streptomycin, griseofulvin and tetracyclines can be found here. The main criticism of the book is that the more physical and kinetic aspects of fermentation have not been dealt with as fully as the biochemistry, although the former are of crucial importance.

The style of the writing is pleasingly concise. One is irritated by the printing of Fig. 9.3, which cannot be read without a magnifying glass, and in chapter 7 by use of the same symbol *W* for both work and width. The book is suitable for degree and postgraduate courses as well as industrial microbiologists, but the standard is uneven; for example, it is gratuitous to give the formula for converting °C to °F and even metres to centimetres.

On the whole, the authors have succeeded in their task of formulating the scientific basis of fermentation. It is a timely contribution to microbiological studies which are now the basis of a large part of the chemical and food industries.

S. J. PIRT

## CALCULATING REACTIONS

### Chemical Relaxation

An Introduction to Theory and Application of Stepwise Perturbation. By George H. Czerlinski. Pp. xiii+314. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1966.) 120s. net.

MEASUREMENT of the rate constants of fast reactions in solution has for a long time exercised the minds of chemical kineticists. Further zest has been added to this from a biochemical and biophysical point of view. Earlier techniques based on stationary state methods are limited to relatively few special cases. In recent years relaxation techniques have increased in importance, the best known being flash photolysis and more recently electron pulse radiolysis. In the wide field of thermal reactions the methods developed by Eigen and de Maeyer have made it possible to investigate a large number of reactions.

We have been promised a monograph by Professor Eigen himself, but as yet there only exists his excellent but rather short article with de Maeyer in *Techniques of Organic Chemistry* (Volume 8, (2) ; 1963). We are therefore indebted to Dr. Czerlinski, one of Eigen's early collaborators, for producing an introductory treatment of this important subject. The monograph deals rather broadly with the elementary theoretical background, and with the techniques of different types of equilibrium displacements, such as temperature jump, field jump, pressure jump, concentration jump as well as rapid flow with temperature jump. The treatment is somewhat uneven with regard to detail and the book is something between a monograph and a text-book; there are a number of problems at the end of each chapter which are suitable for postgraduate students.

The publication of this book will be welcome by all who are interested in this field. It will certainly be very useful until a comprehensive monograph comes from Professor Eigen himself. It is well produced with a number of good illustrations. The price seems rather high for a slim volume of this type.

J. J. WEISS

## FINDING INFORMATION

### Textbook on Mechanized Information Retrieval

By Allen Kent. Second edition. (Library Science and Documentation: a Series of Texts and Monographs, Vol. 3.) Pp. xx+371. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 85s.

Annual Review of Information Science and Technology Vol. 1. Edited by Carlos A. Cuadra. (American Documentation Institute.) Pp. ix+389. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 100s.

WHILE the use of computers for routine library operations can now be considered an accepted fact, it is still a matter for doubt as to how far mechanization will be able to go in the more specialized field of information retrieval. As Professor Kent says in the second edition of his book, "the field is still in a state of flux". It is difficult to write a good text-book in these conditions, and many changes have been made as a result of the experience gained in using the first edition. The volume still remains an excellent introduction to the subject, both for the student and for those faced with the practical problem of developing a mechanized system.

The *Annual Review of Information Science and Technology* takes over from the previous book by attempting to cover, under thirteen separate headings, the research work now being undertaken in the field of information science. It is symptomatic of this activity that more than 95 per cent of the references are to work undertaken in

the United States, and that most of this work is directed towards extending the role of computers. This annual review is the first of the series to appear, and the chapter authors have interpreted their instructions in different ways. In his chapter on evaluation of indexing systems, Bourne has attempted to cover all the work in this field since 1952, and the result is a paper of little merit. In an excellent chapter on man-machine communications, Davis only refers to papers written before 1965 to illustrate particular topics, while Menzel, in writing about information needs and users, makes no reference to earlier papers.

With further excellent chapters by Baxendale on content analysis; by Climençon on file organization; by Simmons on automated language processing; by Black and Foley on library automation; and by Sherrod on national information issues and trends, this volume should be of real value to most serious workers in the field.

C. W. CLEVERDON

## PLEASURES OF THE NEW FOREST

### The New Forest

A Symposium. Contributed by Juanita Casey and nine other authors. Pp. x+201+24 plates. (London: Phoenix House, 1966.) Revised edition. 42s. net.

THIS admirable book is, in effect, a collection of eleven essays covering some of the most endearing aspects of what is, for far too many of us, a pleasant interlude on the journey from London to the South. But for those who are prepared to linger, look and read, the New Forest has great treasures to offer, many of which are revealed here in a way that will appeal both to the student and the casual visitor. Needless to say, the plants and animals receive their rightful treatment (it is particularly good to find a whole chapter devoted to the forest pony). But we are also given glimpses of the historical background and the position of the commoners within it, the gypsies, perambulations and Beaulieu Abbey. Considering its price, the standard of production is disappointing. Several of the photographs are poor (for example, those facing page twenty-three) and indifferently reproduced. There is no index—an infuriating omission for those wishing to study the Forest seriously.

W. H. DOWDESWELL

## OBITUARIES

### Professor R. C. Punnett

REGINALD CRUNDALL PUNNETT, late Arthur Balfour professor of genetics in the University of Cambridge, died on January 3 at the age of 92. He was born in Tonbridge and graduated from Gonville and Caius College (M.A., 1902). From 1899 until 1902 he was a lecturer at the University of St. Andrews, and between 1902 and 1910 he was university demonstrator in comparative anatomy and animal morphology at Cambridge. In 1910 he became professor of biology and from 1912 until his retirement in 1940 he was professor of genetics.

With Punnett died the last of a brilliant group of investigators who at the beginning of this century took an active part in the rebirth of Mendelian genetics, and, with great enthusiasm and penetration, helped the infant science to take its first important strides. By work on invertebrates, especially nemerteans, Punnett had earlier shown himself to be a distinguished comparative morphologist, and at that time he had become interested in problems of sex determination and the sex ratio.

At the beginning of 1904 Punnett joined William Bateson in an association that became a most successful partnership. Investigations of discontinuous variation had already guided Bateson's mind in the direction fore-

shadowed by Mendel's work, and on the rediscovery of this work Bateson set out to gather a team of co-workers of which Punnett became the mainstay. Sturtevant's later remark about the *Drosophila* group at Columbia University that "it was often not only impossible to say, but was felt to be unimportant, who first had an idea" also applied to Bateson's group. It was an ideal solidarity. Intensive work with a variety of plants and animals soon gave much insight into the nature of the hereditary transmission of traits, and served to explain the causes of aberrant segregation ratios. This work also provided Punnett with effective ammunition against arguments raised by the biometrical school. The origin of new and unexpected phenotypes, in first and second hybrid generations, was explained by the interaction of independent factors (genes). A momentous experimental discovery was the finding of partial "coupling", that is linkage, which soon became a crucial tool for the establishment of the chromosomal theory of the gene. By investigating quantitative traits Punnett recognized the important role of multiple genes. Of great practical interest was his "invention" of auto-sexing—the synthetic production of breeds in which the sex of newly hatched chicks could be told from their plumage.

Punnett ranged widely in the choice of problems and of the material he used. He combined, happily, the curiosity of the naturalist and the precision of the morphologist. The clarity of his analytical mind and his great fund of information and critical judgment made him an excellent expositor of his science. His *Mendelism* of 1905 ran through seven editions and was translated into seven foreign languages; his *Heredity in Poultry* of 1923, though addressed to a more restricted audience, was equally lucid. The *Journal of Genetics*, founded by Bateson and Punnett in 1910, which was skilfully edited by Punnett until 1948, was of immense value to all geneticists. In 1912 Punnett was elected a fellow of the Royal Society; he received the Darwin Medal in 1922, and in 1948 he was made an honorary member of the Genetical Society. He was a brilliant, a good and a kind man who faced the exigencies of life with courage and equanimity.

WALTER LANDAUER

### Professor Joseph le Fleming Coy Burrow

PROFESSOR LE FLEMING BURROW, who died on January 20, aged 78, was born at Bowness, Westmoreland, in 1888. He graduated in medicine in 1910 from the University of Edinburgh, where he was president of the Royal Medical Society, a much coveted student honour. After holding several house appointments at the Royal Infirmary, Edinburgh, he moved to Leeds to become medical registrar and tutor to the professor of medicine, Dr. Wardrop Griffith. During the First World War he served in the R.A.M.C. as physician to the Second Northern General Hospital at Beckett's Park, and during the Second World War as consultant physician to the E.M.S. Hospital at Seacroft. He was appointed assistant physician to the General Infirmary at Leeds in 1919, full physician in 1929, and became professor of clinical medicine in the University of Leeds in 1937, an appointment he held until his retirement in 1948. He continued to practise as regional adviser to the then Ministry of Pensions and as consultant to the Leeds Regional Hospital Board until shortly before his death. At the age of 67 he had the courage to leave the United Kingdom for four years, and acted as medical superintendent and director of the Princess Tsahai Memorial Hospital at Addis Ababa.

Professor Burrow was an able clinician whose services were much in demand in the north of England. His handsome figure, elegance of manner and gift for the apt illustration made him a popular teacher. The demands of practice left him little time for research, but he gave loyal and devoted service to the medical school of his adoption.

R. E. TUNBRIDGE



# Gravitation in Flat Space-time

by  
J. C. W. SCOTT

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Einstein's general theory of relativity deals quite differently with gravitational and electromagnetic fields. What follows is a more symmetrical treatment. Unlike Einstein's theory, it predicts that the speed of light remains constant even when measured across a gravitational field.

A NEW antisymmetric tensor theory of gravitation has been developed which is a basic modification of my gravitokinetic theory<sup>1,2</sup>. In this theory space-time is always flat and energy and momentum are strictly conserved.

In analogy to the electrodynamics of moving media the field is now described by a source tensor and a force tensor. The relation between these two tensors depends on the local mass velocity and the direction of the vector potential. Thus the vector potential must be completely determined by the source distribution. If  $\gamma$  is the gravitational constant and  $\rho_0$  the rest density of mass the source tensor equations are

$$\square K^i = -4\Pi\gamma\rho_0 w^i \quad (1)$$

$$K^i_{;i} = 0 \quad (2)$$

which are completely determined when the boundary conditions at infinity are prescribed and impose the field equations

$$w_{ij} \equiv K_{i,j} - K_{j,i} \quad (3)$$

$$w^i_{;j} = -4\Pi\gamma\rho_0 u^i \quad (4)$$

Clearly both the field and its potential are propagated in empty space with the velocity of light.

The vector potential may be resolved into its invariant length  $\phi$  and its direction  $w_i$ . The gradient of its length is the generalized Newtonian field component  $G_i$ . Thus the field of a fixed and constant elementary source reduces to Poisson's or Gauss's law. We have

$$K^i \equiv \phi w^i \quad (5)$$

$$G_i \equiv \phi_{;i} \quad (6)$$

so that for a fixed elementary source

$$\nabla^2 \phi = 4\Pi\gamma\rho_0 \quad (7)$$

$$\nabla \cdot \mathbf{G} = -4\Pi\gamma\rho_0 \quad (8)$$

where  $\mathbf{G}$  is the spatial component of contravariant  $G^i$ .

The relation between the source field and the force field depends on what, in analogy to electrodynamics, we may call the constitutive equation. It defines the characteristic force field velocity  $v^i$  and may be written in the equivalent forms

$$w^a u_a w^i = \frac{1}{2}(u^i + v^i) \quad (9)$$

or

$$v^i = s^i j u_j \quad (10)$$

where

$$s^i j \equiv 2w^i w^j - g^{ij} \quad (11)$$

Its physical meaning is simply that the vector potential bisects the space-time angle between the local mass velocity  $u^i$  and  $v^i$ .

Both the source field  $w_{ij}$  and the relative force field  $G_{ij}$  may be broken into tensor components which depend respectively on the rotation of the vector potential and on its magnitude gradient

$$w_{ij} = \psi(w_{i,j} - w_{j,i}) - (G_i w_j - G_j w_i) \quad (12)$$

$$G_{ij} = w^a v_a \psi(w_{i,j} - w_{j,i}) - (G_i v_j - G_j v_i) \quad (13)$$

When the material velocity has the same direction as the vector potential  $u^i = v^i = w^i$  and  $G_{ij}$  reduces to  $w_{ij}$ . The force has the same form as the electromagnetic force with the rest density of mass replacing the charge density

$$f^i = -\rho_0 G^i u_j \quad (14)$$

Because of its low angular velocity the rotation dipole term of the gravitational field of the Sun is negligible. Assuming the solar field to be constant in time, special co-ordinates may be chosen in which the Sun is fixed at the origin. In these co-ordinates

$$K_i = (0, 0, 0, \psi) \quad (15)$$

$$u^i = (\lambda_u \mathbf{u}, \lambda_u), \quad v^i = (\lambda_v \mathbf{v}, \lambda_v) \quad (16)$$

$$\mathbf{u} = -\mathbf{v}, \quad \lambda_u = \lambda_v$$

and the curl of  $w_i$  may be neglected. If  $\mathbf{K}$  is defined by  $\mathbf{K} = \mathbf{v} \times \mathbf{G}$ , then

$$\mathbf{K} = -\mathbf{u} \times \mathbf{G} \quad (17)$$

and the solar force per unit volume reduces to the simple form

$$\mathbf{f} = \rho(\mathbf{G} + \mathbf{u} \times \mathbf{K}), \quad \dot{\rho} = \rho \mathbf{G} \cdot \mathbf{u} \quad (18)$$

Note that  $\rho$  is the relative mass density so that the force is proportional to the total mass as is required by the experiments of Eötvös and Dicke.

In terms of the vector distance from the Sun, after dividing by the density, the force becomes

$$\mathbf{F} = -\frac{\gamma s m}{r^2} (\mathbf{r}_1 - \mathbf{u} \times (\mathbf{u} \times \mathbf{r}_1)) \quad (19)$$

$$dm/m = -\gamma s dr/r^2 \quad (20)$$

where  $s$  is the mass of the Sun,  $m$  the accelerated mass and  $\mathbf{r}_1$  the unit radial vector.

The correct precession of the orbit of Mercury and the correct gravitational deflexion of a light ray are obtained from these equations in a straightforward manner.

In outline the reduction is as follows. The angular component is

$$2 \frac{\dot{r}}{r} + \frac{\ddot{\theta}}{\dot{\theta}} + \frac{\dot{m}}{m} = \gamma s \frac{\dot{r}}{r^2} \quad (21)$$

which has the integral

$$mr^2 \dot{\theta} = K e^{-\gamma s/r} \quad (22)$$

In terms of the rest mass  $m_0$  and the velocity  $U$  at perihelion we define the constant  $h$  by

$$\frac{1}{h^2} = \frac{m_0^2}{K^2} = \frac{1 - U^2}{R^2 U^2} e^{-2\gamma s/R} \quad (23)$$

where, when the velocity is the invariant velocity of light,  $1/h^2 = 0$ , the radial component reduces to

$$\frac{d^2 n}{d\theta^2} + n = \gamma s \left[ n^2 + \left( \frac{dn}{d\theta} \right)^2 + \frac{m^2}{K^2} e^{2\gamma s n} \right] \quad (24)$$

where  $n = 1/r$ , which leads to the orbital equation

$$\frac{d^2 n}{d\theta^2} + n = \gamma s \left\{ \frac{1}{h^2} [1 + 2\gamma s n] + 2 \left[ n^2 + \left( \frac{dn}{d\theta} \right)^2 \right] \right\} \quad (25)$$

The gravitational red-shift is a consequence of the conservation of energy and the proportionality of photon frequency to its energy.

Equation (20) has the integral

$$m = m_{\infty} e^{\gamma s/r} \quad (26)$$

which, because at the surface of the Sun  $\gamma s/r$  is of the order of  $10^{-6}$ , reduces to

$$m = m_{\infty} (1 + \gamma s/r) \quad (27)$$

and therefore to the corresponding dependence of photon frequency on distance from the Sun

$$f = f_{\infty} (1 + \gamma s/r) \quad (28)$$

The divergence of the energy tensor has the same form as its electromagnetic analogue for material media. The field energy, however, takes the place of Newtonian potential energy which like the latter belongs to the accelerated mass, just as much as does its kinetic energy or its rest energy and depends on its velocity. As in Newtonian theory it is a negative term in the positive total energy of the accelerated mass.

Thus this new theory extends the validity of Einstein's restricted theory of relativity to include the gravitational field on the same basis as the electromagnetic field and makes the complications of Einstein's general theory superfluous.

Moreover, new gravitational phenomena are predicted similar to electrodynamic phenomena. Thus a spinning mass should have a kinetic dipole moment analogous to the magnetic dipole moment of a spinning charge. In general, unless the velocity is large the kinetic component of the gravitational field will be difficult to measure because of the extreme weakness of the gravitational force.

A new radar test of relativity proposed by I. I. Shapiro<sup>3</sup> however, will definitely distinguish between this new theory and general relativity. Because in our theory the speed of light is unchanged by a gravitational field the transit time of a radar signal will depend only on the length of the path. Thus the theory predicts a null result from the experiment whereas a positive result is to be expected if Einstein's general theory is correct.

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## Viral Transformation of Monkey Kidney Cell Cultures

by

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Simian tumour virus SV40 induces transformation of primary culture of cercopithecus monkey kidney cells. The cells become highly vacuolated, complement fixing viral antigen is formed and the cell become resistant to the cytopathic effects of the virus. It is probable that a persistent association develops between the cells and the virus genome.

THE simian virus SV40 induces tumours in hamsters<sup>1</sup> and transforms cell cultures of human, hamster, rabbit, mouse, swine, and bovine origin into cells with neoplastic features<sup>2</sup>.

Although the sera of rhesus and cynomolgous monkeys frequently contain antibodies to this virus, there is no present evidence that SV40 is oncogenic in monkeys. Rabson *et al.*<sup>3</sup> infected muscle, skin and testis cells from rhesus monkeys *in vitro* with SV40, and observed transformation several months later. Autologous implantation of the transformed cells failed, however, to produce tumours. Propagation of SV40 in cercopithecus monkey kidney (CMK) cells usually results in cytopathic changes leading to complete lysis of infected cultures<sup>4,5</sup>. Fernandes and Moorhead<sup>6</sup> established a strain (WGM-1) from CMK, and noted a gradual change in the susceptibility of the cells to the vacuolating effect of SV40 during serial culture. Low multiplicities of virus produced chronic infection in WGM-1 cultures; nuclear inclusions occurred in about 3 per cent of the cells, but no cytoplasmic vacuoles were observed. Morphological transformation occurred 15 weeks after infection.

This article reports the morphological transformation of primary cultures of CMK (PCMK) 6.5–7 weeks after infection with SV40, and although the transformed cell population was characterized by extensive cytoplasmic vacuolization, many of these cells were observed to undergo mitosis and amitotic polynucleate formation.

The SV40 strain used, the methods of preparing and maintaining PCMK cultures and the setting up of coverslip cultures for cytological investigation were as previously described<sup>7</sup>. Titrations of culture fluids for infectious virus were performed in PCMK cultures. In tests for traces of virus, 0.5 ml. of undiluted culture fluid or whole cultures, disrupted by freezing and thawing, were inoculated into

each of between five and eight tubes of PCMK culture. These were incubated at 37° C and observed for virus cytopathic effects over a period of 5 weeks.

Uninfected controls and transformed CMK culture were sub-cultured by dispersal of confluent cell growth with 0.01 per cent crystalline trypsin in Hanks balanced salt solution (pH 8.5). Aliquots of cell suspensions, diluted with LAPAGT growth medium containing 10 per cent cow serum<sup>8</sup>, were inoculated into other tubes or bottles. In comparative complement fixation tests and assay of susceptibility to superinfection with SV40, the cercopithecus monkey strain BSC-1 (ref. 9) was also used.

Complement fixed SV40 tumour antigen was measured by the microtechnique described by Huebner<sup>10</sup>. Antigen were 10 per cent suspensions of tissue cultured cells in veronal buffered diluent, frozen-thawed, and homogenized. For titrating antigens, a pool of sera from hamster bearing tumours induced with SV40 and free of virus was used at a dilution containing 8 units of SV40 tumour antibody.

The first transformed CMK strains were established from PCMK cultures in 16 × 150 mm tubes which had received 0.1 ml. of 10<sup>-4.0</sup> or 10<sup>-5.0</sup> dilution of frozen-thawed culture fluids with an SV40 titre of 10<sup>5.0</sup>–10<sup>6.0</sup> TCID<sub>50</sub>/0.1 ml. The fluids had been removed from cultures of human diploid fibroblasts 2 months after morphological transformation following exposure to SV40 (ref. 7), and were being titrated for infectivity in a series of 30 PCMK cultures. Of these cultures, fifteen inoculated and five uninoculated controls remained 1 month after infection and apparently normal cells were observed to repopulate many of the infected cultures which had earlier shown viral cytopathic effect. Six of these were selected and held together with five controls, for further observation

Growth medium was renewed twice a week. During the next 2 weeks, marked cytoplasmic vacuolation and some cell destruction occurred in all infected cultures, and three of them were lost by complete lysis. Viral titres of fluid at this stage ranged from  $10^{4.0}$  to  $10^{6.0}$   $TCID_{50}/0.1$  ml. About 6.5 weeks after infection, each of the surviving three cultures developed two or more dense foci of proliferating cells and marked acidity of the medium and, when sub-cultured, each produced a strain of cells capable of serial propagation. These were designated the *LCMK*-123 series. Sub-cultures were characterized by rapid acidification of the culture medium and extreme cytoplasmic vacuolation involving 60–70 per cent of the cells, many of which were also giant and polynucleated. Lobated, budding nuclei, similar to those in *SV*40-transformed human cells<sup>7</sup>, were prominent. No nuclear inclusions were apparent. Vacuolated cells were frequently seen in mitosis and undergoing amitotic nuclear divisions. A small percentage of the population evidenced severe nuclear fragmentation, and appeared to be degenerating (Figs. 1 and 2).

The growth rate and efficiency of *LCMK*-123 cultures remained relatively low during passages 1–24. About a million cells were required to initiate 8 oz. bottle cultures, which needed sub-division only once each week. At about the twenty-sixth passage, the growth rate and efficiency became accelerated. Cultures could be initiated with  $3 \times 10^6$  cells, and had to be sub-divided every 3 days. The numbers of cells with cytoplasmic vacuoles began to decrease at this time, and by the thirty-third passage vacuolated cells made up as little as 5 per cent of the population. *LCMK*-123 serial sub-cultures gradually lost virus. Fluids from the first to the third passage cultures contained  $10^2$ – $10^3$   $TCID_{50}/0.1$  ml., while the comparable value for the fourth passage was 10  $TCID_{50}/0.1$  ml. Fluids and cell homogenates subsequent to the sixth passage were usually negative, although an occasional culture yielded trace amounts of virus. On these occasions, one in seven *PCMK* cultures developed *SV*40 infection when inoculated with 0.5 ml. each of undiluted transformed tissue culture homogenate.

Non-infected control cultures were maintained for 8 months. Cells did not change as in infected cultures, and no virus was detectable. After three passages, sub-cultures failed to multiply and were discarded.

To determine whether transformation of *PCMK* cells by *SV*40 can be attributed to a property of virus propagated in human transformed cells, *SV*40 seed virus grown in cultures of *PCMK* and having a titre of  $10^{6.5}$   $TCID_{50}/0.1$  ml. was used to infect another group of *PCMK* tube cultures. Five weeks later, seven cultures containing cells surviving exposure to a  $10^{-6.0}$  dilution of virus and eight non-infected controls were renewed with growth medium. During the following 2 weeks, four of the

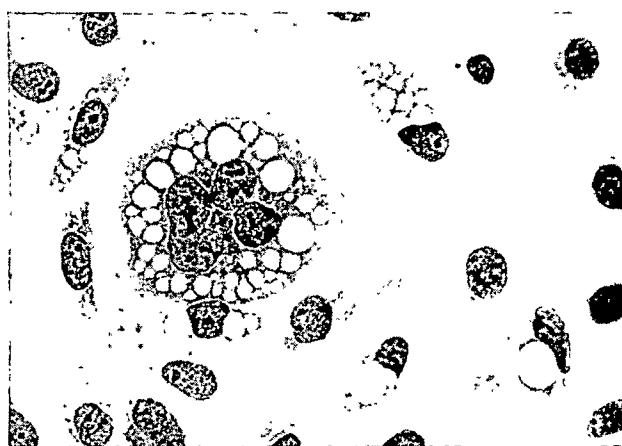


Fig. 2. *SV*40-transformed *LCMK*-123 cells, sixteenth tissue culture passage. Stained with haematoxylin. ( $\times 325$ .)

infected cultures were lost by complete viral cytopathic effects. Beginning seven weeks after infection, each of the three remaining *SV*40 cultures developed excessive acidity of the medium and colonies of transformed cell growth. On subsequent passage these strains, designated the *LCMK*-147 series, exhibited cellular characteristics similar to those described for *LCMK*-123 cultures except that the growth rate was rapid from the start and the numbers of vacuolated cells diminished earlier. Controls were maintained for 4 months, and showed none of the cellular changes seen in infected cultures.

To determine whether prolonged treatment with viral antiserum would accelerate virus loss from the transformed cultures, *LCMK*-147 cells from the second passage cultures were dispersed with trypsin, rinsed three times with five volumes of Hanks solution and exposed at a concentration of  $10^5$  cells/ml. to either Hanks solution or viral antiserum for 2 h at  $37^\circ$  C (rabbit antiserum was used, a dilution of 1:2000 of which neutralized 300  $TCID_{50}$  of *SV*40). Cells exposed to antiserum were further treated with culture medium containing 10 per cent antiserum for 2 weeks of continuous culture. Cultures treated with antiserum were negative for infectious virus after the fourth tissue culture passage, whereas those without antiserum yielded low levels of virus up to the sixty-fourth culture passage after infection. An occasional culture from the progeny of both cells treated with antiserum and untreated cells yielded traces of virus when tested at the tenth to twentieth passages, thus supporting the suggestion that the *SV*40 viral genome may be carried in a non-infectious state, and that maturation of virus occurs only rarely in an occasional cell<sup>11</sup>.

*LCMK*-123 and *LCMK*-147 cells proved to be resistant to the viral cytopathic effects of *SV*40. Replicate  $16 \times 150$  mm tubes were prepared from cultures, free of virus, of each of the transformed *LCMK* lines and also from the *BSC*-1 strain. Serial dilutions of *SV*40 grown in *PCMK* and with a titre of  $10^{6.0}$   $TCID_{50}/0.1$  ml. were inoculated into each tube. During a period of incubation of 4 weeks, the *BSC*-1 cells were destroyed by all dilutions of virus through  $10^{5.0}$ , whereas light microscopic examination revealed no evidence of viral cytopathology in the transformed *CMK* cells. The re-establishment of a persistent virus infection in *LCMK*-123 and -147 cultures was verified by the recovery of infectious virus from fluids for about 2.5 weeks after inoculation. Virus gradually disappeared, however, from all cultures in which superinfection had been attempted.

Tissue culture homogenates of both the transformed *LCMK* series were positive in complement fixation tests for the *SV*40 tumour antigen. *LCMK*-147 cultures at the eighth, eleventh and fourteenth passages reacted with hamster *SV*40 tumour antibody at dilutions of 1:64.

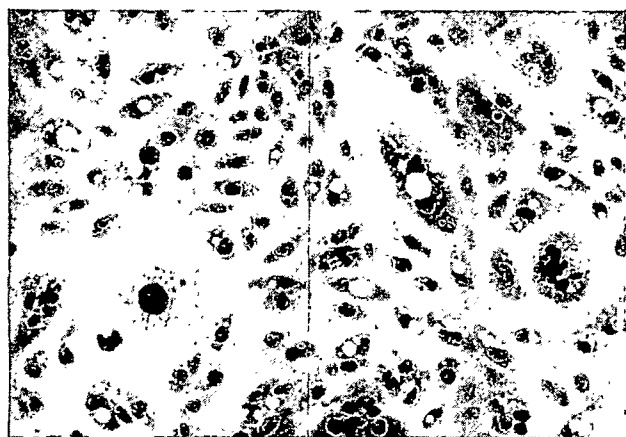


Fig. 1. *SV*40-transformed *LCMK*-123, fourteenth tissue culture passage. Stained with haematoxylin. ( $\times 75$ .)

while LCMK-123 cultures, at the twentieth, twenty-fifth, and twenty-eighth passages, reacted positively at dilutions of 1:32 to 1:64. None of the transformed LCMK cell homogenates reacted with normal hamster serum, and antigens prepared from uninfected CMK or from BSC-1 cells were negative for the SV40 tumour antigen.

SV40 virus in PCMK cells normally enters a replicative cycle ending in complete destruction of the cells rather than the morphological transformation reported here after low doses of virus. Fernandes and Moorhead<sup>6</sup> produced a carrier state in WGM-1 cells infected with SV40, and found their strain not to be susceptible to the vacuolating effect of the virus. They noted transformation 15 weeks after infection, after which there was a lytic phase correlated with the percentage of cells with nuclear inclusions and the viral antigen appeared in the nucleus.

In the present investigation, PCMK cells surviving the lytic effect of low virus doses exhibited marked cytoplasmic vacuolation for 3 weeks before and for several months after transformation. Although 60–70 per cent of the cells contained numerous large vacuoles, many of these were observed to undergo mitosis and to form amitotic polynuclei. Cytological investigations of SV40 infections in patas and cercopithecus monkey kidney cultures<sup>4,5</sup> have indicated an apparent lack of correlation between the development of nuclear inclusions and cytoplasmic vacuolation; each abnormality has been seen without the other, and they appear to be separate phenomena. Our findings indicate that cytoplasmic vacuolation is not associated with the release of a large amount of infectious virus, nor invariably followed by cell destruction. A lytic phase did not follow transformation of the PCMK cells, and although a small percentage of cells with nuclear inclusions may have been present, they were not seen in the transformed populations examined.

It is unlikely that the development of transformed cell lines after SV40 infection of PCMK cultures is the result of the overgrowth of a cell contaminant. The unique cytoplasmic vacuolation of polynucleated cells in LCMK-123 and -147 cultures is a morphological characteristic unlike any observed in cell lines at present maintained in these laboratories, and has not been apparent in human and hamster cells infected *in vitro* with SV40. At the time of these experiments, the only other tissue culture lines in this laboratory were those derived from hamster tumours induced *in vivo* by SV40 and highly tumorigenic for the weanling hamster. In contrast, neither LCMK-123 nor -147 produced tumours in weanling hamsters inoculated subcutaneously in doses of 10–20 million cells.

In the LCMK-123 and -147 lines, the presence of complement fixed SV40 tumour antigen, resistance to viral cytopathic effects of SV40, and the characteristic cytoplasmic vacuolation in a large percentage of the transformed cells point toward some persisting effect of an intimate association between the cells and the SV40 genome, and suggest that the cells were transformed because of this association. Little is known of the mechanisms in cell interactions with cytolytic viruses that can lead to a neoplastic event. The transformation of primary CMK cells by SV40 as described here may have been the result of a gradual change in resistance to the cytolytic action of the virus by certain cells, or to selection of pre-existing cells which differed in their response to infection. Interferon is produced by CMK cells infected with SV40 (ref. 12) and Isaacs<sup>13</sup> has postulated its promoter action in tumour formation by stimulation and selection of cells which obtain their energy by glycolysis. As a result of a repressor action in certain cells of an initial step in the viral vegetative cycle, the virus might enter a sub-viral state in the nucleus of the cell. Stimulation of cell multiplication by the renewal of cultures with growth medium could lead to genetic alteration after intimate contact with viral genome during a susceptible period in the replicative cycle of the cells.

Although interactions leading to cell transformation may be rare in cell populations undergoing viral cytopathic changes, the observations reported here indicate that these events are sufficiently frequent and reproducible in the SV40-CMK system to allow experimental investigation.

I thank Mrs. Susan Kulina for technical assistance and Mrs. E. Osborn for her help in the preparation of this article.

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## A Carrier State of Lymphocytic Choriomeningitis Virus in L Cell Cultures

by

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The establishment of a carrier state for lymphocytic choriomeningitis virus in mouse L cells, without antibodies or a requirement for interferon production, indicates that other self-regulatory mechanisms can balance the simultaneous synthesis of new cellular and viral material.

KNOWLEDGE concerning the basic mechanisms involved in lymphocytic choriomeningitis (LCM)—a virus disease of the mouse<sup>1</sup>—is still scanty despite numerous efforts for more than 30 years<sup>2</sup>. The lack of an *in vitro* cell system, with which to study the interaction between host and parasite at the cellular level, appears to be the main cause of this deficiency. Efforts to fill this gap have indicated that a variety of different cells can support the multiplication of the virus; others are less suitable (unpublished results). This report is concerned with

experiments designed to obtain an LCM virus-carrier state in L cells<sup>3</sup> (kindly supplied by Prof. W. Schäfer).

The L cells were routinely grown in "milk dilution bottles", using Eagle's basal medium (EBM)<sup>4</sup> with no essential amino-acids<sup>5</sup>, supplemented with 10 per cent heated calf serum (CaS). For experimental purposes the cells were seeded into square screw-capped tablet bottles with a surface area of 21 cm<sup>2</sup>. Cells were enumerated by counting crystal violet stained nuclei, released after incubation at 37° C, with a solution of 0.1 per cent

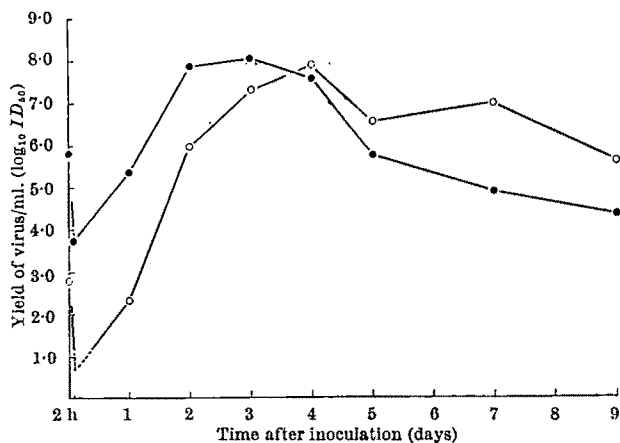


Fig. 1. Growth of LCM virus, strain WE<sub>3</sub>, in cultures of L cells. ●, Multiplicity of infection, 0.1; ○, multiplicity of infection, 0.0001.

'Tween 80' in 0.1 molar citric acid; this is a modification of the method developed by Sanford *et al.*<sup>6</sup>. The WE<sub>3</sub> strain of LCM virus, derived from the WE strain<sup>7</sup>, was provided by Professor W. Scheid. Some of its properties have already been reported<sup>8,9</sup>. Seed virus was prepared from lungs and spleens of infected guinea-pigs<sup>8</sup>. The Armstrong strain<sup>10</sup> was obtained from the American Type Culture Collection. A pool was prepared from mouse brains 5 days after intracerebral infection<sup>8</sup>.

For virus assay, decimal dilutions were made up in balanced salt solution (BSS)<sup>11</sup> containing antibiotics. Portions of 0.03 ml. were inoculated intracerebrally into 4–5 week old mice (five animals per dilution). The 50 per cent lethal dose (LD<sub>50</sub>), based on deaths occurring between the fifth and fourteenth day after infection, was calculated according to Fazekas de St. Groth<sup>12</sup>. The titres presented in the growth curve (Fig. 1) represent 50 per cent infectious doses (ID<sub>50</sub>), determined as previously described<sup>8</sup>. Intracellular virus was set free by means of an ultrasonic drill. Complement-fixing antigen was assayed in tubes using antiserum from a rabbit immunized with monkey kidney-grown virus. Host-specific or non-specific reactions were ruled out by the incorporation of suitable controls. The vesicular stomatitis virus (VSV), strain 'Indiana', was obtained from the American Type Culture Collection. A pool was prepared by inoculating monolayer cultures of L cells using EBM supplemented with 1 per cent heat-inactivated CaS for maintenance. As soon as the cytopathic effect had developed in essentially all cells (which was the case little more than 1 day later) the media were pooled, centrifuged free of cell debris at 4° C, dispensed in ampoules, and stored at –60° C. Titres were determined by inoculating 0.1 ml. of virus, diluted in log<sub>10</sub> 0.5 steps, into L cell tube cultures containing 0.9 ml. of fresh maintenance medium. Five tubes were used for each dilution. Final titres based on the cytopathic effect on the seventh day were calculated<sup>12</sup> and expressed as ID<sub>50</sub>/ml. Tests for the presence of interferon in media from infected L cells were carried out in homologous cell cultures essentially as described by Sellers and Fitzpatrick<sup>13</sup>. VSV was used as the challenging agent. Media were rendered non-infective by ultracentrifugation followed by either dialysis for 24 h against a pH of 2 or by heating at 37° C for 50 h.

After infection of L cell cultures with either high or low doses, newly replicated virus appeared within 24 h and reached maximum titres on days 3 and 4, depending on the initial inocula used (Fig. 1). Thereafter, the virus content in the medium dropped. Throughout the period of observation, infected cultures were morphologically similar to the controls. Furthermore, counts performed repeatedly showed that there was no change in the number of cells present.

The establishment of carrier cultures was achieved in the following way. Bottle cultures of L cells were washed three times with BSS and infected with 0.5 ml. LCM virus, strain WE<sub>3</sub>, at a multiplicity (mouse ID<sub>50</sub> per cell) of about 0.1. After a period of adsorption at 37° C for 60 min, 4.5 ml. of growth medium was added. Forty-eight hours later the media from two cultures were pooled and centrifuged in the cold. The supernatant was dispensed into ampoules and stored at –60° C, until virus titrations were carried out in mice. Cells were washed three times with BSS, dispersed with 0.05 per cent trypsin, diluted to contain about 10<sup>5</sup> cell/ml. with growth medium, and seeded into new culture vessels. At the same time two parallel cultures were prepared with 'Tween'-citric acid to be counted the next day. When the cell sheets were almost complete, that is, 2–3 days after they had been seeded, the cultures were rinsed three times and overlaid with 5 ml. of fresh medium. Again, 48 h later, media from two cultures were pooled and stored in ampoules for assay. The cells were sub-cultured as already described and the cell number was determined from parallel cultures. Results from ten such passages together with uninfected controls are depicted in Fig. 2. The multiplication of the cells was significantly affected by the virus only after the first sub-cultivation. This phenomenon, which has been observed regularly (see also Fig. 4), was accompanied by a cytopathic degeneration affecting about half the cells on the glass. While it is possible that cells infected with virus were still slightly injured during the following two sub-passages (though when compared with the controls there were no visible alterations) no significant differences could be observed thereafter. An interesting, though unexplained, feature of this experiment was the fluctuation with time (or passage numbers) of the amount of infectious virus released into the medium, a fluctuation which was both regular and extensive. Comparable changes of the virus content in the medium of lymph-node cultures infected with LCM had been seen by Traub<sup>14</sup>.

Table 1 summarizes the results of virus titrations from different levels of sub-cultivation. It can be seen that the increases and decreases of virus yields which are so strikingly obvious in Fig. 2 extended to at least the fifteenth cell passage. Later on, however, there appears to be less irregularity of virus release from persistently infected cells (Table 1). (It should be noted that inactivated calf sera, as well as sera from animals representing a variety of other species, have never been found to contain factors which inactivate virus, even if tested undiluted.) While there is no doubt that L cells carrying LCM virus behave very much like their normal counter-

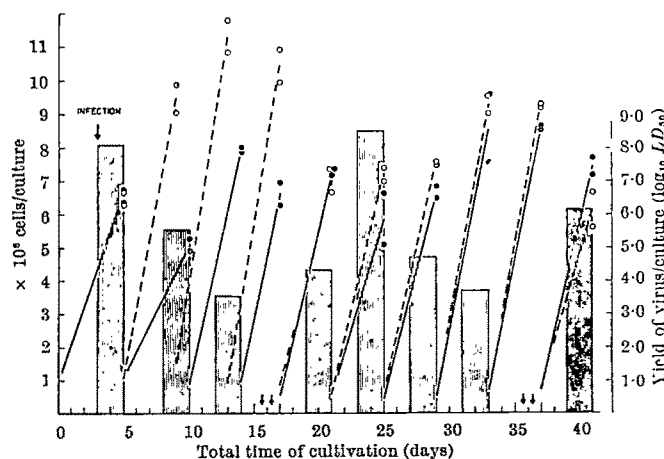


Fig. 2. Initiation of L(WE<sub>3</sub>) carrier cell cultures. Numbers of cells per culture (round symbols) and yields of virus per culture medium (columns) of the first ten passages are represented. ●, WE<sub>3</sub>-infected cultures; ○, control cultures.



Table 1. VIRUS CONTENT OF  $L(WE_3)$  CELL CULTURES DETERMINED AT DIFFERENT LEVELS OF PASSAGE *in vitro*

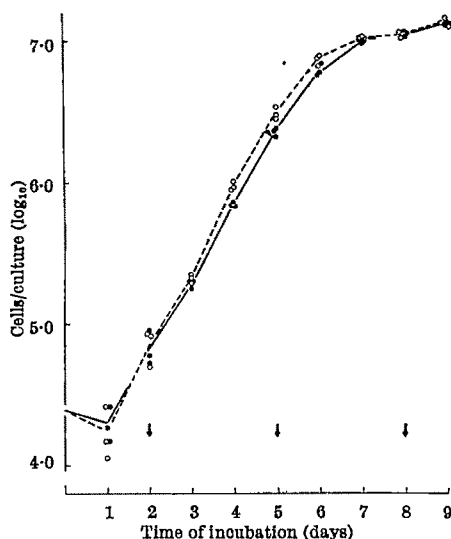
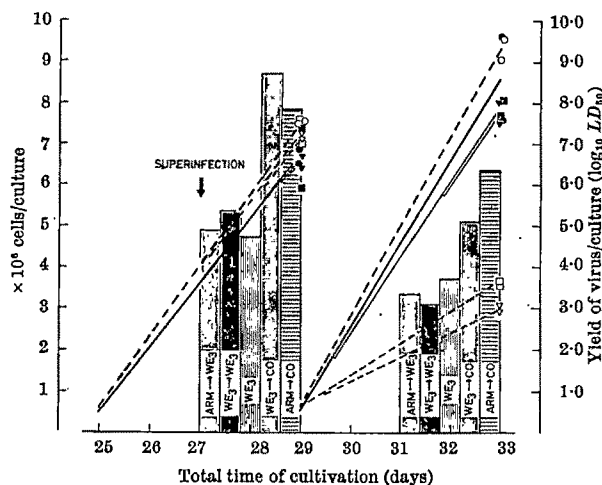
No. of collection	Total time of cultivation	Virus content per culture ( $\log_{10} LD_{50}$ )		Complement-fixing antigen in the medium
		In medium	In cells	
1	2	8.10	N.t.*	
2	7	5.55	"	Tr.†
3	11	3.55	"	
4	14	2.0	"	
5	18	4.35	"	
6	22	8.55	"	
7	28	4.72	"	
8	30	3.72	"	
9	34	2.0	"	
10	38	6.15	"	
11	42	7.10	"	Tr.
12	46	4.89	"	
13	50	2.93	"	
14	54	2.47	"	
15	58	6.72	"	
21	82	5.89	"	
25	100	8.55	"	Tr.
28	113	4.05	"	
30	122	6.85	"	
31	126	4.00	"	
32	130	4.02	"	
33	134	5.10	"	
34	138	7.72	"	Tr.
35	142	5.08	"	
36	146	3.93	3.97	
37	150	3.10	2.51	
38	154	4.51	4.02	
39	158	7.62	7.89	
40	162	4.79	4.72	

\* Not tested.

† Trace.

parts, small differences would have escaped notice with the techniques used. Of all possible ways to assess the overall metabolic state of a cell *in vitro* the determination of its growth rate appears to be the most direct and, at the same time, the most informative procedure. Cells from carrier and from control cultures, respectively, were dispersed by the action of trypsin. After enumeration in a haemocytometer, they were diluted with growth medium to contain 5,000 cells/ml. Five ml. was seeded into 5 cm Petri plates, which were incubated under 5 per cent carbon dioxide in a humidified incubator at 37° C. At regular intervals, three dishes were removed and the cells were counted as already described. The results from three such experiments performed with cells corresponding to the thirty-first, the thirty-third and the thirty-fifth passage, respectively, of which one is presented in Fig. 3, can be summed up as follows: the doubling time of normal *L* cells under our experimental conditions was always close to 13 h. The doubling time for the carrier cells was possibly slightly longer. A difference, however, was seen in two of three comparative trials only and did not reach statistical significance in the individual case. Thus, if any difference exists, it is small.

The question to be answered next concerned the fluctuation of infectious virus titres in the culture media

Fig. 8. Comparison of the multiplication of  $L(WE_3)$  cells (●—●) and *L* (normal control) cells (○---○). (Arrows denote change of medium.)Fig. 4. Superinfection of  $L(WE_3)$  cell cultures with isologous ( $WE_3$ ) and homologous (Armstrong) strains of LCM virus. (Compare with Fig. 2.) ●,  $WE_3$ ; ■,  $WE_3 \rightarrow WE_3$ ; ▼,  $ARM \rightarrow WE_3$ ; ○, CO; □,  $WE_3 \rightarrow CO$ ; ▽,  $ARM \rightarrow CO$ .

at different passages (see Fig. 2 and Table 1). The possible role of interferon-like substances or of homologous interference was investigated. As regards interferon, it suffices to state that neither cells 3 or 7 days after primary infection with  $WE_3$  virus nor carrier cells corresponding to the nineteenth level of cell passage released such a substance; the media, even when tested undiluted, did not noticeably reduce the susceptibility of *L* cells to VSV. Carrier cell cultures at the sixth level of passage were superinfected with the isologous ( $WE_3$ ) and a closely related (Armstrong) virus. Fig. 4 shows that the virus yield after 48 h was low and not altered by the superinfection with either virus. The control cultures infected under identical conditions released large amounts of progeny virus. A further passage of these cells confirmed the observation already reported that infected cells passaged once exhibited a cytopathic effect and a reduced cellular multiplication. Again, superinfected cells could not be distinguished from both types of controls. Thus, while there is marked homologous interference, interferon would not seem to have been responsible for the observation reported here.

The possibility was also considered that the periodically occurring reduction of the infectivity might result from the intermittent production of virus-like but non-infectious particles causing a phenomenon similar to that described by von Magnus for myxoviruses. For want of a haemagglutinin test, the media of all passages listed in Table 2 were investigated for virus-specific material which would fix complement with a hyper-immune rabbit serum. The results presented in Table 2 show that only traces were found in some fluids. Homologous interference was tested by titrating VSV in persistently infected *L* cells in parallel with non-infected controls using five tube cultures for each 3.16-fold dilution step. The results of three representative experiments are given in Table 2. Neither the time of appearance of the cytopathic effect due to VSV nor the final titre on the seventh day was influenced by the carrier state of the cells. Thus while superinfecting identical or similar viruses were inhibited to the same extent as the carrier virus, interference against a heterologous virus could not be detected.

Table 2. COMPARATIVE TITRATIONS OF VSV IN NORMAL AND PERSISTENTLY  $WE_3$ -INFECTED *L*-CELL CULTURES

No. of expt.	No. of cell passages	Titre ( $\log_{10} LD_{50}$ /ml.) of VSV as determined in		$WE_3$ virus ( $\log_{10} LD_{50}$ ) per culture (medium only) at times of VSV titrations					
		$L(WE_3)$	<i>L</i>	$L(WE_3)$		<i>L</i>			
				1*	2	3	1*	2	3
1	25	8.94	8.81	5.2	>3.0	>3.0	<	<	<
2	31	8.94	8.75	3.2	3.7	3.2	<	<	<
3	33	8.71	8.64	>5.7	>6.0	>6.0	<	<	<

\* Three cultures assayed.

These observations partly confirm and extend those by Wagner and Snyder<sup>15</sup>, who have mentioned persistent infection of *L* cells with the LCM virus in the absence of resistance-promoting factors in the medium. They contrast with these authors' finding, however, in that the carrier cultures "exhibited moderately increased resistance to superinfection with vesicular stomatitis virus". Traub and Kesting<sup>16</sup>, who worked with LCM-infected mouse lymph-node cells cultivated on glass, reported marked homologous interference but no suppression of the susceptibility to Eastern equine encephalitis and vesicular stomatitis viruses. Mims and Subrahmanyam<sup>17</sup> used mouse peritoneal macrophages and fibroblasts infected with LCM. Superinfection with the isologous virus did not change the proportion of cells containing antigen as revealed by fluorescing antibodies. As judged by the same criterion, LCM-infected macrophages were slightly resistant to ectromelia virus. Fibroblasts, on the other hand, were fully susceptible to the encephalomyocarditis and Semliki forest viruses. In these investigations interferon was not detected in cultures of cells infected with LCM virus.

In conclusion, cultures of cells infected with LCM are resistant to superinfection with the isologous agent or related strains; they do not release interferon and are, as a rule, not refractory to heterologous viruses. Of the many examples of persistent viral infection of cultivated cells which have been described in the past, more than one has been shown to exhibit similar properties. In fact, not one feature described here is uniquely associated with our carrier cultures. What sets the *L* (LCM) system apart is the faculty of the LCM virus not only to initiate and maintain a persistent infection in cells *in vitro*, but also to induce a status *in vivo*, that is in mice, which is similar in all major aspects. There the same criteria hold and can be listed as follows: (a) persistent viral infection in the absence of antibodies<sup>18-21</sup>; (b) few outward signs of illness and no major pathological alterations of the mouse despite continuous virus multiplication<sup>22-24</sup>; (c) the absence of interfering soluble factors in the tissues of LCM-infected mice<sup>15,17,21,25</sup>; (d) interference with superinfecting identical or closely related strains<sup>17,26</sup>; (e) lack of interference with at least two heterologous viruses, that is ectromelia<sup>17</sup> and yellow fever<sup>21</sup>. (The slight but undoubtedly significant resistance of LCM carrier mice

towards the EEE virus<sup>15,25,26</sup> is also exhibited by mice actively immunized with the LCM virus<sup>25</sup>. This type of refractoriness to a superinfecting virus, therefore, seems to result from some mechanism other than viral interference.)

This overall similarity of the properties of persistently infected mouse cell cultures as compared with the LCM carrier mice is indeed striking. While our primary objective is the immunological relationship between LCM virus and its natural host, a detailed knowledge of the events following the infection of cells maintained under defined conditions appears to be a prerequisite. With this in mind experiments are now being conducted to investigate the interaction of the LCM virus with its host on the cellular level, taking advantage of the availability of an *in vitro* model as has been described.

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## Competence in *Bacillus subtilis* Transformation System

by

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Immediately after uptake of tritiated DNA into the bacterial cell, the label is located adjacent to the cell wall. Cell fractionation studies indicate that the newly acquired DNA is bound to the cytoplasmic membrane, not the cell wall.

THE first step in the process of genetic transformation of cells by deoxyribonucleic acid involves a modification of the cell to permit binding of DNA. The mechanism by which cells develop the capacity to incorporate DNA (competence) still remains to be defined in molecular terms. As long as 20 years ago McCarty *et al.*<sup>1</sup> suggested that "it is not unreasonable to suppose that less extensive reversible alterations at specific sites of the surface of the pneumococcal cells can result from enzymatic actions and that these alterations make possible the adsorption or penetration of specific deoxyribonucleic acid". Extracellular activating factors have been identified in the pneumococcal<sup>2</sup> and streptococcal transformation systems<sup>3</sup>.

In *Bacillus subtilis* there are differences in the chemical composition and enzyme activity of cell walls of the highly transformable strain and mutants which are blocked in the capacity to incorporate DNA<sup>4,5</sup>. The relationship between the mode of action of the activating factors described in the pneumococcal and streptococcal transformation systems and the chemical and enzyme modifications in highly transformable strains of *B. subtilis* remains to be elucidated.

The present work was initiated to ascertain by morphological and chemical methods the location of donor DNA in the recipient cell immediately after irreversible uptake during the process of genetic transformation. The data



demonstrate that the DNA is bound to the surface of the cell in a DNase-insensitive and lysozyme-insensitive form most frequently in association with structures resembling fore-spores. These observations indicate that the DNA could be bound to the cytoplasmic membrane after transformation.

Competent cultures of *B. subtilis* were obtained by growing the highly transformable strain for 5 h in growth medium and for 60–75 min in the transformation medium at 36° C with vigorous aeration as described previously<sup>4</sup>. In these conditions transformation frequencies of 1 per cent to 4 per cent could be obtained for a number of genetic traits. Tritiated DNA was prepared by growing an adenine auxotroph or thymidine auxotroph on adenine-<sup>3</sup>H and thymidine-<sup>3</sup>H respectively<sup>4</sup>. The competent cultures used for the morphological experiments were incubated for 30 min at 35° C with DNA (2.5 µg/ml. specific activity, 1,310 c.p.m./µg of DNA) with vigorous aeration. The reaction was terminated with DNase (50 µg/ml.), and the cells centrifuged at 8,000 r.p.m. for 5 min at 4° C. The pellet was resuspended in cold minimal growth medium and then centrifuged again. This process was repeated twice to ensure the removal of degraded DNA and reversibly bound DNA. The cell pellet was then fixed in osmic acid<sup>6</sup>, processed as described by Kellenberger *et al.*<sup>7</sup>, embedded in methacrylate and in 'Epon' and sectioned on a Porter Blum microtome. Some of the sections were coated with Kodak nuclear track emulsion, stored at room temperature for 2 weeks in the dark and then processed as described by Salpeter and Bachmann<sup>8</sup>. In other experiments thick sections were examined directly. Two predominant forms exist in the population at the time of maximal competence (Fig. 1). One type (A in Fig. 1) has a large pale structure at one end of the cell which is surrounded by a double membrane. This structure in these thick sections is probably analogous to the ovoid form of presporulation (stage III) described by Ryter in a detailed morphological investigation of sporulation in *B. subtilis*<sup>9</sup>. Other cells (B in Fig. 1) are still in the vegetative phase of growth. Approximately 20 per cent of the cells have tracks produced by the disintegration of tritiated DNA. In 88 per cent of the cells examined the disintegration track of tritium was located adjacent to the cell wall–cytoplasmic membrane complex (Fig. 2). These tracks were most frequently seen in association with the pale structures tentatively identi-

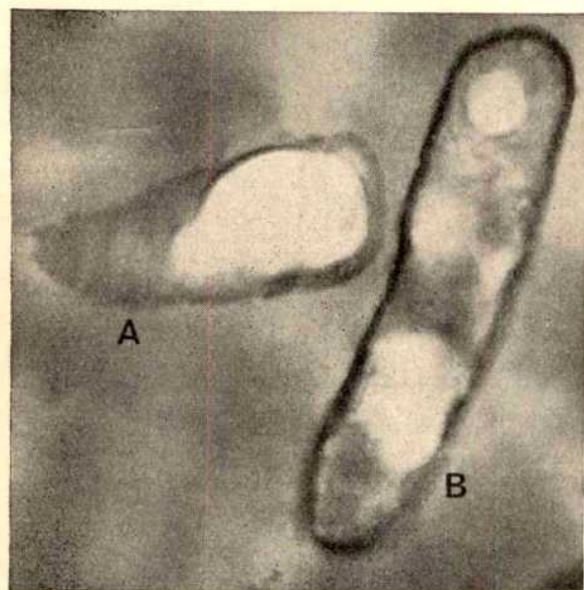


Fig. 1. Heterogeneity of a competent population of *B. subtilis*. Cell A is a cell with a large elliptical structure suggestive of a fore-spore. Cell B is a vegetative cell. (× 44,000.)

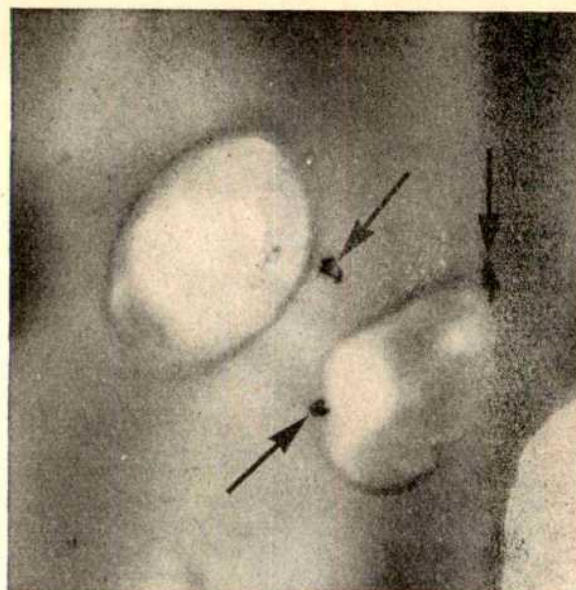


Fig. 2. Autoradiography of *B. subtilis* incubated with tritiated DNA. Disintegration tracks are indicated by the arrows. (× 48,000.)

fied as fore-spores. It is not possible to determine whether the disintegration tracks were related primarily to the cell wall, cytoplasmic membrane or fore-spore membranes.

To ascertain whether the newly acquired DNA was associated with the cell wall or cytoplasmic membrane, lysozyme was added to cultures of transformed cells at various intervals after the irreversible incorporation of DNA. Lysozyme solubilizes almost all of the teichoic acid in the cell wall. The sucrose in the medium stabilizes the protoplasts and prevents their lysis<sup>10</sup>. The addition of lysozyme resulted in greater than 99–96 per cent reduction in the cells which can withstand osmotic shock (residual viable count of protoplasts diluted in water). Table 1 shows that the amount of radioactive DNA in the supernatant liquid and the pellet is similar in the lysozyme-treated and control cultures. Less than 25 per cent of the radioactivity in the supernatant liquid is acid precipitable. Similar results were obtained with cells which were killed with ultra-violet light and by heating before the addition of lysozyme, and so the release of radioactive fragments of DNA during the incubation period with lysozyme apparently results from a transfer of fragments of DNA from the cell to the extracellular medium. The loss of radioactive DNA is not confined to cells incubated in the sucrose medium. A similar loss of radioactive fragments of DNA occurs during incubation of transformed cells in transformation medium. Again only a small portion of this radioactivity is acid precipitable. Lysozyme, therefore, does not liberate a significant amount of the newly acquired DNA immediately following transformation.

Table 1. LOCATION OF TRITIATED DNA AFTER IRREVERSIBLE INCORPORATION IN *B. subtilis*

Time of incubation (min)	Supernatant liquid		Pellet	
	SC (c.p.m./ml.)	LC (c.p.m./ml.)	SC (c.p.m./ml.)	LC (c.p.m./ml.)
0	273	255	690	828
30	152	142	560	523
60	159	177	568	503
120	179	139	395	386
180	122	133	326	308

A competent culture of *B. subtilis* 168 I-C<sup>+</sup> was exposed to tritiated DNA (0.13 µg/ml. specific activity 10<sup>5</sup> c.p.m./µg) for 15 min at 37° C. The reaction was terminated by DNase (10 µg/ml.) and the cells sedimented at 8,000 r.p.m. at 4° C. The pellet was washed three times with transformation medium, resuspended in transformation medium, divided into five aliquots, and incubated for 0, 30, 60, 120 and 180 min at 35° C. These cultures were centrifuged and resuspended in 0.5 molar sucrose<sup>10</sup>. Lysozyme (200 µg/ml.) was added to half of the cultures (LC), the other half received an equal volume of 0.5 molar sucrose (SC) and served as the control. After 20 min at 37° C the cultures were centrifuged at 4° C for 10 min at 9,000 r.p.m. The radioactivity of the supernatant liquid and the pellet was determined in a Packard 'Tri-Carb' scintillation spectrometer.



A more extensive release of radioactivity was observed with lysozyme from cells which had incorporated DNA-<sup>32</sup>P than was noted in the present investigation<sup>11</sup>. Two aspects of the previous investigation must be considered carefully. First phosphorus-32 rather than DNA-<sup>3</sup>H was used as the donor DNA. Second, it is not known whether the radioactive phosphorus released by lysozyme was in macromolecular DNA, oligonucleotides, or inorganic phosphorus. In *Pneumococcus* approximately 50 per cent of the total radioactivity incorporated during transformation exists initially as low molecular weight fragments<sup>12</sup>. One-quarter to one-third of this radioactivity is in inorganic phosphorus<sup>12</sup>, and so the re-utilization of labelled inorganic phosphorus in cell wall biosynthesis could produce teichoic acid labelled with phosphorus-32 which would be released by lysozyme. Thus, the greater release of radioactive material in the study of Miller and Landman<sup>11</sup> may be a result of the combination of transfer of degraded DNA to the extracellular medium (as noted in the present investigation) and the release of teichoic acid labelled with phosphorus-32.

During the penetration of DNA into the cell, the cell wall must be transversed. There are differences in chemical composition of the cell walls of highly and poorly transformable strains. Not only do the highly transformable strains contain more galactosamine than the poorly transformable strains so far investigated<sup>4</sup>, but the galactosamine content of the cell wall increased with growth, reaching a maximum at the time of maximal competence<sup>13</sup>. Galactosamine is isolated with the teichoic acid fraction of the cell wall<sup>5</sup>, and so it is possible that an increased substitution of galactosamine on terminal phosphorus groups could decrease the net negative charge of the cell wall and facilitate penetration of DNA. The modification of the cell wall by its indigenous autolytic enzyme *N*-acyl muramyl-L-alanine amidase might produce focal relaxation and gaps in the cell wall and thus facilitate the penetration of DNA<sup>5</sup>. Two observations strongly indicate that the initial irreversible complex between DNA and the cell does not result from binding of DNA to the cell wall. First, the irreversibly bound DNA cannot be stripped by lysozyme. Second, irreversible complexes cannot be formed between DNA and cell walls or DNA and cell wall polymers. Furthermore, cell wall polymers do not inhibit transformation. The data presented in this communication, however, cannot rule out a complex between the DNA and a lysozyme insensitive portion of the cell wall.

The capacity to sporulate is observed to be frequently associated with competence<sup>14,15</sup>. On the other hand, mutants have been isolated which are blocked in some aspect of sporulation, but are still susceptible to transformation<sup>16,17</sup>. In the absence of detailed morphological work on these organisms it is impossible to ascertain whether the early stages of sporulation occurred. On the basis of the frequent association of the capacity to sporulate with competence, and the morphological observations that the DNA was associated with cells containing structures resembling fore-spores, it is possible that the DNA is segregated after irreversible binding to the cytoplasmic membrane into the fore-spore and thus exists in a form which is not in equilibrium with the remainder of the genome of the cell. This process of segregation of DNA during fore-spore formation could be the driving force for uptake of DNA in a fashion analogous to that proposed for the replication of DNA during conjugation<sup>8</sup>. The subsequent failure of the sporulation processes to go to completion in minimal medium may produce a reversion to vegetative growth and permit complete recombination and expression of the newly acquired genetic information.

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## Metabolic Pathways of Red Blood Cell Copper in Normal Humans and in Wilson's Disease

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One of the symptoms of Wilson's disease (hepatolenticular degeneration) is an increased output of copper in the urine. This now seems to be linked with a defect in the binding of copper to amino-acid in the blood.

In recent years it has become increasingly apparent that the proper balance of the biologically available metals, such as copper, is necessary for the efficient metabolism and growth of human and animal organisms. Copper deficiency rarely occurs in humans<sup>1,2</sup>, and despite its widespread use in everyday life, poisoning by this metal is as unusual as its deficiency<sup>3</sup>. We know only one

hereditary abnormality, Wilson's disease (hepatolenticular degeneration; HLD), in which the inherited defect in the regulation of copper metabolism results in a progressive, fatal copper toxicity<sup>4</sup>.

Copper is essential for erythropoiesis in most mammalian species. There is a well established association between the shortened survival time of erythrocytes of

copper deficient pigs<sup>5</sup>, and anaemia associated with dietary copper deficiency in dogs and man<sup>2,6</sup>. It seems that the state and amount of copper in erythrocytes are important in maintaining the integrity of the cells.

Previous investigators showed that copper enters erythrocytes both *in vivo*<sup>2</sup>, after an oral or intravenous administration of copper, and *in vitro*<sup>7</sup>. It is present in red blood cells both as a minor diffusible and a major non-dialysable fraction<sup>8</sup>. The studies of Bush *et al.* showed the probable existence of these labile and stable copper pools in erythrocytes of normal individuals. It has been suggested that the stable pool is identical with erythrocytein, but the chemical nature of the labile pool has never been described<sup>9</sup>.

The copper enters erythrocytes from the plasma compartment<sup>9</sup>, and it could in theory originate from copper bound either to ceruloplasmin or albumin. Copper has never been shown to be dissociated from ceruloplasmin at physiological pH (7.4) either *in vitro* or *in vivo*<sup>10</sup>, and it was thought until recently that the transport source of copper is the albumin-bound fraction<sup>1,11</sup>. The latter was presumed to be freely diffusible across semi-permeable membranes, red blood cell membrane, glomerular capillaries, placenta<sup>12</sup> and blood brain barrier<sup>13</sup>. A previous report by one of us<sup>14</sup> seems to indicate that there is a small fraction of plasma copper, bound to amino-acids, which may be the physiologically important transport form of copper in blood, capable of diffusing across cell membranes. We have also shown that amino-acids, particularly histidine, facilitate the active transport of copper-64 in several mammalian tissues, for example, rat liver slices, rat kidney slices, Ehrlich mouse ascites carcinoma cells<sup>15</sup>, and human liver slices<sup>16</sup>.

It may therefore be postulated that the physiologically active transport form of copper is bound to amino-acid, which may have an important function in the transport of copper between the plasma and erythrocyte compartments.

The few investigators in the past who have studied the state of copper in red blood cells in patients with Wilson's disease found it to be not significantly different from normal human controls<sup>6,17</sup>. These cases were, however, under treatment with copper-chelating agents at the time of study and the results might have been affected by this.

The present article describes the rate of uptake, the role of amino-acids in uptake, the nature of transport, the chemical nature of the labile pool, and the dynamics and distribution of copper-64 in erythrocytes of an untreated 12 year old boy suffering from Wilson's disease and his 11 year old normal brother. The sibling was considered to be normal because of a normal serum ceruloplasmin and an abnormal rate of incorporation of copper-64 into ceruloplasmin *in vitro*.

Both *in vivo* and *in vitro* studies were carried out using cupric-64 acetate of high specific activity (10–20 mc./ $\mu$ g copper). All samples were counted in a well type scintillation counter of 5 per cent efficiency for copper-64. Standards were counted twice daily, and all counts were corrected for radioactive decay. In all procedures, copper-free needles, syringes and test-tubes were used.

The following experiments were carried out.

(1) *In vivo* uptake of copper-64 by erythrocytes. A normal subject and a patient with Wilson's disease (both males, 11 and 12 years of age, respectively) were given a single intravenous dose of 0.5 mg (1 mc.) of copper-64. The radioactivity was determined serially in 5 ml. portions of whole blood and 2 ml. of plasma. The samples were drawn through an indwelling catheter at 5, 10, 20 and 30 min and 1, 2, 3, 4, 5, 7, 10, 12, 18, 35, 42, 58, 61 and 63 h after injection of the radiocopper.

The radioactivity in erythrocytes was calculated directly by pipetting exactly 5 ml. of blood into counting vials, which were centrifuged for 3 min at 3,000 r.p.m. at 5°C. The plasma was discarded, the cells were washed

twice with ice-cold physiological saline, recentrifuged and counted immediately.

Results were no different when the red blood cell activity was calculated as the difference between plasma and whole blood activity. Haematocrits were determined in random samples, and showed no significant difference on any occasion.

On the first and second day of the experiment, blood was taken from both subjects and the total erythrocyte copper content was determined by the method of Eden and Green<sup>18</sup>.

During the experiment, both experimental subjects were kept on a low copper diet, containing less than 1.0 mg copper per day.

(2) *The effect of amino-acids on the transfer of copper through a semi-permeable membrane.* A 4 g per cent purified human albumin solution in Krebs-Ringer-bicarbonate (KRB) buffer of pH 7.4, with added copper-63 and copper-64 acetate, was dialysed through a semi-permeable membrane against the same albumin solution with no added copper at 37°C with constant shaking.

The twenty naturally occurring amino-acids were added to another portion of the same albumin solution in physiological concentration<sup>19</sup> and the same experiment was carried out.

The percentage of copper-64 dialysed across the semi-permeable membrane was determined serially up to 24 h.

Similar experiments were carried out using fresh serum of a normal donor and of the patient with Wilson's disease. A portion of this serum was first dialysed against five changes of five times the volume of Krebs-Ringer-bicarbonate buffer at pH 7.4 for a period of 3 h each, in order to dialyse out all diffusible constituents of serum, including amino-acids. Following this "predialysis", a mixture of copper-63 and copper-64 acetate was added to the portions of dialysed serum, in amounts to achieve an albumin : copper molar ratio of 1 : 2, and were then dialysed against either the "native" or "pre-dialysed" serum with no added copper.

(3) *The effect of amino-acids on uptake of copper-64 by red blood cells (RBCs).* In order to determine the effect of amino-acids on the uptake of copper-64 by RBCs from plasma, 300 ml. of blood was taken from a healthy donor, 24 h before the experiment, and collected in plastic bags containing 15 ml. of acid-citrate-dextrose solution. The erythrocytes were separated and discarded. The plasma was divided into two aliquots. One was stored at 5°C, the other was dialysed against five changes of five times volume of Krebs-Ringer-bicarbonate solution of pH 7.4 also at 5°C, in order to dialyse out all amino-acids from the plasma.

On the day of the experiment an additional 300 ml. of blood was taken from the same donor, and the red blood cells separated and kept at 5°C for less than 3 h.

5  $\mu$ c. of copper-64 (in 4  $\mu$ moles concentration) was added to 30 ml. portions of native and pre-dialysed plasma, and 0.3 ml. L-histidine was added in equimolar (4  $\mu$ moles) concentration to the different dialysed portions of plasma. This medium was then adjusted to pH 7.4 with 0.1 normal sodium hydroxide. To each 30 ml. portion of plasma 20 ml. of fresh packed RBCs were added. 1 ml. portions were taken at this time to determine the total activity in each sample.

2 ml. portions were withdrawn after 10, 20, 30, 60, 120, 180 and 200 min of incubation in the Dubnoff shaker at 37°C, in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. The erythrocytes were separated by centrifugation at 5,000 r.p.m. for 5 min, washed twice in ice cold 0.9 per cent sodium chloride and assayed for radioactivity. The uptake of copper-64 was expressed as c.p.m./ml. packed cells. In a similar experiment, a double label technique was used by adding <sup>14</sup>C-L-histidine of 0.5  $\mu$ c. activity to determine the possible uptake of histidine into red blood cells with the labelled copper. The change in the plasma level of <sup>14</sup>C-L-histidine was



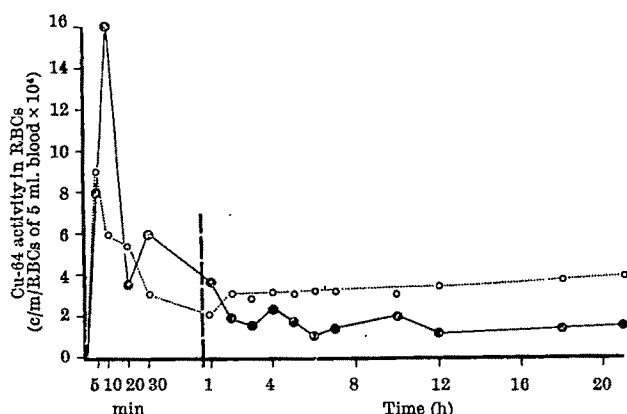


Fig. 1. The *in vivo* labelling of erythrocytes by copper-64 in normal (O) and Wilson's disease (●).

determined serially in 0.2 ml. of portions of plasma by counting it in a  $\beta$ -liquid scintillation counter 10 days after the experiments in order to allow for the disappearance of copper-64 activity.

(4) *The state of copper-64 in the in vivo labelled erythrocytes.* In order to determine whether copper-64 in erythrocytes, labelled *in vivo*, could pass from the cells back into the plasma, serial blood samples (5 ml.) were taken from the experimental subjects at different times after the intravenous administration of copper-64. The erythrocytes were separated by centrifugation at 3,000 r.p.m. for 5 min at 5° C, and the plasma discarded. The cells were washed twice in 5 ml. of ice cold 0.9 per cent sodium chloride, recentrifuged and assayed for copper-64 activity.

3 ml. of normal human plasma was added to the washed red blood cells, and the reconstituted blood was incubated at 37° C for 6 h in the Dubnoff metabolic shaker in an atmosphere of 95 per cent oxygen-5 per cent carbon dioxide. After incubation, 1 ml. of plasma was separated and assayed for radioactivity.

The activity remaining in the erythrocytes after incubation for 6 h was also determined by counting directly the twice washed and centrifuged red blood cells by procedures already described.

The experimental results can be summarized as follows:

(1) *The in vivo uptake of copper-64 into erythrocytes of normal and Wilson's disease subjects (Fig. 1).* In the normal human (O---O), there is an initial rapid uptake of copper-64 into the RBCs in 5-10 min, after which there is a decline in the RBC activity. This decline is followed by a secondary rise in the red blood cell copper-64 activity in 1 h, which is slower than the initial rise, and reaches a near maximum in 18-20 h.

The erythrocytes of the patient with Wilson's disease (●---●) differ from this pattern. Initially, there appears to be significantly greater uptake, followed by a precipitous decline in RBC copper-64 content. This is followed by fluctuations in the activity of red blood cells, which continue for several hours and only after a delay of 10-12 h is there a secondary rise in erythrocyte copper-64 activity, which is, however, much lower than in the normal subject.

The total red blood cell content on two consecutive days was found to be no different in the normal subject and in his diseased sibling, that is, 60, 69  $\mu$ g per cent and 69, 67  $\mu$ g per cent, respectively.

(2) *The effect of amino-acids on the transfer of copper-64 across a semi-permeable membrane.* This was investigated using a purified albumin solution (O---O), normal fresh human serum ( $\Delta$ --- $\Delta$ ), and the serum of the patient with Wilson's disease (x---x) as basic media. The results are shown in Fig. 2.

When a 4 g per cent solution of albumin in Krebs-Ringer-bicarbonate buffer at pH 7.4 with added copper-64 was dialysed through a semi-permeable membrane against the same albumin solution with no added copper, almost

no transfer occurred in 24 h (O---O). The addition of the twenty naturally occurring amino-acids in physiological concentration resulted in a 40 per cent transfer of copper-64 during the same period (●---●). When normal human serum was dialysed before an identical experiment in order to remove most of the amino-acids, it behaved in exactly the same way as purified albumin ( $\Delta$ --- $\Delta$ ), while native serum, with all its amino-acids present, also resulted in a 40 per cent transfer in 24 h ( $\Delta$ --- $\Delta$ ). Serum from the patient with Wilson's disease (x---x) gave similar results.

The results suggest that amino-acids are important in making albumin-bound copper diffusible, and that this action on the transfer of copper-64 across a 'Cellophane' membrane is similar *in vitro*, whether purified human albumin solution, normal human serum or serum of a patient with Wilson's disease is used.

(3) *The transport of copper-64 into erythrocytes as facilitated by amino-acids in vitro (Fig. 3).* The presence of the naturally occurring amino-acids in serum results in a significantly greater uptake of copper-64 into red blood cells (●---●), compared with the same serum after dialysis and removal of most of the amino-acids (O---O).

When 4  $\mu$ molar L-histidine ( $\blacklozenge$ --- $\blacklozenge$ ) is added to medium free from amino-acid, greater uptake of copper-64 results than in the control experiments. There is an initial rapid uptake of copper-64 during the first 5 min, followed by a decline in RBC copper-64 content. This

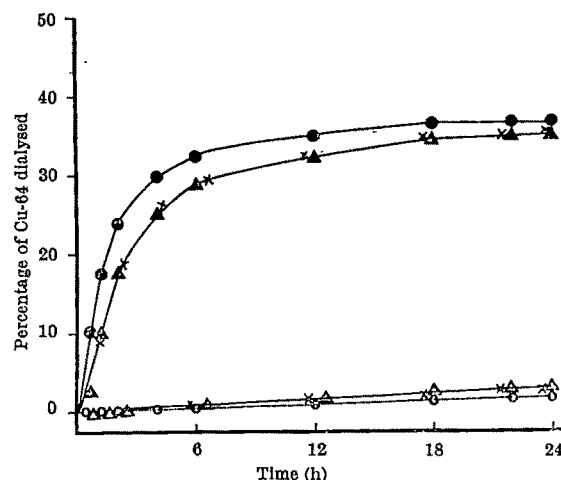


Fig. 2. The effect of amino-acids on the *in vitro* transfer of copper-64 across a semi-permeable membrane. (Copper/albumin molar ratio 2:1, pH 7.4, 37° C.) O, Albumin (human); ●, albumin + 20 amino-acids;  $\Delta$ , human serum;  $\Delta$ , dialysed human serum; x, Wilson's disease serum.

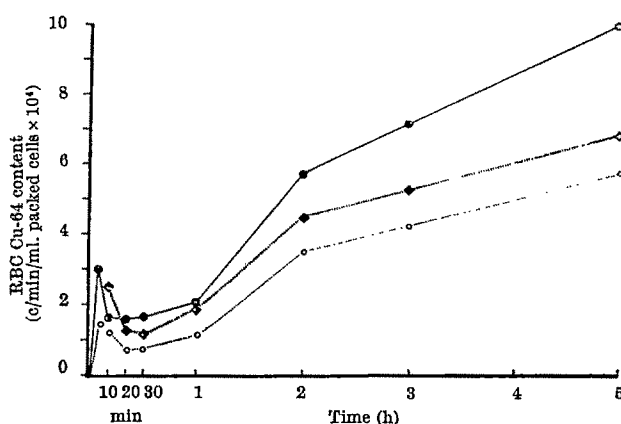


Fig. 3. The effect of amino-acids on the uptake of copper-64 by erythrocytes *in vitro*. (95 per cent oxygen-5 per cent carbon dioxide, pH 7.4, 37° C.) O, Dialysed serum (no amino-acids); ●, native serum (all amino-acids); ◆, dialysed serum + histidine.

rapid initial uptake and decline of copper-64 is followed by a slower rate of uptake of the isotope over the period of the experiment.

These results suggest that amino-acids enhance the uptake of copper by normal erythrocytes *in vitro*.

The ratio of the activity in 1 ml. of packed red blood cells to the activity of 1 ml. of plasma is less than unity. A ratio less than one suggests diffusion<sup>20</sup>, and it appears that histidine and other amino-acids enhance the uptake of copper-64 into red blood cells by making the albumin-bound copper diffusible and that the mode of entry of copper into RBCs is apparently by diffusion. In experiments in which a double label technique was used with <sup>14</sup>C-L-histidine and copper-64, both in 4  $\mu$ molar concentrations (Fig. 4), however, facilitated diffusion is suggested.

The changes in the level of labelled histidine (●—●) in plasma were determined in relation to the uptake of copper-64 (○—○) into the red blood cells (Fig. 4). Initially, there is a rapid uptake of copper-64 into the red blood cells, and a disappearance of histidine carbon-14 activity from the medium. With the counterflux of copper from the RBCs after the initial uptake there is a corresponding increase in the concentration of carbon-14 histidine in the medium. The beginning of the secondary uptake of copper-64 by RBCs corresponds with the disappearance of <sup>14</sup>C-histidine from the medium. At 1 h the plasma <sup>14</sup>C-histidine level rises again, as if transport from then on reaches a steady equilibrium.

These results suggest that the uptake of copper-64 by RBCs is related to a lowering of the histidine concentration in the suspending medium, because copper and histidine are probably moving simultaneously from the plasma compartment into the erythrocyte fraction.

(4) *The state of diffusibility of copper-64 in the in vivo labelled erythrocytes at different intervals of time after the intravenous administration of copper-64 (Fig. 5).* In normal erythrocytes (○—○) the amount of dialysable copper-64 decreases sharply with time, and 35 h after the

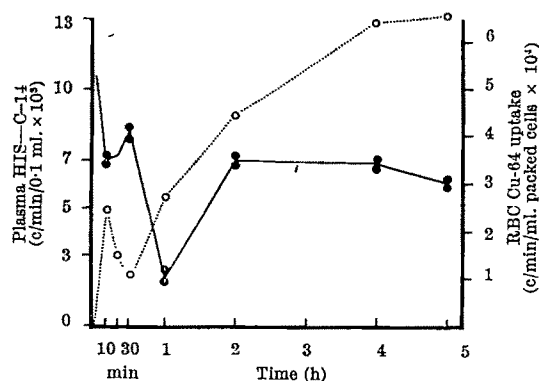


Fig. 4. The *in vitro* relationship of plasma histidine carbon-14 concentration to uptake of radiocopper-64 by erythrocytes. (64  $\mu$ moles, H15-4  $\mu$ moles, pH, 7.4, 37° C). ●, H15 C-14 in plasma; ○, Cu-64 uptake by RBCs.

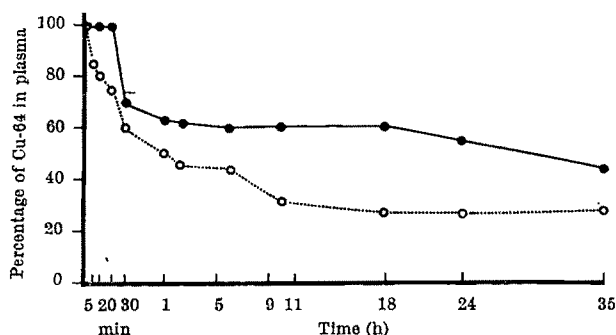


Fig. 5. Total diffusible copper-64 fraction of *in vivo* labelled erythrocytes in normal (○) and in Wilson's disease (●).

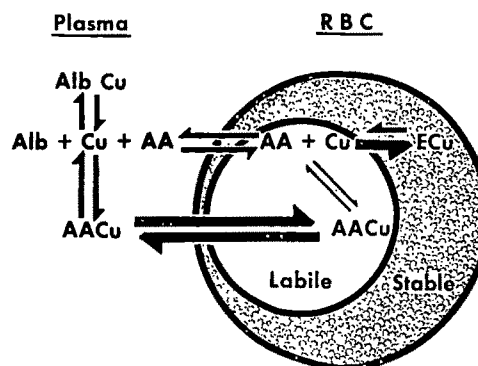


Fig. 6. Hypothetical metabolic pathways of red blood cell copper. Alb Cu, Albumin bound copper; AA, amino-acids; AACu, amino-acid-copper complex; FCu, erythrocyte copper.

injection of radiocopper-64, only 20–30 per cent of the initial activity remains in a labile, dialysable state.

In Wilson's disease (●—●), on the other hand, during the first 20 min after injection all the copper-64 in red blood cells is dialysable, and at 18 h 60 per cent of the erythrocyte copper-64 content can still be diffused. From then on, there appears to be a gradual decline in the total diffusible fraction, but it is still significantly greater than normal at 35 h.

The present studies suggest that amino-acids play a part in the uptake of copper into erythrocytes. Both the *in vivo* and *in vitro* experiments show that there is an initial rapid transport of copper-64 by the erythrocyte fraction in the first few minutes. This flux of copper-64 appears to be followed by a counter-flux of copper out of the cells. A similar phenomenon of transport and counter-transport has recently been reported by Winter and Christensen, who investigated the migration of amino-acids across the membrane of the human erythrocyte<sup>21</sup>.

The exact nature of the copper transport into erythrocytes as facilitated by amino-acids is not fully explained by our experiments. We cannot exclude the possibility that the initial hump is due to adsorption on the cell surface. The entry of copper by simple diffusion through a lipid barrier seems unlikely to account for the phenomenon of counter-transport. Further experiments are in progress to elucidate this problem.

The initial phenomenon of rapid transport and counter-transport of copper-64 is followed by a slower rate of uptake of copper-64 by erythrocytes. This can be demonstrated in both the *in vitro* and *in vivo* experiments. The rate of this secondary uptake of copper-64 is significantly lower than the initial rate of uptake, and reaches a near maximum in 24 h in the *in vivo* experiments.

This secondary uptake of copper-64 into erythrocytes corresponds with the formation of a non-dialysable, stable copper complex in erythrocytes. The rate limiting factor may be the rate of formation of this latter complex. This portion of erythrocyte copper-64 probably represents binding of the radiocopper-64 to a copper protein of RBCs: "erythrocyte copper-64" complex. Although there is no evidence that erythrocyte copper exists as an apoprotein, or one with only a partial complement of copper, this must be seriously considered, unless one postulates the existence of a new stable copper-protein complex.

The erythrocytes of the patient with Wilson's disease seem to differ from the normal, mainly in a quantitative manner. There is significantly greater initial uptake of copper-64 by his erythrocytes. This initial greater uptake cannot be explained by the difference of the serum albumin binding capacity of Wilson's disease, because we have shown (Fig. 2) that it behaves in exactly the same way in normal human serum. Nor is it due to increased serum amino-acid concentrations in Wilson's disease, because these are known to be normal<sup>19</sup>. It seems likely to be

related to the fact that the half-life of circulating albumin-bound copper-64 is usually longer in Wilson's disease. In these experiments it was found to be 13 min in the patient with Wilson's disease, compared with 8 min in the normal subject. In time, however, incorporation of copper-64 into the non-dialysable form will take place in HLD erythrocytes, indicating that the copper bound to "erythrocuprein" is in a slow dynamic equilibrium with the copper present in a labile form in red cells. A similar reduction in RBC stable copper was found in one of two patients with Wilson's disease studied by Shields<sup>24</sup>. This defect in copper-64 incorporation into the stable pool may have a parallel with the hepatic defect in ceruloplasmin formation.

Table 1. CHARACTERISTICS OF RED BLOOD CELL COPPER

Features	Labile pool	Stable pool
Chemical nature	Amino-acid bound	Protein bound ("Erythrocuprein")
Affinity for copper	? Non-specific	Specific
Copper turnover	Fast	Slow
Nature of transport	Bidirectional	Primarily unidirectional
Origin	Diffusion (? facilitated)	Unknown
	Extracellular fluid (plasma)	Intracellular fluid (RBC)
Distribution of copper-64 35 h after intravenous injection		
Normal RBC	20-30 per cent	70-80 per cent
Wilson's disease RBC	50-60 per cent	40-50 per cent

The experiments reported in this article confirm the previous findings of two copper pools in erythrocytes in both normal and Wilson's disease subjects (Table 1).

(1) A dialysable compartment, in which the movement of copper is fast and bidirectional. The chemical nature of this pool is probably copper bound to amino-acids. Its copper originates from the albumin bound copper of plasma, which is made more freely diffusible through cell membranes by amino-acids. This pool has been called the "labile" pool by Bush<sup>6</sup>.

(2) A non-dialysable copper pool which binds copper strongly, and its copper turnover is slow and primarily unidirectional. The chemical nature of this pool is likely copper bound to erythrocuprein, a protein of molecular weight 33,400, which binds two atoms of copper per molecule of protein<sup>23</sup>. In the normal erythrocyte, when this pool is labelled *in vivo*, only 20-30 per cent of the total red blood cell copper-64 is found in this compartment. The exact nature of the transport across erythrocyte membranes has not yet been completely elucidated, but it is likely to be facilitated diffusion. The nature of the transport of copper from the amino-acid pool into the stable pool is unknown. The formation of this stable copper pool in Wilson's disease is much delayed, but we have no evidence at present which indicates that this

may be primary or secondary. The diagrammatic representation of the hypothetical metabolic pathways of red blood cell copper is shown in Fig. 6.

In conclusion, we have studied the uptake and kinetics of radiocopper-64 by human erythrocytes in normal people and in a patient with Wilson's disease *in vivo* and *in vitro*. Amino-acids increase the rate of uptake of copper-64 by erythrocytes, probably by facilitated diffusion. The chemical nature of the "labile" pool in erythrocytes appears to be diffusible copper bound to amino-acid. A phenomenon of transport and counter-transport is described for copper in the human erythrocyte.

Apparent kinetic differences have been observed between the erythrocytes of two normal people and one patient with untreated Wilson's disease. An initial greater uptake and a marked delay in incorporation of copper-64 into the non-diffusible pool were noted. More cases will have to be studied to verify and elaborate on these findings.

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## Cortisol in the Regulation of RNA and Protein Synthesis

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The initial events involved in the regulation of RNA and protein synthesis by the hormone cortisol are rapid. This suggests that steroid hormones act as effector molecules in induction and repression.

HORMONE control of gene expression is clearly demonstrated by morphological evidence which is receiving increasing support from biochemical data (reviewed by Davidson<sup>1</sup>). Interest in the molecular basis by which hormones operate gene functions centres particularly on the way in which they can achieve selectivity of action, causing the induction of some cistrons and the repression of others in the same genome<sup>2</sup>. These selective effects,

which possibly include competitive, antagonistic actions of two or more hormones on the synthesis of a single gene product<sup>3</sup>, may collectively represent a system of hormonal programming of gene expression<sup>4</sup>.

Talwar *et al.*<sup>5</sup> have reported evidence that oestradiol-induced derepression of RNA synthesis in rat uterus is mediated through an oestradiol-binding protein. T'so and Lu<sup>6</sup> observed binding of testosterone to DNA, a finding which could suggest a direct action of the hormone on transcription, but others<sup>7</sup> have failed to obtain evidence

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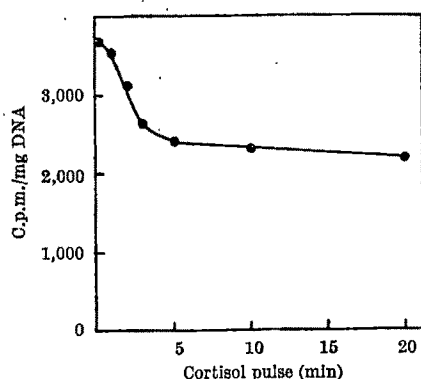


Fig. 1. Rapid effect of cortisol on the synthesis of rapidly labelled RNA in rabbit lymph node cells. Cortisol (5  $\mu$ g/ml.) was added at varying times before a standard 2 min pulse of  $^3$ H-uridine (10  $\mu$ c./ml.). 5 ml. incubation volume.

for DNA-binding of cortisol. Certain data based on actinomycin-inhibition of the induced synthesis of enzymes by hormones suggest that the hormone effect involves the induced synthesis of the RNA template<sup>8</sup>, while other evidence from actinomycin experiments suggests that in some cases a hormone may control translation of the RNA template into enzyme protein<sup>9</sup>.

At the present time it is not possible to visualize a single functional model which will account for the control of RNA and protein synthesis by steroid and peptide hormones, by different hormones in the same class, or even by a single hormone on different genetic loci. Many of the effects of cortisol, for example, have been studied over periods of several hours<sup>7</sup>, times which are long when considering the rapid events involved in the initiation of RNA and protein synthesis. Recently, however, it has been demonstrated that cortisol alters RNA synthesis in seconds or minutes<sup>10</sup>, times which are more in keeping with the expected kinetics of initial events in induction and repression. Before it is feasible to build even preliminary models of hormone action on gene expression it is essential to know whether altered transcriptional (RNA synthesis) or translational (protein synthesis) events occur first and to know whether these alterations may be dissociated in time. The present studies, which examine critically the kinetics of cortisol action on RNA and protein synthesis in short time periods in isolated lymphoid cells, suggest that these events can be dissociated to some extent and that the initial effects are directed at the level of transcription.

Suspensions of lymphoid cells were prepared from mesenteric lymph nodes of two or more rabbits<sup>10</sup> and incubated in Dulbecco medium<sup>11</sup> containing 1 per cent glucose. The cell suspension was divided into a number of aliquots to allow internal controls in each experiment. Cortisol, as hydrocortisone sodium succinate, was added in solution where indicated. RNA was pulse-labelled with  $^3$ H-uridine; protein was labelled with  $^3$ H- or  $^{14}$ C-leucine, or with  $^3$ H- or  $^{14}$ C-phenylalanine. RNA synthesis was expressed per mg DNA and newly synthesized RNA was isolated by a modified<sup>10</sup> two-stage phenol procedure<sup>12</sup>. In experiments measuring protein synthesis, incubation was ended by the addition of ice-cold, unlabelled leucine or phenylalanine (to give a dilution  $> 1/1,000$ ), followed by half a volume of 20 per cent trichloroacetic acid. The precipitate was washed with boiling trichloroacetic acid, cold trichloroacetic acid, ethanol and acetone. Radioactivity was assayed in a liquid scintillation counter; protein was first dissolved in tetraethylammonium hydroxide before adding the liquid phosphor.

In one group of experiments the effect of cortisol on RNA synthesis was assessed in spleen cells from rats adrenalectomized three to five days previously and maintained on saline, without exogenous steroids. Spleens were removed, cell suspensions prepared<sup>10</sup>, and RNA

synthesis measured after incubation in the presence or absence of cortisol.

Cortisol rapidly alters the synthesis of rapidly labelled RNA in lymphoid cells, as illustrated in Fig. 1. In this type of experiment cortisol was given for varying times before a 2 min pulse with  $^3$ H-uridine. Other experiments<sup>10</sup> which have examined the effect of a standard cortisol pulse and varying pulse-times of  $^3$ H-uridine suggest that the initial effect is probably initiated in seconds, becoming readily measurable in 1–2 min. Adrenalectomy accentuates this rapid effect of cortisol on RNA synthesis (Table 1). Basal RNA synthesis was greater in spleen cells from adrenalectomized rats than in cells from controls, and the proportional change in rate of labelling of RNA when incubated with cortisol was greater in cells from adrenalectomized than from control animals. It is evident that in both types of lymphoid tissues—rabbit lymph node cells and rat spleen cells—the net effect caused by cortisol is a decrease in RNA synthesis, as observed by Feigelson and Feigelson<sup>7</sup> during periods of several hours. The method of RNA preparation used excludes terminally labelled rRNA (ref. 10), but the rapidly labelled material may include RNA other than messenger RNA (refs. 10 and 13). The cortisol effect is selective in these tissues as it is in rat liver<sup>2</sup>.

Table 1. EFFECT OF SHORT PULSES OF CORTISOL ON INCORPORATION OF  $^3$ H-URIDINE (5 MIN PULSE) INTO RNA OF SPLEEN CELLS FROM INTACT AND ADRENALECTOMIZED RATS

Treatment	Intact rats (c.p.m./mg DNA)	Per cent decrease + cortisol	Adrenalectomized rats (c.p.m./mg DNA)	Per cent decrease + cortisol
Control (– cortisol)	5,315	–	9,950	–
+ Cortisol (1 min)	4,440	16	7,700	23
+ Cortisol (5 min)	3,540	33	4,900	51

The ultimate effect of cortisol on protein synthesis in lymphoid tissues is also a net decrease<sup>14</sup>. A kinetic analysis of short periods of time, however, indicates that a rather complex series of events precedes this general decrease. Fig. 2 shows the effect of a pulse of cortisol added to the cell culture at various times before a standard incubation of 20 min with radioactive leucine. The eventual decrease in rate of protein synthesis was preceded by a significant rise in the rate of leucine incorporation, which was maximal with a pre-leucine cortisol pulse of about 5 min. When the leucine incubation period was reduced to 5 min a delay was apparent in the appearance of this "burst" of increased protein synthesis (Fig. 3), suggesting that some time (10–15 min) is required before any effect of cortisol on protein synthesis becomes measurable.

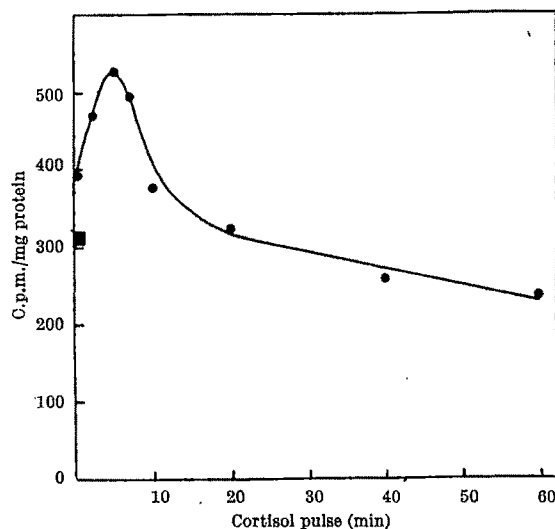


Fig. 2. Effect of cortisol on protein synthesis in rabbit lymph node cells. Cortisol (5  $\mu$ g/ml.) was added at varying times before the addition of  $^3$ H-leucine (5  $\mu$ c./ml.) for 20 min. 2 ml. incubation volume. ■, Control; ●, cortisol.

This observation was verified in another way, by using a standard cortisol pulse (5 min) and varying leucine pulse-times (Fig. 4a). Increased  $^3\text{H}$ -leucine incorporation in the presence of cortisol was measurable only after 15–20 min. A similar result was obtained using  $^3\text{H}$ -phenylalanine (Fig. 4b). If earlier effects are exerted by cortisol on amino-acid incorporation into protein, they are not recognizable by these techniques. The present results do not exclude, of course, qualitative changes in protein synthesis which may be induced earlier by cortisol.

The times of both RNA and protein synthesis effects could be influenced by the rate of uptake of cortisol into the lymphoid cells, a possibility which was investigated by the use of radioactive cortisol. Cortisol-1,2- $^3\text{H}$  was converted to the succinate by incubation with succinic anhydride in the presence of pyridine, the pyridine then being removed under vacuum. The water-soluble  $^3\text{H}$ -cortisol succinate was added to cell suspensions which had been incubated at  $37^\circ\text{C}$  for varying times before they were poured into 20 volumes of ice-cold Dulbecco medium. The cells were washed three times with ice-cold medium to remove non-adsorbed cortisol, then the amount of cortisol taken up by the cells was measured by assaying radioactivity. Uptake of the steroid was linear during the first 2 min and reached a maximum after about 5 min (Fig. 5). Cortisol-induced decrease in the rate of RNA synthesis in lymphoid cells is also maximal after about 5 min<sup>10</sup>. The correspondence of these two times suggests that the extent of the measurable cortisol effects on RNA synthesis is limited by the time required to achieve maximal availability of intracellular steroid. This observation supports the conclusion from previous data<sup>10</sup> that cortisol exerts its effect on RNA synthesis extremely rapidly, probably within seconds of entry into the cell.

The sequence of events which follow the uptake of cortisol into the lymphoid cells thus appears to be: (1) a net decrease in the rate of synthesis of rapidly labelled RNA, occurring in seconds; (2) an initial lag of several minutes before there is any effect on protein synthesis; (3) a "burst" of increased protein synthesis, maximal after 15–20 min; (4) a final net decrease in the rate of protein synthesis. The inference is that transcriptional (RNA synthesis) effects caused by cortisol precede translational (protein synthesis) effects. The transcriptional effects could be induced by direct binding of the steroid to DNA (ref. 6) (but this is unlikely in view of negative evidence for cortisol-DNA association<sup>7</sup>) via protein-bound hormone<sup>8</sup> or even by direct binding of the steroid to RNA. The kinetics indicate, however, that any binding which is operative in the initial stages of cortisol action on RNA synthesis must be between the steroid and pre-existing macromolecules.

A decrease in messenger RNA synthesis would be expected to result in decreased synthesis of the corre-

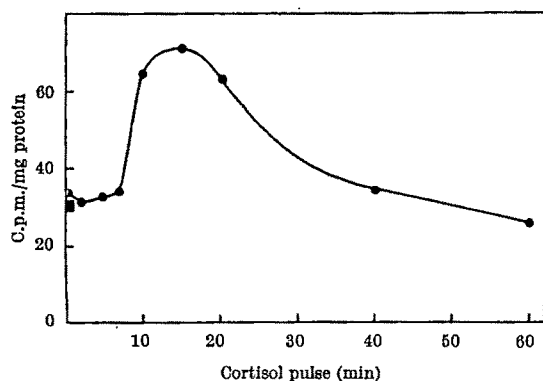


Fig. 3. Effect of cortisol at an earlier stage of protein synthesis in rabbit lymph node cells. Cortisol ( $5\text{ }\mu\text{g/ml}$ ) was added at varying times before the addition of  $^3\text{H}$ -leucine ( $5\text{ }\mu\text{C/ml}$ ) for 5 min. 2 ml. incubation volume. ■, Control; ●, cortisol.

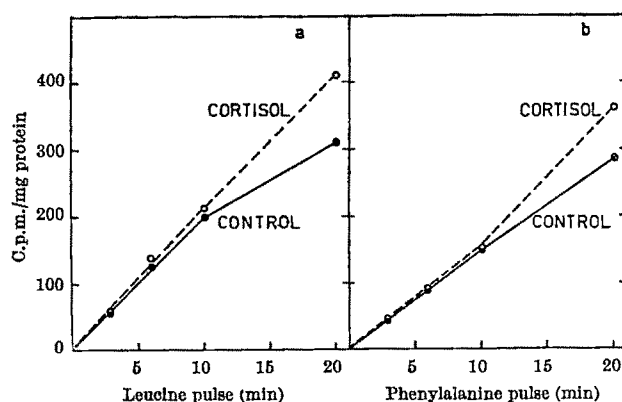


Fig. 4. Effect of cortisol on the incorporation of leucine and phenylalanine into protein in rabbit lymph node cells. A standard 5 min pulse of cortisol ( $5\text{ }\mu\text{g/ml}$ ) was followed by the addition of (a)  $^3\text{H}$ -leucine ( $5\text{ }\mu\text{C/ml}$ ) or (b)  $^3\text{H}$ -phenylalanine ( $5\text{ }\mu\text{C/ml}$ ) for varying time periods. 2 ml. incubation volume.

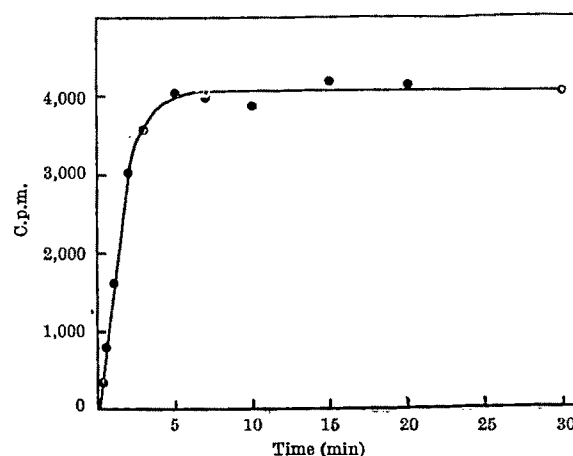


Fig. 5. Uptake of  $^3\text{H}$ -cortisol by rabbit lymph node cells.  $^3\text{H}$ -cortisol ( $5\text{ }\mu\text{C/ml}$ ) was added to 2 ml. volumes of cell culture at zero time, then the culture was poured into 20 volumes of ice-cold medium at varying times to dilute radioactivity and limit further absorption.

sponding proteins, following a lag related in length to messenger stability. The occurrence of an intervening "burst" of increased protein synthesis, however, indicates that a more complex situation exists. The most probable explanation is that the rapidly labelled RNA is comprised of heterogeneous species, and that in addition to messenger RNA and ribosomal RNA precursors, some of this RNA subserves regulatory functions. Assuming that many messenger RNAs are fairly stable<sup>15</sup>, a simple model will explain the present data (Fig. 6). Such a model does not distinguish between direct repressor function of some rapidly labelled RNAs and repressor proteins synthesized on these RNAs as templates. What it does indicate is that repressor RNAs or repressor template RNAs would require to be less stable than the messenger RNAs (coding structural or enzyme proteins) the function of which they regulate. Considered from the point of view of induction or repression of a structural (enzyme) protein, the hormone would actually appear to act at the level of translation rather than transcription<sup>3,9</sup>.

Alternatively, it is possible that some of the "burst" proteins are regulatory in function (Fig. 7). In this model, the binding of cortisol to repressor proteins would lead both to the repression of messenger RNA synthesis and to the increased "burst" synthesis of more repressor proteins. This would imply that repressors regulated their own synthesis; it would also imply that synthesis of such repressors is inducible, an observation which so far seems unlikely in bacteria<sup>16</sup>.



It will not be possible to decide between models of this type or others until more knowledge is available on the nature of macromolecular binding of cortisol relevant to its gene regulatory functions. To account for the observed selectivity of action<sup>2</sup> of cortisol in a given tissue and the differing responses of liver and lymphoid tissues to cortisol<sup>7,10</sup>, the hormone must be able to cause both induction and repression of different regions of the same genome, a possibility which, on theoretical grounds, is most likely to result from the versatility of hormone-protein complexes<sup>17</sup>. What the present data show is that the initial events involved in cortisol regulation of RNA and protein synthesis are extremely rapid, a finding which favours a

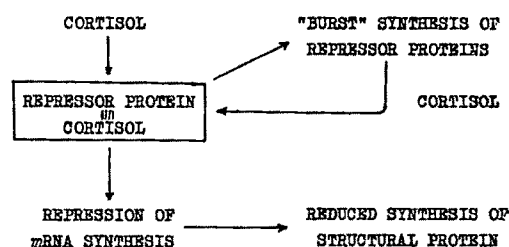


Fig. 7. Alternative model of cortisol regulation of lymphoid RNA and protein synthesis.

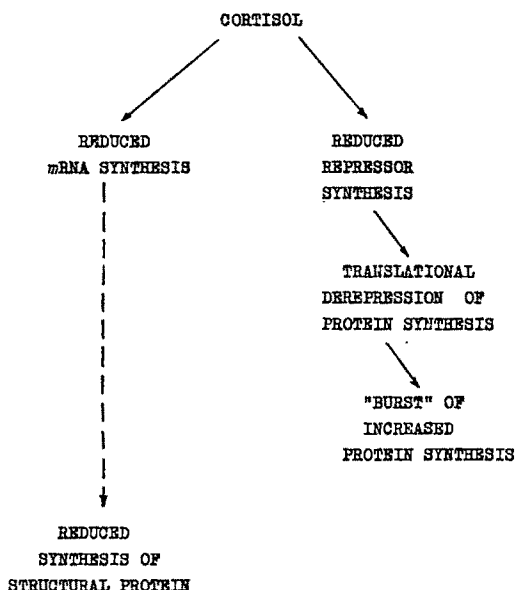


Fig. 6. Model of possible sequence of events in the regulation by cortisol of lymphoid RNA and protein synthesis.

direct role of the steroid hormone as an effector molecule in induction and repression.

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## Sorption and Swelling within Wood Cell Walls

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Rates of swelling of wood cell walls in water and alcohol suggest that the mechanism of uptake of liquid and vapour involves two stages—wetting or adsorption at capillary surfaces followed by molecular penetration and swelling of the solid phase.

MANY years of research have not completely revealed the mechanism by which water enters or leaves the wood cell wall in the course of the swelling or shrinkage of wood<sup>1</sup>. It has generally been assumed that the rate of change of moisture content is controlled by diffusion, and complicated by the intricate cell wall structure and probable occurrence within the cell wall, at least in its saturated state, of capillaries or continuous water pathways. I have shown, however, that so long as specimens are made sufficiently thin, the rates of sorption of water vapour in the absence of air by wood<sup>2</sup>, wood pulp<sup>3</sup>, hemicellulose<sup>4</sup> and regenerated cellulose film<sup>5</sup> are determined by a mechanism which differs markedly from diffusion in its

dependence on the conditions of sorption. As evidence for this conclusion, it was found that (a) below a limiting thickness, sorption rate was independent of material thickness; (b) for moisture increments of a given size, the sorption rate was markedly lower at higher initial moisture contents, whereas the diffusion coefficient is known to be greater at higher moisture contents; and (c) for the same final moisture content, large moisture increments were completed much faster than smaller ones.

Following Bagley and Long<sup>6</sup> and later workers, it was postulated that the rate of sorption by such thin materials was limited by relatively slow molecular re-arrangements

within the swelling substance. It was further suggested that the speed of this re-arrangement was dependent on swelling pressure or change in free energy, because the rate of sorption resulting from a change in relative vapour pressure from  $p_i$  to a final value of  $p_f$  was found to be related empirically to  $\log p_f/p_i$ , for values of  $p_i$  other than zero.

This work has now been extended to measurement of the rates of swelling of wood cell walls after rapid wetting with liquid. It soon became clear that the above mechanism alone could not account for the behaviour observed when liquid replaced vapour as the external phase, nor were the results any more consistent with diffusion as the rate-controlling mechanism than with sorption of vapour.

The experiments were conducted mainly on the wood of klinki pine (*Araucaria hunsteinii* K. Schum. syn. *A. klinkii* Lauterb.) with fibres about 7 mm long, and with walls 3–4  $\mu$  thick. The liquid, namely water, methanol or ethanol, was brought into intimate contact with all cell wall surfaces in a very short space of time, generally less than 0.2 sec and, in some cases, within 0.02 sec. This was achieved by using cross sections of wood approximately 2–3 mm in the fibre direction, that is, shorter than the fibre. Specimens were initially evacuated and either completely dried or conditioned to equilibrium at a selected vapour pressure in the absence of air. De-aerated liquid under atmospheric pressure was then rapidly admitted to the specimen and the course of transverse swelling of the specimen was recorded photographically at up to 8,000 exposures/min. It was assumed that the measured external swelling of the specimen reflected the behaviour of all its fibres swelling simultaneously.

Table 1. RATES OF SORPTION AND SWELLING CHARACTERIZED BY THE RECIPROCAL OF THE HALF-TIME\*

	Sorption of vapour		Swelling in liquid ( $p_f=1$ )		
	Water	Methanol	Water	Methanol	Ethanol
$p_i = 0$	$2 \times 10^{-4}$	$1 \times 10^{-3}$	1.5	$1 \times 10^{-1}$	$1.5 \times 10^{-2}$
$\frac{p_i}{p_f} = 0.7$	$6 \times 10^{-4}$	$1 \times 10^{-4}$	1	$1.5 \times 10^{-3}$	$2 \times 10^{-5}$
$\frac{p_i}{p_f} = 0.8$	$2.5 \times 10^{-4}$	$2.5 \times 10^{-5}$	$2 \times 10^{-1}$	$3 \times 10^{-4}$	$3 \times 10^{-6}$
$\frac{p_i}{p_f} = 0.9$	$3 \times 10^{-4}$	—	$5 \times 10^{-2}$	$2.5 \times 10^{-4}$	—

The measurements (in sec) were made on wood of klinki pine.

\* The "half-time" is the time taken to reach half the change in sorption or swelling occurring between the initial and final equilibrium conditions.

The main observations from these measurements of rate of swelling include the following. (Some of these observations are illustrated by the results in Table 1.) (a) The rates of swelling in liquid (water and methanol) were several orders of magnitude faster than the rates of sorption of the corresponding vapour. (b) Specimens conditioned first to alcohol vapour swelled more slowly on immersion in the corresponding liquid than specimens not conditioned. The swelling rate was strongly dependent on  $p_i$  in a manner closely parallel to the dependence of the vapour sorption rate on  $p_i/p_f$ . (c) Swelling in water showed a similar strong dependence on  $p_i$ , but only for values of  $p_i$  greater than about 0.7. For  $p_i$  of less than 0.7 the swelling rate was little different from that of dry wood. (d) At values of  $p_i$  below about 0.7, the swelling rate in water was strongly dependent on cell wall thickness, for example, ten times faster for samples of balsa (1  $\mu$ ) than for klinki pine (3–4  $\mu$ ) and faster for early wood than for late wood of radiata pine. At values of  $p_i$  above 0.7, that is, when the swelling rate in water was strongly dependent on  $p_i$ , differences caused by cell wall thickness were reduced or disappeared. (e) Swelling rates in methanol and ethanol were about three and five orders of magnitude lower respectively than the corresponding rates of swelling in water (for comparisons at  $p_i$  values of 0.7 or greater).

The following suggestions are now put forward as to a possible mechanism by which water or water vapour is taken up by wood cell walls. In arriving at this mechanism, several facts arising from both the vapour and liquid experiments seemed to be very important. These facts and their possible interpretations are as follows. (a) The uptake of liquid is much faster than that of vapour. This suggests that, in addition to the molecular processes of water transport and swelling within the solid phase, interfacial phenomena play a crucial part at some stage of the process. (b) Despite their difference in magnitude, the rates of uptake of both liquid and vapour are strongly and similarly dependent on the initial conditioning treatment. This suggests that basically similar mechanisms are involved in both liquid and vapour uptake, although modified by interfacial conditions. (c) Rates of uptake of both liquid and vapour are largely independent of material thickness under those conditions in which they are strongly sensitive to the ratio  $p_i/p_f$ . This suggests either that there are no significant gradients in time-dependent properties of the material (for example, concentration of sorbate) or that if gradients are present their boundaries lie at interfaces other than the geometric surface of the specimen. (d) The rate of uptake of liquid by wood initially dry or conditioned to a low vapour pressure was insensitive to previous conditioning but was strongly dependent on the thickness of the cell wall. It is possible that this behaviour results from a transition to a different controlling mechanism, analogous to that observed previously in vapour sorption by thicker wood samples<sup>7</sup>. As a result of that work, the mechanism controlling the vapour sorption rate at low initial vapour pressures was thought to be diffusion across a succession of wood fibres.

Elaboration of these ideas has led to the hypothesis that the uptake of both liquid and vapour involves two consecutive stages or processes. The first stage consists of the intrusion of either the liquid or vapour phase into the cell wall by a number of discrete pathways. It is possible that this consists of flow through pre-existing cell wall capillaries or void spaces in the dry or conditioned wood. For present purposes these need only be large enough to enable their contents, at the end of the first stage, to be regarded as a continuation of the liquid or vapour phase present outside the cell wall. At the same time, the spaces need only be frequent enough to divide the cell wall into regions which are small compared with its total thickness. The initial penetration and filling of capillaries is followed by the second stage—molecular migration into the intervening solid regions at a rate determined by the rate of molecular re-orientation or true swelling of the solid phase.

The first stage is strongly dependent on the thickness of the cell wall, that is the length of the capillary path, but not markedly so on initial conditions. The second stage, which occurs in a large number of regions of the cell wall simultaneously, is much less affected by cell wall thickness, but is markedly dependent on the initial vapour pressure conditions and also on whether it is vapour or liquid which is introduced at the interface.

The resultant rate of uptake is determined mainly by whichever of the two stages is slower in any given conditions. In cases where the molecular re-arrangement or true swelling can take place more rapidly (see below), the resultant rate of uptake is determined by the speed with which the external phase can penetrate the capillary structure during the first stage. This occurs when a critical thickness of the material is exceeded for any given set of otherwise constant experimental conditions. In vapour sorption, as stated above, this situation has been shown, for water vapour only, to be reached when the wood specimens are thick enough to require the penetration of a considerable number of fibres in succession. In immersion experiments, on the other hand, the critical thickness can be exceeded by a single cell wall. This occurs for the

uptake of water by klinki pine for all  $p_i$  values up to approximately 0.7.

With regard to the present series of investigations on small specimens, however, this constitutes exceptional behaviour. Thus, for immersion in water after conditioning to large  $p_i$  values, in alcohols for all  $p_i$  values, and for all vapour sorption experiments involving only single cell walls, the second or molecular swelling process is so slow relative to the first stage that it becomes rate controlling.

It now remains to explain why the rate of the second, or swelling, stage is dependent in the observed manner on both the initial conditioning treatment and the nature of the external phase. Together with the rapid filling of the capillaries with vapour or liquid, there will take place, at the capillary surfaces, adsorption of vapour in one case and surface wetting by liquid in the other. The change in surface properties of the solid at the capillary interface or, alternatively, the incorporation of sorbate within the first molecular layers which constitute the solid surface, leads to the development of a stress between the surface layer and the underlying unswollen material. The magnitude of this stress will depend on the steepness of concentration gradient of sorbate from the interface to the interior of the solid region. In the present context there are two factors which can affect this gradient. Other things being equal, it will be steeper the larger the difference between  $p_i$  and  $p_r$ , but the concentration gradient can also be expected to be larger the faster the quasi-equilibrium condition is established at the interface. It is reasonable to expect this to be much more rapid after wetting than after exposure to vapour.

So far the mechanism proposed for the swelling stage is indistinguishable from diffusion in which the surface concentration is time-dependent and stresses are present within the solid phase. That the controlling mechanism is not diffusion, even after immersion in liquid, is evidenced by, for example, the inordinately long times (up to several days or even weeks) required by the wet cell wall to reach swelling equilibrium when it starts from high initial sorbate contents. It is assumed that the rate of molecular

re-arrangement of the solid phase is the controlling mechanism and that this limits the rate at which additional sorbate molecules can enter the swelling structure. The other key assumption is that the rate of molecular re-arrangement at any point in the solid is determined by the magnitude of the stress produced at that point by the concentration gradient. The magnitude of this stress is related to events occurring initially at the solid surface, and so the complete course of swelling of each of the inter-capillary regions, that is the propagation of the concentration and stress gradients through it, is also determined by the events occurring at the surface. In this way, it is possible to explain the dependence of the measured rates of sorption and swelling on both the change in sorbate concentration, that is, on the values of  $p_i$  and  $p_r$ , and on whether liquid or vapour is used.

A further point of explanation which is necessary concerns the transport of sorbate molecules through the solid during the second or swelling stage. It is presumed that this occurs by a normal process of molecular diffusion, and that it takes place quite independently of the swelling. This is to say that diffusive transport of the additional sorbate, from the interface over the very short distance to any region in the solid where an increment in volume (swelling) is occurring, is potentially capable of proceeding many times faster than is actually required. The diffusion coefficient which should be used to characterize this potential transport rate should be that pertaining to steady-state (non-swelling) conditions. It follows that, in some circumstances, diffusion coefficients determined from non-steady state experiments, on sorbents which swell, may in reality be a measure of the swelling process rather than diffusion.

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## Niche and Species Diversity in Neotropical Bats

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In stable environments there seems to be some overlap of niches occupied by the Chiroptera.

A PERUSAL of the numerous faunal reports in the literature reveals that tropical environments support more species in a unit area than those of temperate zones. This situation has often been explained by the number of "niches" the habitat provides<sup>1</sup>. Until recently, explanations for the presence of a large number of species in an environment have either been unsatisfactory or vague, but an explanation of this phenomenon in terms of individuals of bird species has been dealt with theoretically and quantitatively. Klopfer and MacArthur<sup>2</sup> suggested and Schoener<sup>3</sup> elaborated that the principal factor which causes an increase in the number of birds in the tropics is an increase in similarity of the requirements of coexisting

species, and, as a consequence, an increased degree of niche overlap, that is, less exclusive requirements.

Similar sympatric species may diverge morphologically and show character differences, that is, two closely related species are often morphologically and ecologically most similar when in allopatry, and most dissimilar when in sympatry<sup>4</sup>. Hutchinson<sup>5</sup> demonstrated the measure of how dissimilar are coexisting bird species with similar habitats by observing that such species usually differ in the size of their feeding parts by a factor of 1.2–1.4. Bill length was used to determine ratios of character difference in coexistent tropical bird species of different body size<sup>2,6</sup>. Their extreme mean ratio values of 1.19–1.28 were less

than those found by Hutchinson, and differences in feeding parts were considered to be reduced between most sympatric tropical species, that is, the fewer the differences between species in feeding requirements, the closer to unity is the ratio.

The degree of character difference in a group of tropical phyllostomid bats was as much reduced as in tropical birds. Table 1 shows results which utilize congeneric pairs of bat species netted together in banana groves in Colombia, South America. The appropriate characters used to represent the feeding apparatus were the mandible and the upper tooth row ( $M^3$  or  $M^{2-3}$ ) as an upper jaw approximation. Mean ratio values of larger to smaller species were 1.15 and 1.14 for mandibular and tooth row measurements. MacArthur<sup>6</sup> postulated that the more similar are the food habits, the lower is the ratio. Simpson<sup>7</sup> questioned this postulate, but in only one bat species pair was there divergence in feeding habits. The ratio was high in bats of the genus *Phyllostomus* because of the larger size and highly carnivorous habits of the species *P. hastatus* (Table 1). Schoener<sup>8</sup> suggested that in birds the larger ratios ( $> 1.14$ ) occur when body sizes are large in proportion to the total abundance of food, smaller ratios resulting when species feed on more abundant foods in proportion to their body size. This relation, however, has yet to be established among bat species.

Table 1. CHARACTER DIFFERENCE AMONG MALES OF COEXISTENT BAT SPECIES OF COLOMBIA\*

Species	Mean mandible length	Ratio of large to small	Mean maxillary tooth row lengths	Ratio of large to small	Mean forearm length
<i>Myronycteris megalotis</i>	11.98	1.12	6.95	1.06	30.68
<i>M. nicefori</i>	13.45		7.40		38.55
<i>Phyllostomus discolor</i>	20.24	1.32	9.74	1.41	62.75
<i>P. hastatus</i>	26.73		13.70		86.94
<i>Glossophaga longirostris</i>	15.70	1.15	8.00	1.16	38.15
<i>G. soricina</i>	13.65		6.91		34.30
<i>Anoura caudifer</i>	15.79	1.11	8.03	1.14	35.23
<i>A. geoffroyi</i>	17.53		9.12		43.60
<i>Sturnira lilium</i>	15.83	1.07	6.85	1.10	41.67
<i>S. ludovici</i>	14.80		6.25		42.13
<i>Artibeus jamaicensis</i>	19.40	1.12	9.65	1.13	59.24
<i>A. lituratus</i>	21.64		10.94		67.08

\* Unpublished data.

Data presented here for bats correlate well with data for birds<sup>2,3</sup> and indicate that multiple occupation of niches or niche reduction may be characteristic of coexistent species in regions of high climatic stability where a constant food supply and abundance of roosting or nesting sites are available. The data suggest that habitat coexistent bat species of comparable sizes may have similar ecological requirements, but they do not explain why different sized bat species may also have similar ecological requirements (see forearm measurements, Table 1).

The spectrum of bat species netted in a habitat may be predicted within a relatively small margin of error, but observed populations appear to be controlled by local and temporal processes; simultaneous netting in two similar, adjacent habitats invariably results in some difference in numbers of individuals and species captured. Other than by casual observations, population dynamics of tropical bats have not been investigated, nor have the differences in composition of tropical bat communities been explained. The total probable bat biomass predicted was found to be essentially the same in two different tropical habitats<sup>6</sup>, one in which more kinds of food of similar form and palatability were present than in the other, although the communities observed were significantly different in composition, that is, in one habitat larger numbers of fewer species predominated, whereas fewer numbers of more species were present in the other. Reduction in competition by food partitioning<sup>3</sup> could be

a valid interpretation if this situation is found to be general in neotropical bats.

Coexistence of birds can obtain because each species occupying a habitat can be limited by the availability of a resource (food) in different habitats<sup>9</sup>. This relation has a direct bearing on bat community composition, and Pirlot<sup>9</sup> has asked: Do net captures of bats give an indication of resident populations or of communities coming to feed from other localities? To answer this, the diurnal roosting site of each species would have to be found and marking-recapture or telemetric investigation carried out, but probably in some habitats residential and feeding areas coincide, whereas in others the residential zone (with radius  $R$ ) contains within it one or more patches of feeding area of radius  $r < R$  (ref. 8). Where there are species in high numbers in a region, the residential and feeding areas are bound to overlap and to coincide occasionally (because bats, by the nature of their food source, are opportunistic).

Competition for food is possible in situations where many species in high numbers occur in a feeding area<sup>8</sup>, and actual food eaten indicates that coexistent bat species have certain common foods. Tropical bats constantly contend with "patchy" food sources, that is, scattered foods alternating in availability<sup>10</sup>, and consequently these bats do not specialize to feed on one or the other and are susceptible to irregular activities. These activities, however, are "convex", as the word is used by MacArthur and MacArthur<sup>7,9</sup>—the species is adapted to a scale of intermediate activities between the extremes of adaptive specialization (to a single food and to all palatable foods). Most neotropical phyllostomid bats (more than 51 per cent of the bat species in regions of high climatic stability in South America eat fruit or visit flowers to suck nectar and are only incidentally insectivorous or carnivorous) are convex in their activities, because many rather than a few foods are variably utilized<sup>11,12</sup>. (The usual classification of food habits is difficult to apply to bats because of their wide adaptation to different animal and plant foods. For example, many species which visit flowers in search of food in the tropics also eat fruit; some which visit flowers and eat fruit also use insects as a source of food and are omnivores.) Feeding sites, on the other hand, may or may not be convex, depending on the time of year (a habitat through which a bat could fly and in which it could feed would be convex). The number of bat species in a habitat, therefore, would be determined considerably by the convexity of the habitat. Unlike birds, the species diversity of which appears to be controlled largely by the structural diversity of the habitat<sup>10</sup>, the diversity and density of bats in a given habitat are controlled primarily by the amount of food (the number of convex habitats in the residential zone) and the number of available roosting sites. (Habitat structural diversity, for example, feeding in the canopy versus feeding at ground level, probably plays an important but unknown part in determining the diversity of bat species; capture by nets gives a biased sample, chiefly of fruit and nectar feeders flying near the ground.)

The roosting site factor is probably more important than the food factor to explain differences in composition of different communities of bats. If roosting sites are available, then the species will forage in a given residential zone. Conversely, even if food is present in the zone, the species may be absent because of a lack of roosting sites<sup>8</sup>. A considerable modifying factor of community components would be numbers of solitary species, for which roosting sites are abundant, and numbers of gregarious species, for which roosting sites are relatively less abundant. Tropical bats do not delimit territories, and the majority have a broad tolerance to a variety of roosting sites, nor is a nursery roost required for most, because the young can be carried with the female parent on foraging flights. These interactions of density dependent factors further contribute to the reduction in niche

restrictions and may explain considerably the flexibility in adaptations of bats in tropical regions.

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## Growth Curves in Inbred Mice

by

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The application of a mathematical model to the growth of inbred mice and their  $F_1$  hybrids makes it possible to analyse the inheritance of growth.

STUDIES of gene effects on postnatal growth have usually depended on either quantitative comparisons of body-weight at a specified time after birth, or qualitative comparisons of the plotted growth curves. Use of these methods has permitted the identification of certain genetic influences on growth, such as the gene pygmy, *pg*, in mice<sup>1</sup> and the dwarfing mutation induced by methylcholanthrene<sup>2</sup>.

Crary and Sawin<sup>3</sup> used a fundamentally different approach to the study of the inheritance of growth and body size in rabbits, based on comparison of the absolute growth rates at significant times during growth. The absolute growth rate increases rapidly after weaning to reach a rather sharp maximum, and it then falls, at a slower and decreasing rate, to approach zero as an asymptote. Crary and Sawin found that when they classified rabbits on the basis of the magnitude and age of the maximum growth rate, and the age of reaching maturity, four hereditary classes of animals could be distinguished. They also identified a single gene as responsible for a sex difference in growth among dwarf animals.

This article outlines a new method of analysis of the inheritance of growth, based on the observation that the specific growth rate tends to undergo an exponential decay with increasing age<sup>4</sup>. When this observation is formalized in a simple growth model, a form of the Gompertz equation, the observed growth of an organism can be analysed by deriving the least squares fit of this equation to a set of growth data by means of a computer<sup>5-7</sup>. Three parameters are solved for: one is the specific growth rate at the start of the period of observation, the second measures the rate of exponential decay of the specific growth rate, and the third is the computed initial size of the animal. The mathematical nature of the growth equation is such that these parameters specify, for the theoretical curve, the asymptotic size approached at maturity, the age and the magnitude of the maximum daily growth rate, and the weight of the animal at any time selected for study by the observer. These quantitative characteristics of growth can be solved for by simple algebraic manipulation of the growth equation, and it is evident that they include the characteristics of growth used in previous studies of the inheritance of growth, and particularly those shown

by Crary and Sawin<sup>3</sup> to be of practical significance in defining hereditary differences among rabbits.

The data described here were collected between 1938 and 1945 in the Department of Genetics, McGill University. Stocks were derived from animals supplied by the Roscoe B. Jackson Laboratory or by L. C. Strong, and continued by brother-sister mating. The mice were housed, fed and managed throughout the period of observations under conditions which were made as uniform as possible. To reduce variation arising from the effects of litter size on postnatal growth, only four young of each litter were kept. They were chosen to represent the heaviest and lightest of each sex. A study of the sex-ratio at birth in strains A, dba, C<sub>57</sub>Black and I, and their hybrids, has been published<sup>8</sup>.

Young mice of eight inbred lines\* were weighed at birth and weekly thereafter for 10 weeks. Four of the lines (A, dba, C<sub>57</sub>Black and I) were chosen for further investigation. They were crossed in all combinations including reciprocals, and samples of the twelve types of hybrid animals were weighed as before. At the same time, fresh samples of young of the parent lines were weighed. The succession of weekly mean weights for each sex of each line constituted the growth data to which the curve described here was fitted. The number of animals of each sex weighed at each interval varied from 37 to 82 for the hybrids and their parental inbred strains; for the other inbred strains the number varied from 20 to 39, with the exception of the Jk strain, for which between ten and twenty animals of each sex were weighed.

For the mathematical analysis of the growth data, we used a Gompertz equation of the form

$$W = W_0 \exp \left( \frac{A_0}{\alpha} (1 - \exp(-\alpha t)) \right)$$

where  $W$  is the weight at time  $t$ ,  $W_0$  is the initial weight for the period of study, and  $A_0$  and  $\alpha$  are constants<sup>5</sup>.  $A_0$  is the initial specific growth rate, and  $\alpha$  measures the rate of exponential decay of  $A_0$ . Only the growth measure-

\* The standard nomenclature for the strains described in this paper is now: A, DBA, I, C57BL, C3H, JK, C57L, CBA. At the time the data were collected, the nomenclature had not been standardized.



ments from the second to the tenth weeks were fitted, because semi-log plots of the specific growth rate suggest that the rate of exponential decay characteristic of post-natal growth starts at about 14 days of age. Because  $t_0$  was set at 14 days, the values obtained for  $A_0$  and  $W_0$  are those characteristic of this age and are designated  $A_{14}$  and  $W_{14}$  in Tables 1-5.

The growth curve was fitted by a computer programmed to determine the Gompertz curve giving the least squares fit to the data, by an iterative fitting procedure. The standard errors on the parameters were derived using the coefficients from the inverted matrix<sup>9</sup>. Each point was weighted by the reciprocal of the variance. Points of biological interest on the growth curve, such as the asymptotic size approached by the early growth process studied here (see later), the age at which the inflexion point (the point of maximum absolute growth rate) was reached, and the instantaneous rate of growth at this time, were calculated using the transform equations derived in a previous study<sup>6</sup>.

The asymptote of this early growth process is not the size finally reached by the mice. As the Gompertz growth process slows at approaching maturity, a second, linear process becomes evident in mice and many other species (to be published). This linear process has relatively little effect on the early part of the growth curve studied here.

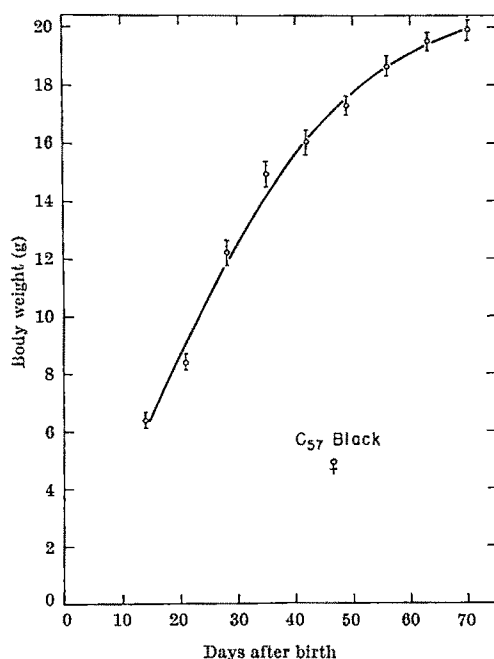


Fig. 1. Theoretical Gompertz growth curve fitted to the mean weights of  $C_{57}$  Black female mice by the method of least squares. The vertical bars are two standard errors of the mean.

Fig. 1 illustrates the theoretical Gompertz growth curve fitted to the mean weights of a representative set of animals. To the limited extent that one can judge by eye, it is evident that the fit is good. The mean deviation, without regard to sign, for these data was 2.5 per cent, and the deviations of the nine experimental points were, in order, 4.7, -7.1, 3.3, 5.2, -0.3, -1.5, 0.1, 0.5, and -0.2 per cent of the computed weights. The largest deviation for nearly all the sets of data occurred, as in Fig. 1, at the 21-day point, and it was always negative. For some of the curves the 28-day point was also below the curve. This characteristic tendency of the observed weights to lie below the curve at 21-28 days is almost certainly due to the minor malnutrition associated with weaning; it has been observed in other growth studies of

mice<sup>10</sup>. The effects of brief periods of malnutrition are known, however, to be only temporary, and afterwards growth takes place unusually fast until the animal has returned to its normal growth curve<sup>11</sup>.

In Tables 1 and 2 are given the parameters of the fitted growth curves, with their standard errors, for eight inbred strains of mice and for the twelve reciprocal crosses among four of them. The parameters differ only slightly from one another, and their standard errors overlap. Nevertheless, the biological significance of the small differences that do exist can be evaluated by the use of a sorting procedure based on sex, inbreeding versus hybridity, and the maternal and paternal strains.

Table 1. GROWTH CONSTANTS FOR INBRED STRAINS

Strain	$A_{14}$ *	$\alpha$ *	$W_{14}$ *
dba, m	0.049 $\pm$ 0.006	0.030 $\pm$ 0.005	6.58 $\pm$ 0.31
dba, f	0.050 $\pm$ 0.009	0.043 $\pm$ 0.007	6.05 $\pm$ 0.44
$C_{57}$ Black, m	0.070 $\pm$ 0.004	0.051 $\pm$ 0.003	6.58 $\pm$ 0.17
$C_{57}$ Black, f	0.067 $\pm$ 0.007	0.054 $\pm$ 0.005	6.09 $\pm$ 0.31
CBA, m	0.072 $\pm$ 0.009	0.049 $\pm$ 0.006	6.30 $\pm$ 0.36
CBA, f	0.061 $\pm$ 0.005	0.047 $\pm$ 0.004	6.47 $\pm$ 0.22
JK, m	0.052 $\pm$ 0.008	0.035 $\pm$ 0.006	6.37 $\pm$ 0.38
JK, f	0.042 $\pm$ 0.006	0.034 $\pm$ 0.006	6.58 $\pm$ 0.27
$C_{57}$ H, m	0.060 $\pm$ 0.010	0.035 $\pm$ 0.007	5.34 $\pm$ 0.42
$C_{57}$ H, f	0.054 $\pm$ 0.006	0.037 $\pm$ 0.005	5.77 $\pm$ 0.37
$C_{57}$ leaden, m	0.077 $\pm$ 0.013	0.048 $\pm$ 0.008	5.87 $\pm$ 0.51
$C_{57}$ leaden, f	0.051 $\pm$ 0.007	0.042 $\pm$ 0.006	6.80 $\pm$ 0.33
I, m	0.052 $\pm$ 0.006	0.035 $\pm$ 0.005	6.15 $\pm$ 0.31
I, f	0.051 $\pm$ 0.007	0.038 $\pm$ 0.005	5.88 $\pm$ 0.38
A, m	0.065 $\pm$ 0.009	0.049 $\pm$ 0.007	6.96 $\pm$ 0.38
A, f	0.063 $\pm$ 0.007	0.052 $\pm$ 0.005	6.85 $\pm$ 0.30

\*  $A_{14}$  and  $W_{14}$  are respectively the computed values of  $A_0$  and  $W_0$  in the growth equation when  $t_0$  is set at 14 days after birth. The value computed for  $\alpha$  is independent of where  $t_0$  is set.

Table 2. GROWTH CONSTANTS FOR RECIPROCAL HYBRIDS

Cross (mother $\times$ father)	$A_{14}$ *	$\alpha$ *	$W_{14}$ *
$C_{57}$ Black $\times$ dba, m	0.078 $\pm$ 0.006	0.055 $\pm$ 0.004	7.13 $\pm$ 0.30
$C_{57}$ Black $\times$ dba, f	0.074 $\pm$ 0.007	0.063 $\pm$ 0.005	6.95 $\pm$ 0.30
dba $\times$ $C_{57}$ Black, m	0.064 $\pm$ 0.005	0.046 $\pm$ 0.004	7.30 $\pm$ 0.27
dba $\times$ $C_{57}$ Black, f	0.061 $\pm$ 0.006	0.056 $\pm$ 0.005	7.59 $\pm$ 0.27
$C_{57}$ Black $\times$ I, m	0.081 $\pm$ 0.008	0.060 $\pm$ 0.006	7.34 $\pm$ 0.34
$C_{57}$ Black $\times$ I, f	0.075 $\pm$ 0.007	0.065 $\pm$ 0.005	7.38 $\pm$ 0.35
I $\times$ $C_{57}$ Black, m	0.077 $\pm$ 0.008	0.054 $\pm$ 0.005	7.15 $\pm$ 0.39
I $\times$ $C_{57}$ Black, f	0.067 $\pm$ 0.006	0.059 $\pm$ 0.005	7.41 $\pm$ 0.27
$C_{57}$ Black $\times$ A, m	0.078 $\pm$ 0.008	0.059 $\pm$ 0.005	7.87 $\pm$ 0.37
$C_{57}$ Black $\times$ A, f	0.069 $\pm$ 0.007	0.065 $\pm$ 0.005	8.04 $\pm$ 0.46
A $\times$ $C_{57}$ Black, m	0.080 $\pm$ 0.010	0.069 $\pm$ 0.007	8.04 $\pm$ 0.46
A $\times$ $C_{57}$ Black, f	0.079 $\pm$ 0.012	0.076 $\pm$ 0.009	8.32 $\pm$ 0.54
dba $\times$ A, m	0.074 $\pm$ 0.006	0.050 $\pm$ 0.004	6.84 $\pm$ 0.31
dba $\times$ A, f	0.071 $\pm$ 0.005	0.054 $\pm$ 0.004	6.80 $\pm$ 0.22
A $\times$ dba, m	0.081 $\pm$ 0.006	0.059 $\pm$ 0.004	7.62 $\pm$ 0.25
A $\times$ dba, f	0.079 $\pm$ 0.007	0.066 $\pm$ 0.005	7.52 $\pm$ 0.27
A $\times$ I, m	0.081 $\pm$ 0.006	0.064 $\pm$ 0.004	7.89 $\pm$ 0.30
A $\times$ I, f	0.078 $\pm$ 0.006	0.070 $\pm$ 0.005	7.70 $\pm$ 0.29
I $\times$ A, m	0.071 $\pm$ 0.008	0.051 $\pm$ 0.005	7.31 $\pm$ 0.36
I $\times$ A, f	0.069 $\pm$ 0.007	0.058 $\pm$ 0.006	7.31 $\pm$ 0.34
dba $\times$ I, m	0.061 $\pm$ 0.005	0.045 $\pm$ 0.004	7.67 $\pm$ 0.25
dba $\times$ I, f	0.057 $\pm$ 0.005	0.049 $\pm$ 0.004	7.37 $\pm$ 0.21
I $\times$ dba, m	0.075 $\pm$ 0.005	0.055 $\pm$ 0.003	6.90 $\pm$ 0.24
I $\times$ dba, f	0.074 $\pm$ 0.008	0.065 $\pm$ 0.006	6.96 $\pm$ 0.33

\* Table headings as for Table 1.

In Table 3 are summarized the results of sorting the animals by sex. With the single exception of  $W_{14}$ , the parameters of the growth curves and such other characteristics as the computed asymptote, and the age and magnitude of the maximum daily growth rate, all show a statistically highly significant tendency to differ in the two sexes. Because  $A_{14}$  is nearly always larger for the male and  $\alpha$  smaller, the ratio  $A_{14}/\alpha$  is larger for him, and the computed asymptote is higher (asymptote =  $W_{14} e^{A_{14}/\alpha}$ ). Furthermore, because  $\alpha$  is larger for the female,

Table 3. SEX DIFFERENCES IN GROWTH CONSTANTS

Growth constant*	Relation male to female	Incidence
$A_{14}$	Male greater	19/20
$\alpha$	Male less	17/20
$W_{14}$	Male less	10/20
Asymptote	Male greater	20/20
Max. growth rate	Male later	20/20
Age, max. growth rate	Male greater	19/20

\* Symbols for growth parameters as in Tables 1 and 2.

her growth curve is more compressed in time ( $\alpha$  is the inverse factor giving the relative duration of corresponding segments of the growth curve<sup>6</sup>). This means that she matures earlier, and reaches her maximum growth rate at a younger age than does the male; however, her maximum rate is less. The computed weight at 14 days shows no consistent difference between the sexes; this fact reflects the tendency of the growth curves to cross one another at about this time.

Table 4. RELATION OF GROWTH CONSTANTS TO HYBRIDITY

	Maternal strain	Paternal strain	Both parental strains
$A_{14}$ —male	12/12	11/12	11/12
female	11/12	10/12	9/12
$\alpha$ —male	12/12	11/12	11/12
female	12/12	12/12	12/12
$W_{14}$ —male	12/12	11/12	11/12
female	12/12	11/12	11/12
Maximum growth rate:			
Age, days—male	11/12	8/12	7/12*
female	11/12	10/12	10/12
g/day—male	12/12	12/12	12/12
female	12/12	12/12	12/12
Asymptote (g)			
Male	8/12	9/12	6/12
Female	11/12	10/12	9/12

\* Four of five exceptions are offspring of dba or I strain mothers.

The effects of hybridity on the properties of the growth curve are shown in Table 4. It is clear that with very few exceptions, the parameters for the hybrids are higher than those of the inbred mice of either parental strain. These differences have the effect that the hybrids reach their maximum growth rate about 1–6 days earlier, and the growth rate at this age is usually 25–60 per cent higher in the hybrids. The few exceptions are usually to be explained by the tendency of the offspring of dba or I mothers to have very low values for all the parameters

that affect it. In so doing, we have demonstrated differences in the growth curve and its parameters associated with sex, hybridity, and a maternal influence, in mice. Such effects have long been known in general terms, but the fitting of a growth equation and the determination of its parameters and mathematical properties permits the differences to be described with much greater precision than heretofore. Furthermore, the formalization of growth in an equation makes it possible to relate changes in the growth pattern to changes in the physiological determinants that are represented by the parameters of the growth equation. It is clear that a sigmoid curve of some sort is essential to describe the full growth curve of normal animals and their parts<sup>13</sup>. The Gompertz model has been shown to offer a satisfactory fit to the prenatal and postnatal growth of the guinea-pig over a 10,000-fold range<sup>5</sup>, a much more extensive range than is accounted for by another sigmoid curve, the logistic<sup>5</sup>. Regardless of the growth model used, however, species differences in the growth pattern must be genetically controlled. When animals as distantly related as cows, mice and chickens are compared with respect to their growth curves, it becomes evident that the growth patterns are superimposable<sup>14</sup>. Species differences are apparent only in the scale differences in the time and weight axes. Thus the hereditary process must act on the physiological parameters that are responsible for the dimensions of the scale. We have already shown in a study of the mathematical properties of the Gompertz growth equation<sup>5</sup> that the three parameters,  $W_0$ ,  $A_0$  and  $\alpha$ , are sufficient for complete specification of the two scales of the growth pattern. It is therefore reasonable to suggest that the three parameters may represent genetically determined, species-specific determinants of growth: the initial specific growth rate ( $A_0$ ), the rate of exponential decay of this growth rate ( $\alpha$ ), and the initial mass ( $W_0$ ). Within a species, these parameters can be modified slightly by

Table 5. MATERNAL EFFECT IN RECIPROCAL CROSSES

Cross	$A_{14}$ *		$\alpha$ *		$W_{14}$ *		Maximum growth rate				Asymptote	
	m	f	m	f	m	f	Age (days)	f	m	f	m	f
$C_{57}Bl \times dba$	0.078	0.074	0.055	0.063	7.13	6.95	20.3	16.7	0.60	0.52	29.4	22.7
$dba \times C_{57}Bl$	0.064	0.061	0.046	0.056	(7.39)	(7.59)	21.1	15.5	0.50	0.47	29.6	(22.5)
$A \times dba$	0.081	0.079	0.059	0.066	7.62	7.52	19.2	16.6	0.60	0.60	29.7	24.6
$dba \times A$	0.074	0.071	0.050	0.054	6.84	6.69	22.0	19.1	0.56	0.50	30.4	25.0
$C_{57}Bl \times I$	0.081	0.075	0.060	0.065	7.34	7.38	18.9	16.2	0.63	0.56	28.2	23.4
$I \times C_{57}Bl$	0.077	0.067	0.054	0.059	7.15	(7.41)	20.7	16.2	0.60	0.50	30.0	(23.1)
$A \times I$	0.081	0.078	0.064	0.070	7.89	7.70	17.6	15.5	0.66	0.60	27.9	23.4
$I \times A$	0.071	0.069	0.051	0.058	7.31	7.31	20.5	16.9	0.55	0.51	29.4	24.0

\* Headings as in Table 1. Exceptions in parentheses.

(see later). The differences in the computed asymptotes are generally quite small, and show no consistent effect of hybridity. A similar effect of hybridity on early growth rate rather than final size has been shown in chickens<sup>12</sup>.

Table 5 shows the regular differences observed in the parameters and properties of the growth curves of reciprocal crosses. To compare the respective crosses it is necessary to note from Tables 1 and 2 that the constants for the parental strains  $C_{57}Black$  and  $A$  are distinctly higher than for the strains  $dba$  and  $I$ , while within each of these pairs the values are closely similar. For  $A_{14}$  and  $\alpha$ , and for the age and magnitude of the maximum growth rate, there is no exception to the rule that the young of mothers from a strain characterized by high values for these constants will have higher values than the reciprocal hybrids. Thus the pattern of growth characteristic of the strain of the mother, whether early and fast or later and slower, is reproduced in the young. No consistent pattern was noted in the distribution of the values for the asymptote or for the computed weight at 14 days.

The principal purpose of this article has been to demonstrate the practical value of using a growth equation to analyse the pattern of the growth curve and the factors

such environmental factors as hormone status, nutrition, etc., as shown in the present investigation.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Radio Diameter Measurements with Interferometer Baselines of One Million and Two Million Wavelengths

MEASUREMENTS of the angular sizes of radio sources made

with a 21 cm interferometer between Jodrell Bank and the Royal Radar Establishment, Malvern, over a baseline of more than half a million wavelengths have already been published<sup>1,2</sup>. Nine sources were shown to be smaller than 0.1 sec of arc in at least one dimension. During 1966 the performance of the digital fringe speed machine was greatly improved and, in addition to further measurements at 21 cm, observations were made at the shorter wavelengths of 11 cm and 6 cm, the radio telescope Mark II being used in place of the Mark I at Jodrell Bank. At the shortest wavelength the maximum effective baseline is greater than two million wavelengths.

Table 1. SUMMARY OF OBSERVATIONS AT WAVELENGTH  $\lambda$ 

Source	Identification and red-shift ( $Z$ ) <sup>§</sup>	$\lambda = 21$ cm. Max. baseline $= 0.6 \times 10^6 \lambda$	$\lambda = 11$ cm. Max. baseline $= 1.1 \times 10^6 \lambda$	$\lambda = 6$ cm. Max. baseline $= 2.1 \times 10^6 \lambda$	Notes on the observations <sup>†</sup>
3C 48	Quasar ( $Z = 0.367$ )	<i>F</i>	<i>F</i>	—	Resolved at most hour angles but results not yet fully interpreted
0202+14 (NRAO 91)		<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
CTA 21		<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
3C 84	Seyfert galaxy ( $Z = 0.0172$ )	<i>F</i>	<i>F</i>	<i>F</i>	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.025''$
NRAO 140		<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
NRAO 150		<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
0403-13	Quasar ( $Z = 0.571$ ) <sup>†</sup>	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
3C 119	Quasar ?	<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
0430+05	Galaxy ( $Z = 0.03$ ) <sup>†</sup>	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
0440-00 (NRAO 190)	Quasar	—	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
3C 138	Quasar ( $Z = 0.759$ )	<i>F</i>	<i>F</i>	—	Double. Component separation $< 0.2''$
3C 147	Quasar ( $Z = 0.545$ )	<i>F</i>	<i>F</i>	—	Double. Component separation $< 0.2''$
3C 161	Galaxy	<i>F</i>	<i>NF</i>	—	Dimensions $> 0.2''$
LHE 210		<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
0834-20	Quasar <sup>†</sup>	—	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
3C 237	Galaxy	<i>F</i>	—	—	Double. Component separation $\sim 1''$
1055+01	Quasar	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
1127-14	Quasar ( $Z = 1.187$ )	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
1148-00	Quasar ( $Z = 1.982$ )	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
3C 273B	Quasar ( $Z = 0.158$ )	<i>F</i>	<i>F</i>	<i>F</i>	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.025''$
3C 274	Galaxy ( $Z = 0.00430$ )	<i>F</i>	<i>F</i>	—	Unresolved core, $\theta < 0.05''$ , containing small percentage of the total flux
1245-19	Quasar <sup>†</sup>	—	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
3C 279	Quasar ( $Z = 0.540$ )	<i>F</i>	<i>F</i>	<i>F</i>	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.025''$
3C 280		<i>F</i>	—	—	—
3C 287	Quasar ( $Z = 1.055$ )	<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
3C 286	Quasar ( $Z = 0.849$ )	<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
3C 295	Galaxy ( $Z = 0.460$ )	<i>F</i>	<i>F</i>	—	Low fringe visibility at minimum resolution
3C 298	Quasar ( $Z = 1.436$ )	<i>F</i>	<i>NF</i>	—	—
3C 309-1	Quasar ( $Z = 0.904$ )	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
1508-05		—	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
1510-08	Quasar ( $Z = 0.361$ )	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
3C 343		<i>F</i>	—	—	See text
3C 343-1		<i>F</i>	—	—	See text
3C 345	Quasar ( $Z = 0.595$ )	<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
NRAO 530		<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
3C 380	Quasar ( $Z = 0.693$ )	<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
1938-15		<i>F</i>	<i>F</i>	—	Double. E.-W. separation $\sim 2.5''$
3C 405	Galaxy ( $Z = 0.0570$ )	<i>F</i>	<i>F</i>	<i>NF</i>	Low fringe visibility at minimum resolution at both 21 and 11 cm
3C 410		<i>F</i>	<i>NF</i>	—	Dimensions $> 0.1''$
3C 418		<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
2127+04		<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
2145+06	Quasar	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
2203-18	Quasar	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
3C 446	Quasar ( $Z = 1.402$ )	<i>F</i>	<i>F</i>	—	Unresolved.* $\theta \lesssim 0.05''$
CTA 102	Quasar ( $Z = 1.037$ )	<i>F</i>	<i>F</i>	—	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.05''$
3C 454-3	Quasar ( $Z = 0.88$ ) <sup>†</sup>	<i>F</i>	<i>F</i>	<i>F</i>	No significant change in fringe amplitude with hour angle. Unresolved. $\theta < 0.025''$

*F*, indicates that fringes were observed. *NF*, indicates that fringes were not observed. —, indicates no observations.

\* Not observed at all hour angles, but shows no change in fringe amplitude between observations at substantially different resolutions.

§ All the identifications and red-shift measurements given here have been published, except those marked <sup>†</sup> which were kindly communicated to us by Professor Bolton in advance of publication. The red-shift parameter  $Z = \frac{\lambda - \lambda_{em}}{\lambda_{em}}$  where  $\lambda$  and  $\lambda_{em}$  are the wavelengths at which the radiation was observed and emitted respectively.

† The angular diameter,  $\theta$ , of each source is given for the shortest wavelength at which the source was observed.

The data at 11 cm were normalized and calibrated by means of frequent observations of the sources 3C 273B, 3C 279, 3C 286 and 3C 454.3. At the beginning of the observation period these sources were studied several times at all the hour angles at which they were more than  $10^\circ$  above the horizon. For these sources, as for sources at most declinations, the resolving power of the interferometer changes appreciably with hour angle as the sources move across the sky. The fringe amplitudes observed for the four normalizing sources did not change with hour angle and were approximately in the ratio of their flux densities. It was therefore concluded that all four of these sources were unresolved and so have sizes smaller than 0.05 sec of arc.

About fifty sources, for which there was evidence of small angular size and flux densities greater than 3 flux units at 11 cm, were next selected for study with this instrument. The evidence of small angular size came from previous interferometric work, from scintillation observations or from unusually flat radio spectra. These sources were observed in the first instance at times when the resolution was near minimum. Thirty-five of the sources selected showed significant fringe patterns. These sources were then observed for a second period at a time when the resolution was near maximum and the position angle of the radio source relative to the interferometer baseline was different. For most of these sources the fringe amplitudes in both cases were approximately those which would have been predicted from the published values of flux density at 11 cm combined with the calibration factors derived above.

Twelve of the sources were then studied in more detail by observing them continuously at all times when their elevation was greater than  $10^\circ$ . For eight of these sources no changes of fringe amplitude with hour angle were detected and it is concluded that they are also unresolved. The remaining four sources gave fringe patterns with a visibility significantly less than 1.0 at some or all hour angles.

Because so many of the sources observed at 11 cm appeared to be unresolved, it was decided to extend the observations to a wavelength of 6 cm, although the telescope efficiencies were lower at this short wavelength and the pointing errors likely to be more important. In ten days of observations fringes were observed from four sources, 3C 84, 3C 273B, 3C 279 and 3C 454.3, and these were studied at all the hour angles at which they were above  $10^\circ$  elevation. There is no evidence of any significant changes in their fringe amplitude. It is concluded that these sources, unresolved by a baseline of 2 million wavelengths, are smaller than 0.025 sec of arc. In the case of 3C 273B previous measurements<sup>1</sup> had suggested that the fringe amplitude changed with hour angle, but this change has since been traced to a tracking error<sup>2</sup>. Full analysis of all the data on 3C 273B, which includes additional measurements on 21 cm, leads to the conclusion that the source is unresolved at any of the wavelengths or baselines studied so far.

In addition to repeating the measurements of 3C 273B, several more sources were studied at 21 cm during 1966. All sources which gave fringe patterns in the combined programme are listed in Table 1, which also contains the results obtained in 1965. Of the forty-six sources listed, twenty-five have been identified with quasars and seven with radio galaxies. The remainder have not yet been optically identified, but it seems reasonable to predict that the majority are also quasars. Caution, however, should be exercised in attempting to make any statistical deductions because the sample of sources studied was not random.

It should be noted that the fringe amplitudes observed for the galaxies 3C 274 (Virgo A) and 3C 405 (Cygnus A) correspond to only a small fraction of the total flux density. 3C 274 appears to have an unresolved component which contains a few per cent of the total emitted flux at

both 21 cm and 11 cm. This component must have a linear dimension of less than 3 pc. A more detailed analysis of the results may show whether it is associated with the nucleus or with the jet of M87.

The fringes obtained at 21 cm from the sources 3C 343 and 3C 343.1 are of particular interest. Moffet<sup>3</sup> has shown that these sources, separated by 28.8 min of arc, are so remarkably similar that they are probably physically connected. Our measurements show that both of them are also among the sources that have angular dimensions of less than 0.1 sec of arc, corresponding to a ratio of separation to component size greater than 17,000 to 1. These dimensions are consistent with those deduced from the theory of synchrotron self-absorption<sup>4</sup>.

A number of sources are known to be variable at radio wavelengths, a characteristic generally considered to be associated with small dimensions. Table 1 shows that the ten radio variable sources listed by Pauliny-Toth and Kellermann<sup>5</sup> are all unresolved. These measurements have also permitted provisional interpretations of the radio structures of two quasars, 3C 138 and 3C 147, which both appear to have two separated components.

The interferometer observations described here demonstrate the remarkably high angular resolution that can be achieved at radio wavelengths. The results place new upper limits, by direct measurement, on the angular dimensions of the important class of radio sources characterized by small size and exceptionally high brightness temperature. Perhaps the most surprising result of the observations is the discovery that such a large number of sources have these small dimensions, and that this may be a general feature of most of the quasars. Any theory to account for these sources must explain the large radiating power being generated from such a small volume. For example, assuming the usual cosmological interpretation of their red-shifts, the upper limits to the linear dimensions of the quasar 3C 273B and the Seyfert galaxy 3C 84 are about 40 and 6 parsecs, respectively.

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## Magnetic Fields in the Solar Corona

It has been proposed by Sturrock<sup>1</sup> that the frequency splitting sometimes observed in type II solar radio bursts may be caused by excitation of plasma oscillations by supra-thermal electrons moving through the solar corona in the presence of density irregularities and a weak magnetic field. He showed that in these circumstances

electromagnetic waves may be produced at two frequencies, one at the local plasma frequency  $f_p$  and one at frequency

$$f_2 = (f_p^2 + f_H^2)^{1/2} \simeq f_p + \frac{f_H^2}{2f_p}$$

where the local electron gyrofrequency  $f_H \ll f_p$ . He envisaged the possibility of using the observations to measure the magnetic field intensity, although the utility of type II frequency splitting as a technique for magnetic field measurements appears to be limited by its relatively infrequent occurrence. More recently, however, Ellis and McCulloch<sup>2</sup> have reported the observation of large numbers of a new type of solar bursts in which frequency splitting occurs regularly. These have been observed mainly between 20 Mc/s and 50 Mc/s, and usually appear as a pair of bursts separated in frequency by 100–200 c/s and lasting for about 2 sec.

It is of some interest, therefore, to examine whether these bursts have the properties expected from the theory of plasma radiation. These have been worked out in some detail by Tidman *et al.*<sup>3</sup>, who have found that for electrons with isotropic velocity distribution the observed wave intensity at  $f_p$  should be much less than at  $f_2$ , mainly through self absorption in the plasma near  $f_p$ . Where the radiation occupies a non-zero bandwidth centred on  $f_p$  and  $f_2$ , it might also be expected that the observed bandwidth near  $f_2$  would be greater than at  $f_p$ , again through self absorption for  $f < f_p$ . On the other hand, some non-isotropic electron velocity distributions lead to comparable radiation intensity at both frequencies, although still with different bandwidths.

A further property of bursts showing frequency splitting expected with the plasma radiation theory is that the frequency interval  $\Delta f = f_2 - f_p$  should increase with frequency. The coronal plasma frequency in the vicinity of the radio emission regions has been observed<sup>4</sup> to vary with radius approximately according to

$$f_p = 2.8 \times 10^4 (1.55\rho^{-6} + 2.99\rho^{-16})^{1/2}$$

where  $\rho = R/R_\odot$  (ref. 3), while if the sunspot magnetic field source is taken to be an extended magnetic pole at the level of the photosphere, the field on the spot axis is given by<sup>5</sup>

$$f_H = f_{H_0} \left( 1 - \frac{\rho - 1}{[(\rho - 1)^2 + (b^2)^{1/2}]^{1/2}} \right)$$

where  $f_{H_0}$  and  $b$  are the gyrofrequency and magnetic pole radius at the photosphere.

That is

$$f_H = f_H(\rho) = f_H(\rho_p) \text{ or } \Delta f = \Delta f(f_p)$$

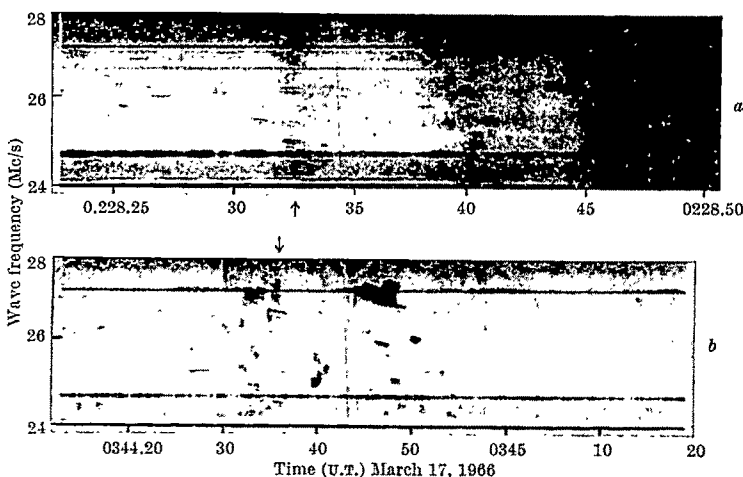


Fig. 1a and b. Spectrograph records showing bursts with frequency splitting in which the frequency interval between the components increases with frequency. A type III burst can be seen in the latter part of the record of Fig. 1a.

With a spot field of, say, 2 kG at the photosphere, and with  $b \sim 1/40$ , the frequency interval for  $f_p$  near 20 Mc/s is approximately 100 kc/s, increasing to 300 kc/s for  $f_p = 30$  Mc/s.

Figs. 1a and b show bursts of the type in which frequency splitting was observed by Ellis and McCulloch. These records are chosen to show two or more pairs of bursts occurring at about the same time, and they illustrate clearly that the frequency interval between the components of a pair increases with increasing wave frequency. An analysis of the change in frequency interval with frequency for a number of bursts is shown in Fig. 2. It can be seen that the magnitude of  $\Delta f$  and its range of change with frequency match closely that expected for the plasma theory of the emission. Fig. 1b also shows the characteristically smaller intensity of the lower frequency component of the burst pairs. A microphotometer scan of the spectrographic record of a typical burst (Fig. 3) illustrates this latter property more clearly. It also shows that, as well as being weaker, the low frequency component has a narrower bandwidth, again as would be expected.

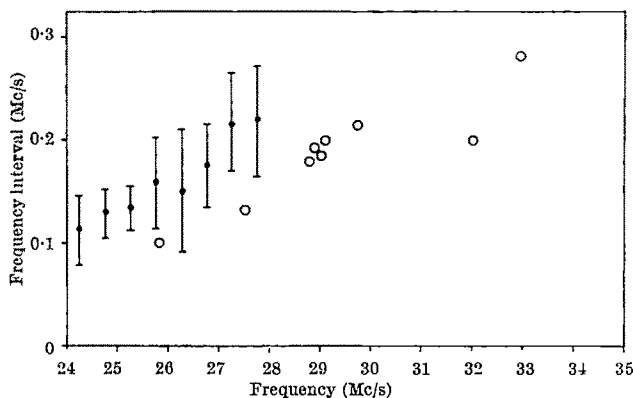


Fig. 2. Variation of frequency interval with frequency for 191 bursts (solid circles) observed on March 17, 1966, and 9 bursts (open circles) observed on September 20, 1966.

The evidence therefore suggests that the pairs of bursts observed by Ellis and McCulloch may well be caused by the frequency splitting of plasma radiation in the presence of a magnetic field. In this case, we can use the results of Fig. 2 to calculate the magnetic field intensity corresponding to different values of plasma frequency which are then given by the lower frequency component of each burst pair. In the absence of positional measurements of the source of the radiation in the solar corona, it is necessary to locate the region for which the magnetic field is measured from the estimated variation of plasma frequency with radial distance such as has been given by Wild *et al.*<sup>4</sup>

Fig. 4 shows the variation of gyrofrequency in the corona with radius obtained in this way from Fig. 2. Also shown for comparison is the theoretical variation of gyrofrequency for a unipolar spot field of 2,000 G ( $b = 1/40$ ), and it can be seen that the values derived from the observations vary with radius at a rate which might be expected with this type of magnetic field configuration. The radial distance at which the derived values of  $f_H$  occur depends strongly on the assumed plasma frequency variation. Use of the Baumbach–Allen model would, for example, give  $\rho = 1.5$  for  $f_H = 3$  Mc/s, instead of  $\rho = 2.47$  found using the model illustrated in Fig. 4. This uncertainty could be overcome by measurement of the positions of the sources of the bursts.



The rate of occurrence of the burst pairs appears to be sufficiently high to permit their use in the routine measurement of coronal magnetic fields. During the solar radio outbursts of March 17–21, 1966, for example, a total of 310 groups of bursts were observed, where a group is defined as several bursts occurring at almost the same time. In the following six months, burst pairs were observed on the following dates: June 3, July 20, August 1, August 30, September 6, September 20, even though the high speed records needed to detect them were made only on occasions when solar radio emissions of other types were occurring.

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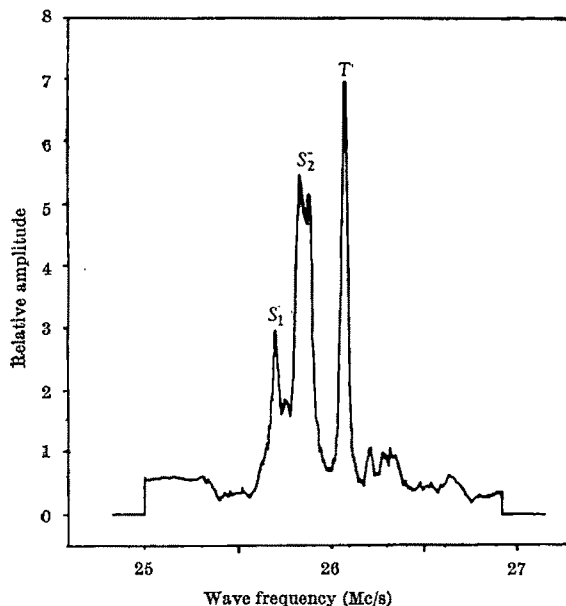


Fig. 3. Microphotometer scan of the two components  $S_1$  and  $S_2$  of a burst pair. A transmitting station is shown at T.

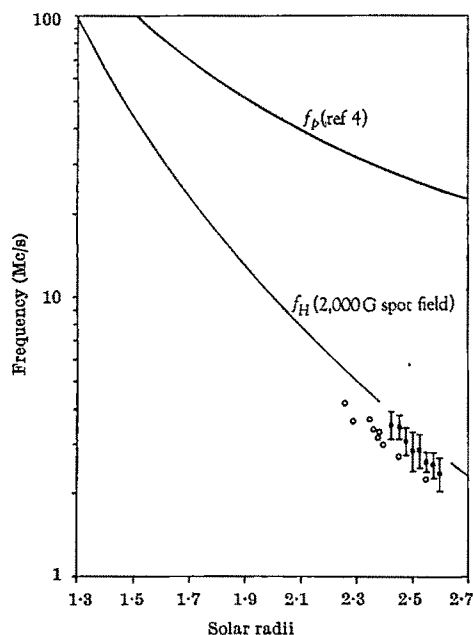


Fig. 4. Variation of gyrofrequency with radius in the solar corona calculated from the observations of Fig. 2 and using the plasma frequency curve shown to locate the source positions. A theoretical gyrofrequency curve for a 2,000 G unipolar spot model is shown for comparison.

## PLANETARY SCIENCE

### Revision of the Calcite–Aragonite Transition, with the Location of a Triple Point between Calcite I, Calcite II and Aragonite

CONFLICTING reports have been published for the position of the calcite–aragonite phase transition, and two factors contributing to this are the slow solid state reaction kinetics<sup>1</sup> and the problems of quenching the stable assemblage<sup>2</sup>. Bell and England<sup>2</sup> concluded from their experiments that aragonite which formed stably within a wide pressure range (above 600° C) inverted to calcite during the quench. We have recently extended the phase relationships involving a liquid phase in the system calcium oxide–carbon dioxide–water from 4 kbars pressure<sup>3,4</sup> to 40 kbars<sup>5</sup> using a piston-and-cylinder high-pressure apparatus. The liquids in this system are very reactive media, and no problems have been encountered in reaching equilibrium in this and a variety of related systems<sup>6</sup>. Across the calcite–aragonite boundary, the ternary phase assemblage calcite + liquid + vapour undergoes a transition to aragonite + liquid + vapour. In runs completed at pressures well above and well below the phase boundary, aragonite and calcite crystals in the quenched assemblage calcium carbonate + liquid + vapour were positively identified by X-ray and optical properties. The morphology of aragonite crystals coexisting with the ternary liquid is distinct from that of the calcite crystals, and the original shape of the equilibrium phase, aragonite or calcite, becomes frozen into the surrounding liquid during the quench and is thus preserved for microscopic examination. The shape of the carbonate crystals in the quenched assemblage calcium carbonate + liquid + vapour was therefore used to bracket the calcite–aragonite transition boundary down to the solidus temperature at 580° C. X-ray powder diffraction and refractive-index measurements demonstrate that crystals with the morphology of aragonite, in runs close to the transition boundary, have inverted to complexly twinned, biaxial calcite with optic axial angles ranging from near zero up to an estimated 20° (2V for aragonite is 18°). The implications of this observation have been discussed elsewhere<sup>7</sup>.

The morphology of quenched crystals also served as a criterion for distinction between calcite and aragonite when the experiments were extended into the subsolidus region of the system calcium oxide–calcium carbonate–water between 580° C and 400° C. X-ray diffraction patterns were not satisfactory criteria, because aragonite quenched from positions near the transition boundary tended to invert to calcite. Slow reaction rates prevented a satisfactory determination below 400° C in a length of time reasonable for the apparatus. Three reversals were completed across the curve shown in Fig. 1.

The purpose of this communication is to present our revised phase diagram for the system calcium carbonate (Fig. 1), to report the location of a triple point, and to indicate briefly the implications of our results in connex-

ion with the metastable crystallization of aragonite at low pressures. The positions of our runs plotted in Fig. 1 require that the calcite-aragonite boundary changes slope at a pressure of 9.4 kilobars at 480° C. This point lies almost on the best estimate of the boundary for the transition calcite I-calcite II, drawn between the determination of Boeke<sup>8</sup> (point B at 975° C) and Bridgman's<sup>9</sup> determination of the metastable transition at 14 to 15 kbars pressure (points Br). We therefore interpret the change in slope of our experimentally determined calcite-aragonite transition as a triple point between the three phases calcite I, calcite II and aragonite. The aragonite-calcite II transition is closely bracketed by our runs, but considerable variation is possible for the aragonite-calcite I curve between our limited runs at the lower temperatures. We have therefore extended this boundary down through the point determined carefully by Crawford and Fyfe<sup>10</sup> at 100° C.

Previous reports have presented the calcite-aragonite transition boundary in linear, or almost linear, form. The marked change in slope of the calcite-aragonite transition curve at the triple point shown in Fig. 1 can probably be explained by the entropy change involved in the transition of calcite I to calcite II. Assuming that calcite II differs from calcite I by having anion disorder<sup>11</sup>, calculation of the ideal configurational contribution to entropy yields a value of  $\Delta S = 1.4$  Cal/mole °C. This compares well with the value of 1.0 Cal/mole °C required by the change in slope using the Clapeyron equation, assuming that  $\Delta V$  for calcite I  $\rightleftharpoons$  aragonite equals  $\Delta V$  for calcite II  $\rightleftharpoons$  aragonite.

Aragonite forms metastably at the surface of the earth in several environments, and metamorphic aragonite (or calcite inverted from aragonite) has been reported from glaucophane schists<sup>12-14</sup>. It has been assumed that under metamorphic conditions aragonite would not form within the calcite stability field, and the experimentally determined calcite-aragonite transition boundary has therefore been used as a geobarometer; this confirms the conclusion that glaucophane schists were formed at great pressures. Vance<sup>15</sup>, however, has recently discovered widely distributed metamorphic aragonite in pumpellyite-prehnite facies rocks of north-west Washington, and he contends that these rocks could not possibly have been buried to the depths required to produce conditions which correspond to the aragonite stability field shown in Fig. 1. This implies either that the experimentally located calcite-aragonite transition boundary does not represent stable equilibrium, or that considerable tectonic overpressures are developed during metamorphism, or that aragonite

can grow metastably in the calcite field, even under metamorphic conditions. The revised phase diagram presented in Fig. 1 does provide a large pressure-temperature area for the metastable crystallization of aragonite, which includes the near-surface conditions where metastable aragonite is known to grow and also the low-grade metamorphic conditions where Vance reports the formation of aragonite. This is the area between the stable aragonite-calcite I curve and the metastable extension of the aragonite-calcite II curve, which would intersect the temperature axis (at zero pressure) at about 150° C. This large region for metastable aragonite at moderate temperatures and low pressures strengthens Vance's query (personal communication) about the validity of the aragonite-calcite I transition boundary as a geobarometer.

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## PHYSICS

### Formation of Alloys at Room Temperature

THE formation of alloys at room temperature was observed by Hund and co-workers, using co-reduction of metal salts by alkaline formaldehyde solution in the case of silver-gold<sup>1</sup>, gold-mercury<sup>2</sup> and silver-mercury<sup>3</sup>. Kulifay<sup>4</sup>, by giving a large number of examples, showed that the formation of intermetallics and other compounds by co-reduction at boiling point, using hydrazine or hypophosphorus acid, is a general phenomenon. Holt<sup>5</sup> also showed that alloys are formed by co-reduction at 50° C using sodium borohydride in the case of platinum-gold, platinum-iridium, palladium-gold, platinum-cobalt, platinum-nickel and platinum-copper.

The purpose of this communication is to show that alloys can also be formed when a metal foil displaces a more noble metal from solution. When silver or copper foil is immersed in certain solution complexes of noble metals it is found that after immersion for 1 or 2 days at room temperature a thin metallic skin forms on the surface of the foil, which exhibits poor adhesion and can be stripped off the foil by means of adhesive tape. The skin can be examined by both X-ray diffraction and X-ray fluorescence spectroscopy. In this way alloy formation was demonstrated in the following examples, in all of which the existence of a continuous series of solid-solution alloys is possible.

Silver foil immersed in a 0.002 molar solution of  $\text{Na}_2\text{PdBr}_4$  and 4 molar sodium bromide for 2 days gave a metallic skin with fluorescence counting rates in a ratio of  $\frac{K\alpha(\text{Ag})}{K\alpha(\text{Pd})} = 1.44$ , and a lattice parameter  $a = 4.00$  Å (pure silver,  $a = 4.0862$  Å; pure palladium  $a = 3.8898$  Å).

In a similar experiment using a 0.002 molar solution of  $\text{Na}_2\text{PtBr}_4$  and 4 molar sodium bromide, the metallic skin

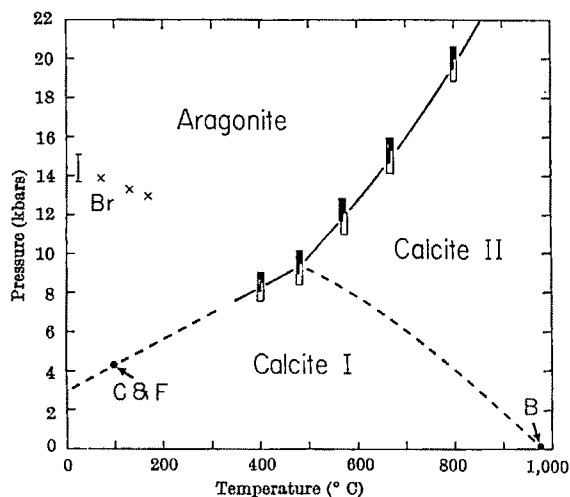


Fig. 1. Phase diagram for the system calcium carbonate. The heavy line passes through our experimental points. The aragonite-calcite I transition passes through our points and the point (C and F) determined by Crawford and Fyfe<sup>10</sup>. The calcite I to calcite II transition was located at point (B) by Boeke<sup>8</sup>, and metastably at points (Br) by Bridgman<sup>9</sup>.

gave counting rates in a ratio  $\frac{K\alpha(\text{Ag})}{L\alpha_1(\text{Pt})} = 4.66$ , and a lattice parameter  $a = 4.03 \text{ \AA}$  (pure platinum,  $a = 3.9231 \text{ \AA}$ ).

In the case of silver and a 0.002 molar solution of  $\text{Na}_3\text{Au}(\text{S}_2\text{O}_3)_2$ , the skin had the appearance of metallic silver and gave counting rates in the ratio  $\frac{K\alpha(\text{Ag})}{L\beta_1(\text{Au})} = 4.71$ .

No measurements of lattice parameters were made because of the close similarity of the values for silver and gold (difference in  $a = 0.0076 \text{ \AA}$ ).

Copper foil immersed overnight in a 0.002 molar solution of  $\text{Na}_2\text{PdBr}_4$  and 4 molar sodium bromide gave a metallic skin with counting rates in the ratio  $\frac{K\alpha(\text{Cu})}{K\alpha(\text{Pd})} = 4.07$ , and lattice parameter  $a = 3.71 \text{ \AA}$  (pure copper,  $a = 3.6150 \text{ \AA}$ ; pure palladium,  $a = 3.8898 \text{ \AA}$ ).

From the lattice parameters it is found that the alloys contain more than 50 atom per cent of the less noble metal.

Attempts to obtain alloys of copper-silver failed, the skins invariably consisting of pure silver. This is not surprising, as the solubility of copper in silver is only 0.3 atom per cent at  $100^\circ \text{C}$  (ref. 6).

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### Influence of Oxygen on Photoconduction in Organic Solutions

WE wish to give further results of our work on photoconduction in organic compounds at room temperature. We have already proposed<sup>1</sup> that photoconduction in solutions of triphenylamine in benzene and *n*-hexane requires the absorption of two quanta by the solute. Pilloff and Albrecht have recently shown a similar phenomenon in solutions of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) in 3-methylpentane<sup>2</sup>. Furthermore, our observations show that the magnitude of the photocurrent can be greatly reduced by changing the solvent from benzene to *n*-hexane. We have also described the influence of oxygen on the response of the pure solvent<sup>3</sup>.

Using the same experimental conditions, that is direct current measurements with a 'Teflon' and quartz cell operating with field strengths up to 20,000 V/cm, work has been carried out on solutions of triphenylamine saturated with dry oxygen. In benzene, the photocurrents were reduced to approximately 60 per cent of their usual values, while in *n*-hexane (where photocurrents are about two orders of magnitude less) there appeared to be no significant changes.

Analogous experiments have been carried out using dry nitrogen free from oxygen. In this case, with benzene, the photocurrent values increased by approximately 10 per cent. Again, in *n*-hexane there were no significant changes.

Under all these conditions, it was found that the photocurrent was proportional to the light intensity and to the square root of the triphenylamine concentration, that is, that two photons were required for photoconduction, in agreement with the previous results and our interpretation of them.

The earlier work<sup>1</sup> has also been extended to solutions of anthracene in benzene and *n*-hexane. Apart from an overall reduction in the observed photocurrents to about

5 per cent of the values obtained with triphenylamine, the results already stated have been reproduced. Oxygen and nitrogen saturation produced effects of a different magnitude from those given for triphenylamine, but the results are otherwise similar.

The main features of the photoconductivity of organic solutions are therefore similar when chemically different solutes are used; this has important implications in a more detailed explanation of the process.

As has been suggested elsewhere, for example by Brocklehurst *et al.*<sup>4</sup>, it is reasonable to consider the triplet state as a possible intermediate in a two-photon ionization process, especially in view of its long lifetime. In our work, flash photolysis confirmed our expectation that the oxygen-saturated solution showed no absorption by triplet state triphenylamine, whereas the solution saturated with nitrogen showed a strong triplet absorption. As already mentioned, the photocurrents were nevertheless only in the approximate ratio of 1:2. If the triplet state had been a rate determining intermediate species, we should have expected a far greater reduction of photocurrent on saturation with oxygen.

A further argument against the participation of the triplet state is derived from our observation that, on saturating with nitrogen a solution of triphenylamine in equilibrium with air, the time taken for the triplet concentration after flashing to decay to half its initial value increased from 15  $\mu\text{sec}$  to 340  $\mu\text{sec}$ . If absorption of a photon by the triplet state were needed for ionization, we might therefore expect a very large increase in photoconduction, which was not observed.

These results are also consistent with the observation that, in equally concentrated solutions of triphenylamine in benzene and *n*-hexane in equilibrium with air, the concentration of triplet state triphenylamine after flashing was approximately the same in both solvents, although the photocurrents differed by two orders of magnitude. Regarded in isolation, this change could be ascribed to a corresponding change of mobility or lifetime of the charge carriers in the two solvents. The possibility of attributing part of the change to this cause is not, of course, excluded.

It is interesting to compare these results with those of Johnson and Albrecht<sup>5</sup>, who studied the photoconduction of TMPD in rigid glasses at  $77^\circ \text{K}$ . They, too, deduced a mechanism involving two photons and, among other interesting results, showed that saturation with oxygen reduced the photocurrent to roughly half its value in a helium-saturated rigid matrix. On this basis Johnson and Albrecht concluded that the triplet state of TMPD was not a rate determining step in the production of charge carriers.

On the evidence presented here it seems likely that the triplet state of the solute does not play an important part in photoconduction. The identification of the intermediate, and in particular its lifetime, is an important question. Johnson and Albrecht have suggested that the solvent is involved, and certainly our results for *n*-hexane and benzene could be interpreted as supporting this proposal. There remain, however, many uncertainties which we hope to resolve by further experiment.

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### Limits to Resolving Power in Photoelectron Spectroscopy

THE energy spectra of electrons ejected in gaseous photoionization by undispersed radiation from rare gas discharges—a process known as molecular photoelectron spectroscopy<sup>1,2</sup> or, better, spectrometry—have been found to be a rich source of fresh electronic and vibrational data on the states of the resultant ions. As usual when a new technique is being explored, improvements in experimental detail have steadily increased the amount of information accessible in a given case<sup>1-4</sup>. Alternatively the study of more complex substances has been made feasible<sup>1,2</sup>.

During the past 4 years several groups of workers have improved the energy discrimination of the retarding field analyser from the original  $\sim 0.5$  eV to  $\sim 0.1$  eV<sup>2,5-7</sup> and, recently, to  $\sim 0.05$  eV<sup>8,9</sup>.

With this it is possible to discern vibrational fine structure corresponding to the formation of more or less vibrationally excited ions of diatomic molecules of nitrogen, oxygen, carbon monoxide and nitric oxide and of certain excited states of polyatomic molecular ions where only one vibrational mode is excited. When combinations of different vibrational modes occur this resolution is insufficient to separate the various component lines.

We may ask what fundamental restrictions prevent the attainment of better resolution. A practical limit will be set by line broadening in the light source associated with self reversal. Added to this will be the combination of Doppler spread in the emission line ( $\sim 2 \times 10^{-4}$  eV for helium) and in the ionization of the target molecule ( $\sim 5 \times 10^{-5}$  eV) and the contribution that thermal velocity of the ionized molecule makes to the velocity of the ejected electron ( $\sim 2 \times 10^{-3}$  eV). Apart from the reversal effect these may amount together ( $\sim 2 \times 10^{-2}$ ;  $16 \text{ cm}^{-1}$ ) to less than the magnitude of some rotational quanta in X-H type molecules, and we predict that rotational fine structure is in principle capable of being observed in photoelectron spectra, provided that self reversal in the light source does not occur to such an extent as to cause emission line broadening of more than  $10^{-3}$  eV.

Watanabe and Inn<sup>10</sup> have reported reversal in the 584 Å line obtained from a helium discharge leading to two peaks separated by about 1 Å (that is,  $\sim 0.03$  eV). If this applies to the sources used in the present work, clearly no further improvement in resolution is to be expected.

The fractional energy resolving power  $E/\Delta E$  of  $\sim 100$  achieved in the present retarding field analysers has, however, been insufficient to enable us to decide whether

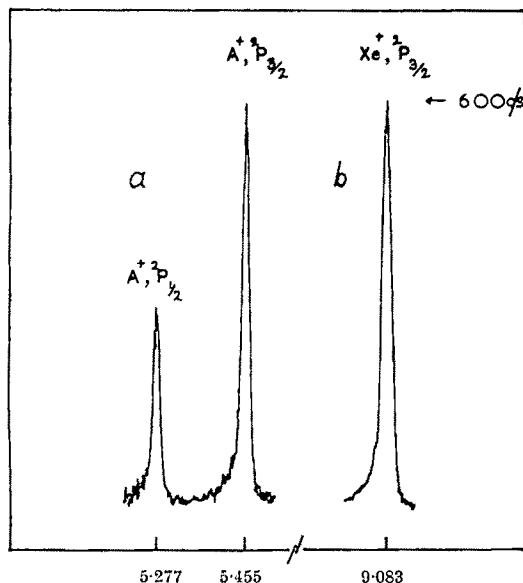


Fig. 1. Photoelectron spectrum excited in (a) argon, (b) xenon, by 21.21 eV photons. Ordinate, counts/sec; abscissa, electron energy (eV).

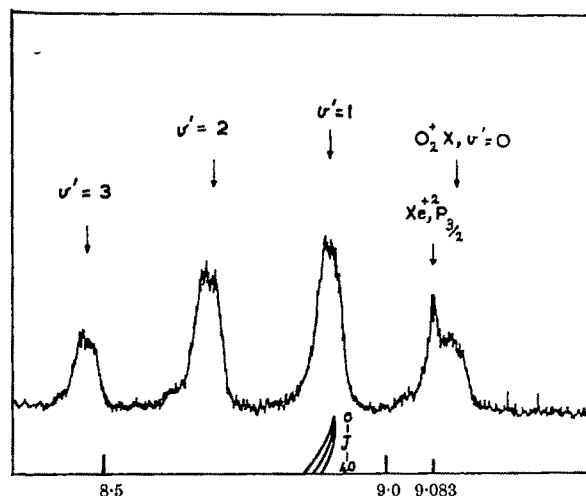


Fig. 2. A portion of the photoelectron spectrum excited in oxygen gas by 21.21 eV photons. The peaks shown are the first three vibrational components of the highest electron energy band ( $O_2^+X, {}^2\Pi \leftarrow O_2X^+ \Sigma_g^-$ ). The sharper peak superimposed on the  $v' = 0$  component is due to added xenon ( ${}^2P_{3/2}$ ).

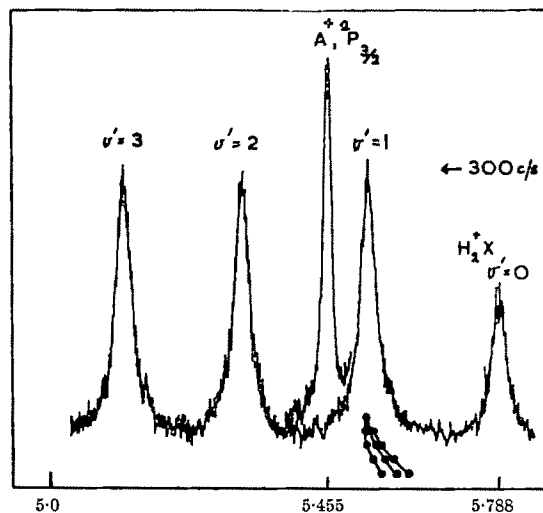


Fig. 3. Part of the photoelectron spectrum excited in hydrogen gas mixed with argon by 21.21 eV photons. Ordinate, counts/sec; abscissa, electron energy (eV).

we have reached the limit and still falls far short of the best achieved in other fields of electron velocity analysis<sup>11</sup> (for example,  $\sim 10^6$  using a Wien filter). There seems to be a special problem associated with grid analysers at low electron energies associated especially with surface potential variation over the extensive grid surface.

To avoid this difficulty we have investigated the design of deflexion analysers for photoelectron spectrometry so as to restrict to small slits the metal surface actually in close proximity to the electron flux. The most recent design uses deflexion by  $\pi/\sqrt{2}$  in a radial electrostatic field with a principal orbit radius of 10 cm, all internal surfaces being coated with a uniform layer of benzene soot.

A resolving power  $E/\Delta E$  of 450 was obtained at 9 eV as measured from the photoelectron spectrum of xenon (Fig. 1b). The smallest width at half peak height (0.015 eV) achieved for the peak for argon  ${}^2P_{3/2} \leftarrow {}^1S_0$  (Fig. 1a) is smaller than that which unresolved rotational fine structures should confer on spectra for polyatomic molecules ( $\sim kT$ , 0.028 eV). This result also implies that the light source is substantially free from self reversal effects.

The expected rotational broadening has now been observed in a number of diatomic molecules. In Fig. 2 a portion of the photoelectron spectrum for oxygen has been expanded to show the first four vibrational levels of the  $O_2^+X, {}^2\Pi \leftarrow O_2X^+ \Sigma_g^-$  ionization for which  $r_e$  decreases to  $1.1127 \text{ Å}$  from  $1.2076 \text{ Å}$ . It can be seen that in comparison with the peak due to added xenon the oxygen

peaks are broadened asymmetrically to lower electron energy. This is more than the symmetrical broadening due to spin-orbit splitting (0.024 eV).

The peaks in the hydrogen photoelectron spectrum (Fig. 3) also show marked asymmetrical broadening in this case to higher electron energy consistent with an increase in dimension on ionization ( $r_0$  1.06 Å ← 0.74 Å).

In the case of hydrogen, however, the thermal velocity effect must give a spread of ~20 mV comparable with that expected from rotational energy.

It can be seen from this that large dimensional changes can be expected to be detected from the "shading" of vibrational bands in molecular photoelectron spectra.

Carbon K shell photoelectron spectra have recently been reported by Axelson *et al.*<sup>12</sup> These were obtained using solid samples so that at least part of the line width noted (~1 eV) can be ascribed to lattice effects.

We may expect to find more detailed fine structure associated with small changes in chemical environment in the case of complex substances if the corresponding molecular photoelectron spectra can be obtained. Extensive vibrational fine structure should be absent since the electrons removed may be essentially "non-bonding". Small differences in electron binding energy (0.1 eV) should in principle be readily detectable and lead to a valuable general method for structural diagnosis.

The development of the deflexion analyser was supported by a grant from the Paul Instrument Fund of the Royal Society and will be described in detail elsewhere.

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## Antimony-125 Contamination in Antimonial Lead

LEAD containing 4 per cent of antimony (B.S.3909 : 1965) is commonly used for radiation shielding, and when the objective is the reduction of the background response of high sensitivity counting equipment, it is desirable that no radioactive contamination be present. Examples of contamination by <sup>210</sup>Pb (ref. 1) and by <sup>110m</sup>Ag (ref. 2) have been reported.

We have now confirmed the presence of antimony-125 (half-life 2.78 yr) in newly manufactured antimonial lead. Measurements were made with a sodium iodide (TI) crystal with two different cylindrical lead collimators. The first of these consisted of two parts weighing 15.8 kg and 7.5 kg, which were supplied in 1964 and 1965 respectively. The second was in one piece (21 kg) and was also manufactured in 1965. Gamma-ray spectra of the backgrounds of the crystal with the two collimators are shown in Fig. 1 (curves 1 and 2 respectively); the spectrum of the difference of 98 c.p.m. is also shown (curve 3). The two well defined peaks were identified as being due to the pairs of gamma-rays at about 0.43 MeV and 0.6 MeV respectively, which are characteristic of antimony-125. This inference was confirmed by the use of a standardized source of the radionuclide which was measured between pieces of lead 0.1 in. thick, assembled as a mock-up of part of the collimator. The gamma-ray spectrum obtained is shown in curve 4.

Comparison of the counting-rates in the peaks of curves 3 and 4 led to an estimated antimony-125 concentration

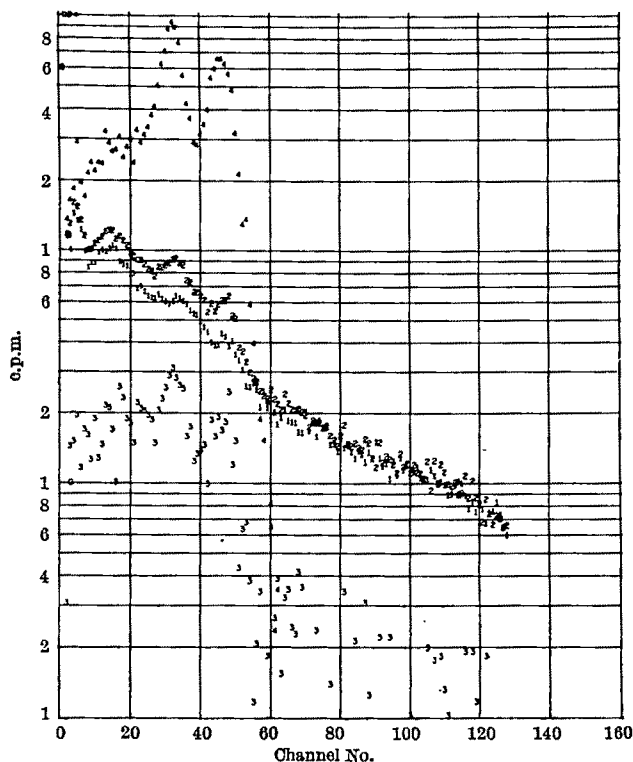


Fig. 1. Computer produced plots of gamma-ray spectra. Curves 1 and 2 are background spectra taken with the two collimators, curve 3 is the difference between them, and curve 4 is the spectrum of 13.6 mc. Sb-125 in a lead mock-up of the lower part of one collimator. The logarithmic counting-rate scale covers the range  $10^{-1}$ – $10^2$  c.p.m.

in the 21 kg collimator of  $(3.6 \pm 0.2) \times 10^{-14}$  curie/g of lead or  $(0.90 \pm 0.05) \times 10^{-12}$  curie/g of antimony. The uncertainties shown are those due only to the statistical errors of counting; there may be a systematic error due to the method of calibration, possibly as much as 50 per cent.

The origin of the contamination is unknown. Neither the manufacturers of the collimators nor their suppliers of antimonial lead used antimony-125 in any of their processes. One possible source might be radioactive fall-out from nuclear test explosions; antimony-125 has been measured at concentrations in the range 0.004–0.06 pc./kg of air, and 2–40 pc./l. of rain in the United Kingdom during 1963–65 (refs. 3 and 4). We could not detect antimony-125 in samples of pure antimony supplied in early 1964, although the levels in air and rain during 1963 were quite similar to those during 1964 (refs. 3 and 4), and we have evidence of antimony-125 in antimonial lead bricks which were received in late 1963.

Antimony-125 in lead shielding for low background gamma-ray spectrometers is considerably more of a nuisance than the naturally radioactive lead-210 found in some samples<sup>1</sup>, because of the higher energy radiation emitted. It is also more annoying than ruthenium-103 which has been observed in steel, because of its much longer half-life (2.78 yr compared with 40 days), and somewhat more of a nuisance than ruthenium-106 which has been found in some steels at roughly the same concentration<sup>2</sup>.

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## CHEMISTRY

## Complex Formation, Isolation and Carcinogenicity of Polycyclic Aromatic Hydrocarbons

THE carcinogenic activity of some of the polycyclic aromatic hydrocarbons<sup>1,2</sup> present in common materials has focused considerable attention on the properties and isolation of this group of compounds. Many methods for their isolation have been reported which are based, in general, on procedures involving partition<sup>3-6</sup>, adsorption<sup>7-9</sup> or complex formation<sup>9,10</sup>, used either singly or in combination.

The present attempt to develop a more satisfactory method of isolating polycyclic aromatic hydrocarbons stems from the work of Weil-Malherbe<sup>11</sup> who, on the basis of an observation by Brock, Druckrey and Hamperl<sup>12</sup>, investigated the quantitative aspects of the solubilization of a number of polycyclic aromatic hydrocarbons in many different purine solutions. 1:3:7:9-Tetramethyluric acid (TMU) and caffeine were shown to be the most effective purines. Although the phenomenon is not specific to polycyclic aromatic hydrocarbons<sup>13-17</sup>, other compounds such as aromatic amines and heteropolycyclics, which form complexes and are often found in hydrocarbon mixtures, present no difficulty to an attempt to isolate polycyclic aromatic hydrocarbons.

Silica impregnated with caffeine has been used<sup>18,19</sup> in a thin layer chromatographic technique to separate the components of a mixture of polycyclic aromatic hydrocarbons; the preparation of a concentrate of polycyclic aromatic hydrocarbons from petroleum products with high boiling points using aqueous caffeine has been described<sup>20</sup> and counter current distribution with a solvent system containing TMU has been used to separate certain polycyclic aromatic hydrocarbons<sup>17</sup>.

From a consideration of the suggestions put forward for the structure of complexes formed between polycyclic aromatic hydrocarbons and purines<sup>11,21-24</sup> and between polycyclic aromatic hydrocarbons and nitro aromatic compounds<sup>25,26</sup>, it seemed likely that the complexes might migrate under the influence of an electrical potential.

Using curtain paper electrophoresis with platinum electrodes in troughs at the top and bottom of the paper, complexes of polycyclic aromatic hydrocarbons with both caffeine and TMU have been found to migrate readily. Complexes were formed before spotting on the paper; the solvent was a solution of 2 g purine in 90 ml. water-10 ml. ethanol-2 ml. ammonia and the potential and current were 30-35 V/cm and 3-4 m.amp/cm. The twenty-five complexes of polycyclic aromatic hydrocarbons examined all migrated as discrete spots which completely left the baseline. Using the same method, it was later shown that these polycyclic aromatic hydrocarbons could be separated from twelve times their own volume of 'Vaseline'.

In large scale applications of the method, as much as 1 g of hydrocarbon mixtures containing polycyclic aromatic hydrocarbons has been adsorbed on 80-100 g silica and the residue packed into a column 38 mm in diameter. By applying a potential of 1,200-1,500 V to the column and slowly eluting with the solvent already mentioned, almost all the species which could be detected by electron capture during gas-liquid chromatography have been separated from the bulk of the mixture which remained on the column. Furthermore, in experiments in which radioactively labelled polycyclic aromatic hydrocarbons were incorporated in the hydrocarbon mixture, 97-98 per cent of the labelled polycyclic aromatic hydrocarbons were isolated. The exact experimental details of the method, which is now being used with most encouraging results to examine the condensate of cigarette smoke, will be published in the near future.

The nature of the purine/hydrocarbon complexes suggested by Liquori *et al.*<sup>22</sup>, namely a dipole-induced dipole interaction between the polar component (caffeine) and the polarizable component (polycyclic aromatic hydrocarbons), is consistent with the phenomenon which has been demonstrated and used in the present work.

Many attempts have been made to relate the complexing ability of polycyclic aromatic hydrocarbons to their carcinogenic properties, but none has so far been successful. Weil-Malherbe<sup>11</sup> could find no direct relationship but suggested that an affinity between hydrocarbons and the purines in nucleoproteins might cause interference with nucleic acid metabolism. Booth and Boyland<sup>13</sup> also found that carcinogenic polycyclic aromatic hydrocarbons did not differ from non-carcinogenic compounds in their reaction with purines. Liquori *et al.*<sup>22</sup> and Van Duuren<sup>24</sup> have also examined the problem. The rates of electrophoretic migration of all the complexes of polycyclic aromatic hydrocarbons with caffeine and TMU examined are similar and the slight visible differences cannot be related to any carcinogenic properties of the hydrocarbons. It is nevertheless possible that the complexing property of polycyclic aromatic hydrocarbons makes an important contribution to their carcinogenicity, the differences in biological properties resulting from a combination of factors such as molecular size, geometry and general reactivity. Thus the absence of carcinogenic activity in the majority of the polycyclic aromatic hydrocarbons might arise from diffusion and transport difficulties within the cell, lack of ability to adapt to a favourable configuration at the site of action in the cell, or destruction in other biochemical reactions before reaching the site of action. As suggested by Booth and Boyland<sup>13</sup>, the protective action of purines in carcinogenesis may result from their competition with the site of action for the polycyclic compounds.

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## BIOCHEMISTRY

## An Error in Model Building

ABOUT a year ago Dr. S. R. Pelc and Miss M. G. E. Welton claimed<sup>1,2</sup> that "it is possible to fit amino-acids stereochemically to their codons". They described how they built models using Courtauld space-filling components, but it was not possible to tell from their very brief descriptions whether their models were stereochemically acceptable. I therefore corresponded with Dr. Pelc and he was kind enough to show me some of their models.

Dr. Pelc produced several examples for me to examine, but I will comment here only on their model of lysine fitted to AAG, as illustrated in the photograph of Fig. 2 of the first of their two papers<sup>1</sup>.

This model is stereochemically unacceptable for the following reasons. (1) The terminal  $-\text{NH}_3^+$  group of the lysine was built as  $-\text{NH}_2$ . (2) In two places (one in the amino-acid, one in the triplet) adjacent methyl groups were in the eclipsed rather than the staggered configuration. (3) In two cases an  $>\text{NH}$ , which should either make a satisfactory hydrogen bond, or at least be free to make one to a water molecule, was pointing directly at a hydrophobic group.

Further inspection revealed that Dr. Pelc and Miss Welton had built all their polynucleotide sequences backwards\*. Their AAG was in fact GAA (which codes glutamic acid). This mistake can be detected by a very careful study of Fig. 2 of ref. 1.

I conclude that the models of Pelc and Welton do not support their hypothesis.

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\* For the standard convention see, for example, ref. 3. The triplet AAG can also be written as ApApG, the convention being that pG signifies a phosphate attached to the 5'-hydroxyl of the guanosine.

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Sir John Randall, Director of the Medical Research Council Biophysics Research Unit at King's College, London, wishes to state that he has read Dr. Crick's letter in manuscript, that he agrees with its conclusion, and that he had so informed Dr. Pelc and Miss Welton at the time of their original publication.—EDITOR, *Nature*.

## Adsorption of Octapeptide Hormones on to Lipid Monolayers

THE interaction between lipid monolayers spread on the surface of water, and oxytocin and vasotocin (arginine vasotocin) in the substrate, has been investigated, using a Langmuir surface trough, by studying the changes in pressure produced on injection of various quantities of the polypeptide solution under the film. This technique is similar to that used for the study of the interaction between protein in the substrate and lipid monolayers<sup>1,2</sup>.

In order to avoid contamination the paraffin wax used to coat the trough was freed from surface active impurities by heating with hot 1 normal sodium hydroxide followed by hot 1 normal hydrochloric acid. After thorough washing the wax was recrystallized twice from light petroleum (40°–60°). The water was redistilled with alkaline permanganate, and nitrogen was passed over the covered trough during the experiments.

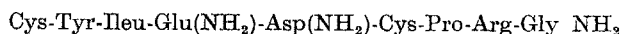
Samples of cholesterol, stearic acid and lauric acid were all recrystallized to constant melting point using light

petroleum (40°–60°) which itself was 'AnalaR' reagent redistilled. The samples of oxytocin and vasotocin were used as supplied.

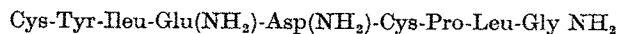
Solutions were made of cholesterol (19.3 mg/100 ml.) in benzene, stearic acid (14.4 mg/100 ml.) in light petroleum (40°–60°), lauric acid (10.0 mg/100 ml.) in light petroleum (40°–60°), oxytocin (16.0 mg/100 ml.) in water and vasotocin (10.0 mg/100 ml.) in water.

Using an 'Aglar' microsyringe  $12 \times 10^{16}$  molecules of lipid were injected on to the water surface in the Langmuir surface trough, and the initial pressure of the film was adjusted to 10 dynes/cm in the case of cholesterol, and 2 dynes/cm in the case of the fatty acids. The polypeptide solution was injected through the lipid monolayer and the pressure of the film increased, reaching a maximum value after 50 min. This increase in pressure was measured by a horizontal torsion wire, which was sensitive to 0.02 dynes/cm, and was used to obtain isotherms for the adsorption of peptide on to the lipid monolayer (Fig. 1). All isotherms obtained were similar, showing two marked discontinuities which are believed to represent stable surface structures (Fig. 2). Details of the results obtained are listed in Table 1.

Structure A, which is proposed for the final structure of the adsorbed film, is based on the fact that the chemical structures of oxytocin and vasotocin contain rings with six peptide bonds:



Vasotocin



Oxytocin

Previous work<sup>1,2</sup> supports the idea that polar groups of the lipid associate with peptide links in protein. It is therefore reasonable to suppose that the final structure indicated by a 6 : 1 lipid to polypeptide ratio represents a ring of six lipid molecules adsorbed at each peptide link in the ring of the hormone—the ring structure being one of the essential structural features for physiological activity<sup>3</sup>.

Structure B is proposed as the intermediate structure based on a 21 : 1 lipid to polypeptide ratio, found for both cholesterol and the fatty acids. Because these two types of lipid are very different in size, the association must

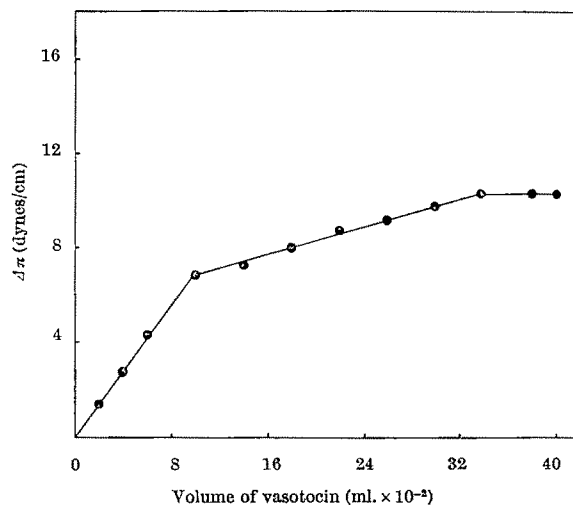


Fig. 1. The variation of surface pressure increase  $\Delta\pi$ , with volume of vasotocin solution injected through a monomolecular layer of cholesterol ( $12 \times 10^{16}$  molecules) at a surface of water.

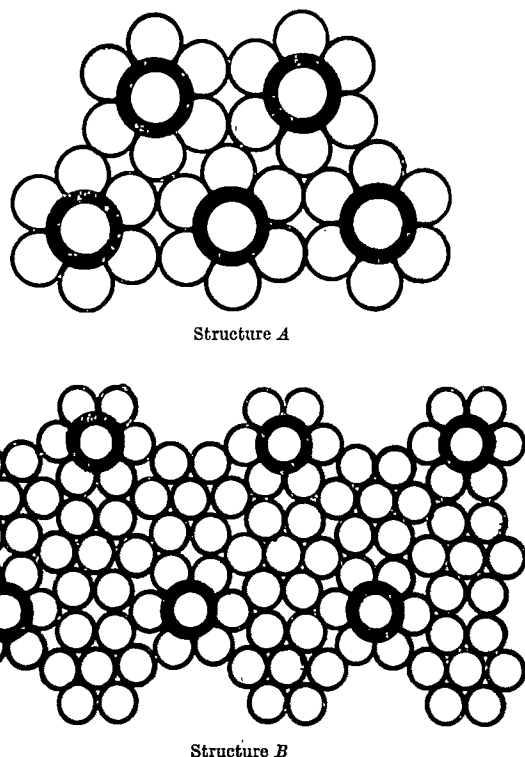


Fig. 2. Proposed surface structures of octapeptide hormones adsorbed on to lipid monolayer.

depend on a definite array of lipid rather than a network of peptide underneath.

The initial adsorption, which accounts for the highest increment of pressure, seems to impose the hexagonal unit structure on the lipid monolayer, possibly as a result of adsorption of both ring and tail of the hormone. The three peptide long tail unit could occupy the space between each polypeptide ring unit. The additional increase in pressure probably results as lipid molecules are removed from the centre of each hexagonal unit to form new units as the final structure forms.

If the lipid monolayer is taken as a suitable basic model of cell membranes, then it may be supposed that the effect of such structures forming in cell membranes would be to provide effective "pores" to facilitate the movement of water and other small molecules. Such biological effects of the octapeptide hormones have been demonstrated<sup>4,5</sup>, which are consistent with those expected if "pore" size had been changed.

Table 1

A, Cholesterol/oxytocin (starting pressure = 10 dynes/cm)				
	First break lipid : hormone	Pressure (dynes/cm)	Second break lipid : hormone	Pressure (dynes/cm)
1	21.05 : 1	15.3	6.01 : 1	20.82
2	18.80 : 1	15.3	6.28 : 1	20.11
3	23.98 : 1	13.5	6.88 : 1	17.70
B, Stearic acid/oxytocin (starting pressure = 2 dynes/cm)				
1	20.23 : 1	6.9	6.08 : 1	11.81
2	20.23 : 1	7.3	6.04 : 1	12.18
3	22.72 : 1	4.9	5.83 : 1	8.28
C, Lauric acid/oxytocin (starting pressure = 2 dynes/cm)				
1	19.63 : 1	7.5	6.05 : 1	10.64
2	20.88 : 1	6.8	6.25 : 1	11.93
D, Cholesterol/vasotocin (starting pressure = 10 dynes/cm)				
1	21.98 : 1	16.8	6.36 : 1	20.40
E, Stearic acid/vasotocin (starting pressure = 2 dynes/cm)				
1	23.04 : 1	7.8	6.49 : 1	11.80
F, Lauric acid/vasotocin (starting pressure = 2 dynes/cm)				
1	20.57 : 1	7.9	6.31 : 1	12.13
Mean	21.19		6.19	

The sample of synthetic vasotocin was provided by Dr. B. Berde, of Sandos, Ltd. This work was supported by a grant from the U.K. Science Research Council.

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### Sterols from the Fruit and Seed of Tomato

THE quantity of carotenoid that a tomato fruit can synthesize depends on its genetic background. The total for a red,  $r^{+}t^{+}$ , or tangerine,  $r^{+}t$ , may run as high as 200  $\mu\text{g/g}$  of fresh fruit if the colourless polyenes, phytoene and phytofluene, are included. The ghost genotypes, for example  $r^{+}t\ gh$ , may even run higher. By contrast, comparable figures for yellow,  $rt^{+}$ , range from 2 to 5  $\mu\text{g}$ .

In the early stages of carotenoid and sterol synthesis, intermediates are the same, available to both unless different sites are involved. We were therefore interested to determine whether some of the numerous genes affecting carotenoid production would likewise affect the sterols. So far as we are aware, apart from the related tomatidine, there have been no studies of the tomato sterols. They are not listed by Stoll and Jucker<sup>1</sup> in their chapter "Phytosterine, Steroidsaponine und Herzglykoside". Of the common phytosterols, the presence or absence of ergosterol can be ascertained from the ultra-violet absorption spectrum, and the most frequently found are the sitosterols and stigmasterol (compare Heftmann and Mosetig<sup>2</sup>). As Shoppee<sup>3</sup> has noted, the sitosterols are usually difficult to purify and identify.

Fruit and seed of the following tomato genotypes were examined separately: red, yellow, tangerine, apricot, red-ghost, yellow-ghost, tangerine-ghost.

Samples of fruit (500 g) and seed (5–10 g) were exhaustively extracted with acetone, and the digitonides were prepared in accord with the outline given by Stoll and Jucker<sup>1</sup>. The precipitates were dried under vacuum and weighed. Results are shown in Table 1.

The free sterols were then obtained by boiling in xylene, and the digitonin removed by centrifugation. The xylene supernatant was then evaporated to dryness, and the residue was acetylated.

Thin-layer chromatography gave spots indistinguishable from those for sitosterol and stigmasterol, but these two could not be effectively distinguished from each other. Adsorbents tested included alumina, kieselguhr G and silica gel<sup>4,5</sup>.

Gas liquid chromatography was more effective on the acetylated products. The acetates of stigmasterol and  $\beta$ -sitosterol melted at 144–5° and 118°, respectively; those of seed and fruit sterols varied from (111–12°) to (116–20°). Samples of about 0.4 mg in 0.04 ml. were injected in a 'Loenco' gas chromatograph. A 5 ft. column of 10 per cent silicone grease 'DC' on 'HMDS' treated 'Chromosorb W', 80/100 mesh, was operated at 283°. The helium flow at 20 lb. pressure was about 75 ml./min. Slower flow rates or lower temperatures did not give sharp peaks.

Table 1. DIGITONIDES FROM FRUIT AND SEED OF TOMATOES OF DIFFERENT GENOTYPES

Tomato	Fruit mg/100 g	Seed mg/g
Red	5.2, 5.0, 8.6, 10.2, 10.8, 11.7	3.9, 3.8
Yellow	9.0, 10.5, 11.6, 8.1	6.4
Tangerine	9.4, 7.6, 11.0, 9.5, 7.8, 12.1	5.6
Apricot	14.1, 12.8	3.9, 3.3, 4.7
Red-ghost	9.2	—
Yellow-ghost	8.1	—
Tangerine-ghost	13.1	—

Under the foregoing conditions, all tomato samples gave three sharply defined peaks with retention times of 140, 165 and 180 sec. Authentic samples of ergosterol and soybean sitosterol, stigmasterol and  $\beta$ -sitosterol were acetylated and used for comparison.

The soybean sitosterol also gave three peaks, the main one indistinguishable from that for stigmasterol and the second peak of the tomato. The  $\beta$ -sitosterol co-chromatographs with the third peak.

Characteristically, all fruit samples gave peak heights  $\text{II} > \text{III} > \text{I}$ , whereas the seeds gave the order  $\text{III} > \text{II} > \text{I}$ . Peak II was enhanced by the addition of stigmasterol, peak III by  $\beta$ -sitosterol.

It seemed likely, therefore, that peak III was caused by  $\beta$ -sitosterol; peak II might be stigmasterol; and peak I might (we speculated at first) be caused by dihydro  $\beta$ -sitosterol, a common minor component of the sterol mixture<sup>1</sup>, but it is more probably a campesterol<sup>6,7</sup>.

Stigmasteryl acetate readily forms a characteristic tetrabrom derivative and crystallizes within a few minutes, melting point 195–6°. The tomato sterol derivatives crystallized much less rapidly; the fruit sterol melted at 184–5° after recrystallization, the seed sterol at 182–3°. Bromination was therefore inconclusive.

Samples of the free sterols were analysed in the mass spectrometer and comparisons were made with stigmasterol,  $\beta$ -sitosterol, soybean sterols and dihydro  $\beta$ -sitosterol prepared by hydrogenation of  $\beta$ -sitosterol.

As would be anticipated, stigmasterol gave a prominent peak, for a molecular ion of mass 412;  $\beta$ -sitosterol, 414; dihydro  $\beta$ -sitosterol, 416. The  $\beta$ -sitosterol sample also yielded a small peak at 400, suggestive of campesterol, and a still smaller one at 416. The soybean sample gave peaks at 412, 414 and a smaller one at 400.

The main peaks for the tomato fruit and seed sterols were at 414 and 412; additional peaks were noted at 400, and also at 416 and at 428 and 426. The presence of  $\text{C}_{30}$  sterols of mass 428 and 426 raises an interesting question as to their relationship to the  $\text{C}_{28}$  sterols of mass 414 and 412.

The two principal sterol components of the tomato fruit in all probability correspond with stigmasterol and  $\beta$ -sitosterol, the former comprising 60–70 per cent of the mixture. In the seed, the same two components are found with the proportions reversed. Campesterol is probably responsible for the first peak from the gas liquid chromatography column, and there is evidence for traces of  $\text{C}_{28}$  sterol of mass 416, and  $\text{C}_{30}$  sterols of mass 426 and 428.

It may be added that squalene was isolated from the unsaponifiable fractions of all extracts by both thin-layer and gas chromatography. It was present in concentrations of about 1 p.p.m.

As shown in Table 1, the genes controlling carotenoid synthesis have no effect on the sterols, either in the fruit or in the seed.

We thank the U.S. National Science Foundation for a grant. We also thank Professors A. L. Burlingame and W. G. Dauben for their help in securing and interpreting the mass spectra, and Mr. J. Jen for preparing the dihydro  $\beta$ -sitosterol and for other assistance.

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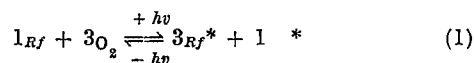
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## Energy Terms of Oxygen and Riboflavin— a Biological Quantum Ladder?

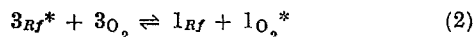
DHRE AND CASTELLI<sup>1</sup> reported the delayed emission of riboflavin at approximately 6000 Å and described it as a phosphorescence. Szent-Gyorgyi<sup>2</sup> found that if the phosphorescence was to be seen dissolved oxygen had to be present. Steele<sup>3</sup> was able to measure the emission spectrum of the riboflavin phosphorescence, maximum at 6030 Å, only in the presence of dissolved oxygen. Shiga and Piette<sup>4</sup> using electron paramagnetic resonance spectroscopy confirmed the oxygen effect and suggested that the phosphorescent emission was prolonged by an inhibition of the non-radiative triplet-singlet transition. As this effect of oxygen, increasing the lifetime of a triplet energy term, is in direct contrast with its well known marked quenching of molecular phosphorescences by shortening the lifetimes of the triplets, presumably by paramagnetic perturbation of the triplet-singlet transition, we have looked for an explanation of the paradoxical increase in the triplet lifetime of riboflavin in the presence of oxygen.

Ogryzlo<sup>5</sup> has discussed the work which assigns the strong visible absorption bands of liquid oxygen as due to a single photon simultaneously elevating two electrons on different oxygen molecules to excited singlet states. A similar double transition event has been reported by Dijkgraaf<sup>6</sup> in a solution of naphthalene and oxygen in which a 3500 Å photon was found simultaneously to excite oxygen to its lowest metastable singlet state (half life  $3.6 \times 10^3$  sec for the unperturbed oxygen ( $^1\Delta_g$  molecule<sup>7</sup>) and naphthalene to its lowest metastable triplet state,  $^3B_{2u}$ . The mechanism converse to the simultaneous absorption of one photon by two molecular species has now been well documented<sup>8</sup> as applying in many chemical reactions which evolve molecular oxygen in excited singlet states. In these instances a pair of excited oxygen molecules react to emit the sum of the excited state energies of the two molecules as one photon of light.

As an extension of these observations we suggest that absorption of light by a riboflavin-oxygen system, at low temperatures, results in a simultaneous electronic transition, induced by one photon, of riboflavin to its lowest excited triplet level and of oxygen to its lowest excited singlet level; equation (1):



We suggest then that the 6030 Å emission from the riboflavin-oxygen system, designated until now as a phosphorescence, is in fact a bimolecular phosphorescence, recombining the energies of the excited metastable states of oxygen and riboflavin in one photon, the reverse of equation (1). In addition, the reaction of equation (2) would account for the apparent prolongation of the riboflavin triplet lifetime by oxygen<sup>4</sup>, that is, the excited singlet oxygen with its extremely long lifetime, functions as an energy reservoir.



This mechanism does not require the formation of any specific riboflavin-oxygen complex, but depends only on the match of the electronic energy terms of the two species. Conceivably, a similar match of energy terms could account for the prolonged riboflavin triplet lifetime induced by cupric ions reported by Shiga and Piette<sup>4</sup> on the basis of electron paramagnetic resonance experiments.

We consider these reflexions to have important implications for biology. If the proposed mechanism is valid it means that the actual triplet energy term of riboflavin lies 1 eV lower than its present assignment of 2 eV. This places it at an energy level equivalent to, or "resonant" with, the lowest excited singlet term of molecular

oxygen, namely, 1 eV. This is a potential drop that can be attained in biological redox energetics and provides a possible mechanism for the generation of excited molecular oxygen. Furthermore, the standard free energy available from perhydroxyl radical interactions,  $2 \cdot \text{O}_2\text{H} \rightarrow \text{HOOH} + \text{O}_2$ , of 1.62 eV (37.5 kcal, ref. 9) equals that of the higher excited energy term of molecular oxygen,  $^1\Sigma_g^+$ , and provides a *raison d'être* for double flavin interactions at the intermediate semiquinone redox level in biological reactions<sup>10-12</sup>. The generation of excited molecular oxygen may account for the ubiquitous distribution of catalase in the body, particularly in the microbodies associated with the endoplasmic reticulum<sup>13</sup>. Support for this thesis is inherent in the observation of Steele<sup>3</sup>, who obtained flashes of light when catalase was injected into an HOOH-riboflavin solution.

It appears probable that biochemical mechanisms exist for the generation of one or the other of the excited molecular oxygen species,  $^1\Delta_g$  or  $^1\Sigma_g^+$ , which may then react in concert by the energy term combinations described by Khan and Kasha<sup>14</sup> to provide a spectrum of available energies an order of magnitude larger than the standard free energy of hydrolysis of adenosine triphosphate. As suggested by Stauff<sup>15</sup> and Khan and Kasha<sup>14</sup>, excited molecular oxygen species may provide the energetics for the display of bioluminescent emissions throughout the visible spectrum. Certainly the phenomenon of bioluminescence is the evidence *par excellence* that biological systems can mobilize energies of such magnitude. The high quantum yields of bioluminescent emissions<sup>16</sup> indicate that the excited molecular oxygen species, if so generated, are "coupled" mechanistically to the molecular oscillators (luciferins) to which the energy is transferred.

In summary, by the generation of electronic excitation states from the oxidation exothermicities of substrates produced by photosynthesis, photochemical energies of the near ultra-violet-visible-near infra-red electromagnetic spectrum, by way of the oxygen quantum ladder, are made accessible, independent of sunlight, to biological systems in the animal kingdom. The ability of these biological systems to mobilize accessible potentials of 1 eV to levels as high as 3.25 eV seems to be evidence for this mechanism.

Critical experiments suggested by this theoretical analysis are in progress.

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## Synthesis of a Plant Hormone by the Salivary Apparatus of Plant-sucking Hemiptera

As a result of investigations into the galls and other plant deformations caused by Hemiptera, the salivary glands of aphids have been claimed to convert tryptophan to  $\beta$ -indolyl acetic acid (IAA) *in vitro*<sup>1</sup>, and their saliva has been shown to contain varying amounts of this plant hormone<sup>2</sup>. Experiments have demonstrated the ability of a mirid and a leafhopper to transfer ingested IAA to the salivary glands and saliva<sup>3</sup>, but the salivary system of all the phytophagous Hemiptera may have the requirements for the synthesis of IAA from endogenous metabolites<sup>4</sup>. Thus tryptophan is known to be converted to IAA by quinones<sup>5</sup> such as would arise by the action of the insects' own salivary polyphenol oxidase<sup>6</sup>; and both tryptophan and a substrate for the enzyme have been found in the secretions of the salivary glands<sup>7</sup>. The following is an account of experiments designed to test the possible synthesis of salivary IAA in Hemiptera. Two insects were used: *Eumecopus punctiventris* Stål (Pentatomidae) and *Elasmolomus sordidus* (F.).

Injection of 1.5  $\mu\text{C}$  of DL-3-phenylalanine-1-<sup>14</sup>C in 3  $\mu\text{l}$ . of Martignoni and Scallion's salt solution<sup>8</sup> (without ascorbic acid) into the haemolymph of larvae of *Eumecopus* at the fifth instar resulted in the appearance in the salivary glands, 1-1.5 h later, of subequal quantities of labelled phenylalanine and other radioactive compounds with the same  $R_F$  as dihydroxyphenylalanine (DOPA). This was revealed by autoradiography of thin layer chromatograms (silica gel G (Merck), developed in butanol/acetic acid/water (77:6:17): entire, rinsed glands were placed on the origins). Most of the radioactivity had disappeared from the salivary glands or been incorporated into insoluble compounds 2 h after the injection, but a labelled compound with the same  $R_F$  as tyrosine could still be identified. The haemolymph, at this time, still showed the presence of labelled phenylalanine, with only traces of derivatives. The results of this experiment support an earlier suggestion that DOPA is the substrate for the salivary polyphenol oxidase<sup>7</sup>; and it indicates that phenylalanine is a precursor.



Fig. 1. Sunflower plant showing swollen and split stem, epinasty, and distortion, caused by feeding by *Elasmolomus sordidus* adults after they had been injected with tryptophan and phenylalanine; untreated insects produced slight distortion at most.



Further experiments were done with the much smaller *Elasmolomus*, the adults of which have a volume of haemolymph, estimated by dye dilution<sup>9</sup>, of about 6  $\mu$ l. After injection of the adults with 0.5  $\mu$ c. labelled phenylalanine, thin layer chromatography with methylethylketone/propionic acid/water (3:1:1) indicated that tyrosine was the main oxidation product in the salivary glands. In another experiment, 0.5  $\mu$ c. DL-tryptophan(methylene-<sup>14</sup>C), in 1  $\mu$ l. salt solution containing 2  $\mu$ g L-phenylalanine (unlabelled), was injected into each of thirty *Elasmolomus*. About 3 h after the first injection, the salivary glands (including the accessory gland and ducts) were dissected out, rinsed in distilled water, and immediately homogenized at 35° C. The dissections were completed in 1 h, and the accumulated brei was then immediately placed on a chromatogram, which was developed in methyl-acetate/isopropanol/25 per cent ammonia (45:35:20). Autoradiography revealed that, in the brei, a small amount of the tryptophan had been converted to a labelled compound with the same  $R_F$  as IAA.

At the pH of the brei (6.5) spontaneous conversion of tryptophan to IAA could not be demonstrated, so we assumed that some of the labelled tryptophan was converted to IAA under the influence of quinones produced from tyrosine or DOPA in the salivary glands by the polyphenol oxidase released from the accessory gland<sup>6</sup> during homogenization. Chromatography of the haemolymph of untreated insects showed that free tryptophan and phenylalanine are normal constituents; thus we consider it likely that synthesis of IAA occurs naturally during the salivation of these insects. Because amino-acids injected into the haemolymph appear in greater concentration in the salivary glands and saliva of Heteroptera shortly afterwards (unpublished data), it seemed possible that insects injected with tryptophan and phenylalanine would produce more salivary IAA than normal ones. In order to test this prediction, small flakes of dried agar, containing tryptophan and phenylalanine (about 3  $\mu$ g of each per insect), were inserted into the abdomens of *Elasmolomus*, and these were confined on sunflower seedlings for 3 days. Over the next 2 weeks, these seedlings developed swelling and splitting of nodes, epinasty, and crooked stems (Fig. 1), that is, all the symptoms that could be simulated by localized applications of IAA. Untreated insects and insects with inserts of agar alone produced no such obvious abnormalities.

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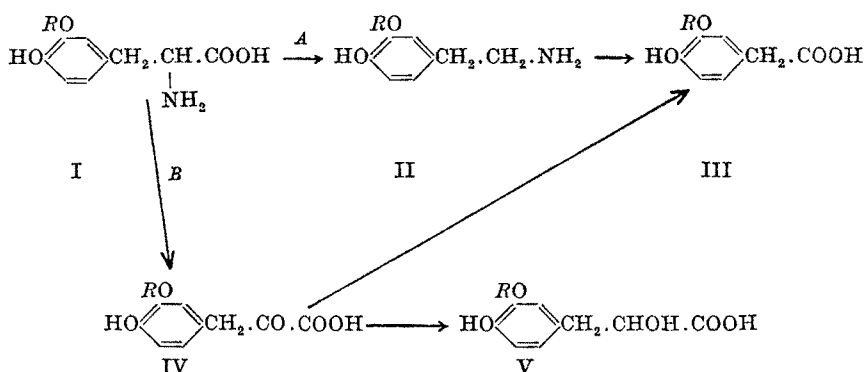
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### Metabolism of Dihydroxyphenylalanine in Human Subjects

THE metabolism of dihydroxyphenylalanine (DOPA; I,  $R=H$ ) may be very complex. By analogy with other amino-acids, degradation may involve decarboxylation (route A) or transamination (route B), with methylation of the 3-hydroxy group possible at any stage. The principal metabolite in normal human urine appears to be homovanillic acid (HVA; III,  $R=CH_3$ ), and excretion of small

amounts of 3-methoxytyramine (II,  $R=CH_3$ ) clearly points to the operation of route A.

Recent observations<sup>1,2</sup> suggest, however, that route B is also operative, particularly in pathological conditions. The main evidence in support of this is the occasional excretion of 4-hydroxy-3-methoxyphenyllactic acid (VLA; V,  $R=CH_3$ ), particularly by subjects producing excessive quantities of DOPA. A search for this acid in normal urines, even using new techniques (unpublished) of greatly enhanced sensitivity, has not so far been fruitful. The corresponding pyruvic acid (VPA; IV,  $R=CH_3$ ) appears, however, to be invariably excreted in small quantities. It has been suggested<sup>2</sup> that the excretion of VLA and VPA in pathological cases involves not only the degradation of larger quantities of DOPA but also an impairment



to route A (or possibly to the conversion of IV to III) leading to an unusual dependence on route B.

In order to throw further light on this subject, I have investigated the metabolism of DOPA and some allied compounds. Urines collected at intervals of 2 h before and after oral administration of suitable doses (50–200 mg) to normal male subjects were examined by paper chromatography. I sought increases in the excretion of methylated compounds (II–V,  $R=CH_3$ ) only; previous workers<sup>3</sup> have detected increased excretion of HVA after administration of DOPA.

Ingestion of DOPA led to the excretion of much apparently homogeneous 3-methoxytyramine. A mixture of acids was formed which could not be resolved completely, but which appeared to consist of HVA heavily contaminated with the isomeric 3-hydroxy-4-methoxy compound. Only doubtfully significant increases in VPA, two or three times the single control values, were observed.

When VPA was fed, much was excreted unchanged, but a moderate increase in HVA was observed and VLA could easily be detected, although it was obviously formed in very small amounts. The increase in HVA was observed in urines heated under acid conditions to destroy VPA, which decomposes to give some HVA when chromatographed. That HVA was formed directly from VPA, rather than by reamination of the pyruvic acid and hence by route A, was indicated by the lack of increase in the excretion of 3-methoxytyramine. Ingested VLA was mainly excreted unchanged, but moderate increases in VPA and HVA were also noted.

It is clear from the formation of the HVA isomer after DOPA that feeding experiments cannot be counted on to reflect normal metabolic processes accurately; urinary HVA usually appears to be homogeneous. The experiments do, however, support the view that route A is the predominant pathway in normal subjects and the rather poor yield of HVA from VPA suggests that the former acid is derived almost entirely from DOPA by way of this route. The experiments also confirm the formation of VLA from VPA. This reaction is reversible, however, and the yield of VLA from VPA was surprisingly low. This observation is compatible with the detection of VPA but not VLA in normal urines. It seems probable

that the reduction of VPA occurs much more readily in pathological urines, but it is also possible that direct conversion of amino-acid (I) to lactic acid (V) might occur, or that VLA might be derived predominantly from 3:4-dihydroxyphenylpyruvic acid (IV,  $R=H$ ).

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### Identification of L-Ornithine and $\delta$ -Amino-succinyl Ornithine in Cell Wall Hydrolysates of *Lactobacillus cellobiosus*

UNTIL recently, 2,6-diaminopimelic acid and lysine were the only diamino-acids known to occur in the murein (mucopolysaccharide) of bacterial cell walls<sup>1,2</sup>. Other diamino-acids have now been found to occur in the cell walls of several micro-organisms. Diaminobutyric acid<sup>3</sup> and 3-hydroxy-2,6-diaminopimelic acid<sup>4</sup> are present in the cell walls of *Corynebacterium tritici* and *Ampullariella regularis*, respectively, and ornithine, both D- and L-configuration, in the plant pathogenic corynebacteria *C. betae*, *C. flaccum-faciens*, *C. poinsettiae*<sup>5</sup>, in *Micrococcus radiodurans*<sup>6</sup>, and in *Lactobacillus bifidus* var. *pennsylvanicus*<sup>6</sup>.

In agreement with earlier investigations<sup>7,8</sup>, the examination of the cell wall composition in thirty species of the genus *Lactobacillus* showed that diaminopimelic acid (in *L. plantarum* only), lysine, alanine, glutamic acid and aspartic acid were present as constituent amino-acids of the murein. In *L. cellobiosus* ATCC 11739, however, ornithine was found instead of lysine.

The cell walls were prepared by shaking the cells with glass beads (homogenizator of Bühler, Tübingen) and by digestion with trypsin. For the removal of teichoic acid the cell walls were extracted with trichloroacetic acid in the cold, and the polysaccharides were extracted by heating with formamide at 160° C for 2 h. The resulting murein

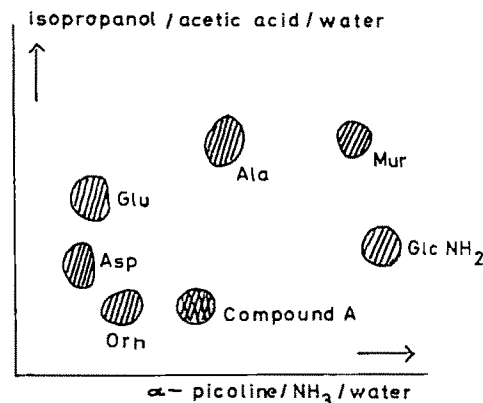


Fig. 2. Two-dimensional paper chromatogram of a hydrolysate (4 normal hydrochloric acid, 16 h, 100° C) of *L. cellobiosus* cell walls in the solvent system (a) and (b).

was about 80 per cent pure, the main impurities being polysaccharides.

Based on glutamic acid, the molar ratio of the amino-acids and amino-sugars in this preparation is: MurNAc: GlcNAc: Ala: Glu: Orn: Asp = 1:1:1,7-1,8:1:1:1.

Ornithine has been identified by column chromatography (amino-acid analyser—Bender u. Hobein, München) and by paper chromatography in the following solvent systems: (a) isopropanol:glacial acetic acid:water 75:10:15; (b)  $\alpha$ -picoline: ammonia (specific gravity 0.88):water 70:2:28; (c) methanol:water:pyridine:hydrochloric acid 32:7:4:1, a solvent system especially suitable for the separation of diamino-acids<sup>9</sup>; (d) butanol:glacial acetic acid:water 4:1:5, upper phase; it was also identified by determination of the absorption spectrum of the reaction product of ornithine with acid ninhydrin<sup>9</sup>. For this purpose, the hydrolysate of the cell walls was chromatographed on paper using system (c), and ornithine was eluted with water after being localized by spraying a strip of the chromatogram with ninhydrin. It was then re-chromatographed in solvent system (b) to remove pyridine hydrochloride which originated from solvent system (c). An authentic sample of ornithine and the eluate were treated identically with acid ninhydrin according to Work<sup>9</sup>, and the absorption spectra were measured in a recording spectrophotometer. As shown in Fig. 1, both absorption spectra are identical and show maxima at 472 and 516 m $\mu$  respectively.

The configuration of ornithine was determined enzymatically. L-Ornithine was converted to L-citrulline in the presence of carbamoyl-phosphate and ornithine carbamoyl-transferase using a cell-free extract of *Streptococcus lactis* which was grown in arginine medium as an enzyme<sup>10</sup>. The assay was performed essentially in the way described by Work<sup>5</sup>. On the basis of this estimation, 92 per cent of ornithine was converted to citrulline, thus indicating that ornithine from *L. cellobiosus* cell walls has the L-configuration.

On two-dimensional paper chromatograms of hydrolysates (4 normal hydrochloric acid, 100° C, 16 h) of the murein of *L. cellobiosus* in systems (a) and (b), a hitherto unknown spot appeared in addition to the usual amino-acids and amino sugars (Fig. 2, compound A). Compound A has been identified as a derivative of ornithine. When cell walls of *L. casei* are hydrolysed under the same conditions  $\epsilon$ -(aminosuccinyl)-lysine is found in the hydrolysate. This compound is formed during acid hydrolysis by cyclization to a succinimide derivative, when aspartic acid is bound by one of its carboxyl groups to the  $\epsilon$ -amino group of lysine<sup>11</sup>. Under more vigorous conditions of hydrolysis, for example, 6 normal hydrochloric acid, 110° C, 18 h,  $\epsilon$ -(aminosuccinyl)-lysine is hydrolysed yielding lysine and aspartic acid<sup>12</sup>. Correspondingly, compound A yielded aspartic acid and ornithine. Under mild alkaline conditions of hydrolysis,

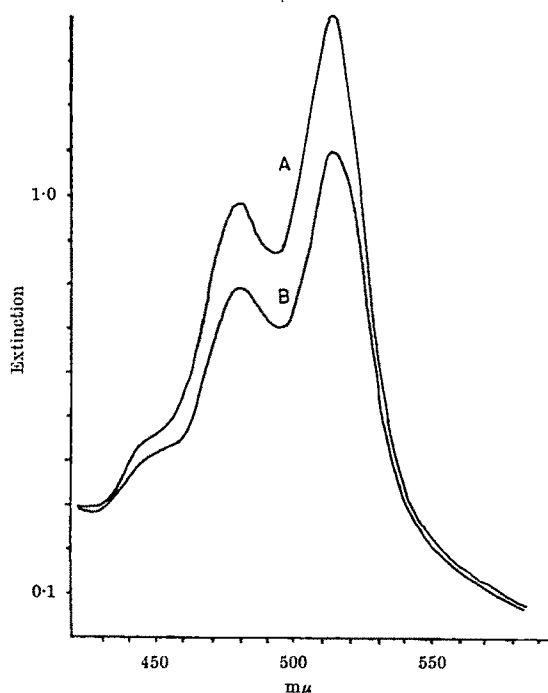


Fig. 1. Absorption spectra of the reaction products of authentic ornithine (curve A) and ornithine from *L. cellobiosus* cell walls (curve B) with acid ninhydrin.

the succinimide ring of  $\epsilon$ -(aminosuccinyl)-lysine is split under the release of the two peptides  $N^6$ -( $\alpha$ -aspartyl)-lysine and  $N^6$ -( $\beta$ -aspartyl)-lysine<sup>12</sup>. If compound A is a homologue of  $\epsilon$ -(aminosuccinyl)-lysine, alkaline treatment of the compound should yield  $N^5$ -( $\alpha$ -aspartyl)-ornithine and  $N^5$ -( $\beta$ -aspartyl)-ornithine. Compound A was therefore incubated for 1 h at room temperature in 0.8 normal barium hydroxide, the solution was neutralized with normal sulphuric acid, and the precipitate was centrifuged off. Chromatography of the supernatant in systems (b) and (d) showed the presence of two ninhydrin-positive compounds which were both different from compound A. One of these compounds was chromatographically identical with  $N^5$ -( $\alpha$ -aspartyl)-ornithine, which has been synthesized for comparison by the method described for the synthesis of  $N^6$ -( $\alpha$ -aspartyl)-lysine by Swallow *et al.*<sup>12</sup>. It is therefore likely that compound A is identical with  $\delta$ -(aminosuccinyl)-ornithine and is probably formed during acid hydrolysis as described for  $\epsilon$ -(aminosuccinyl)-lysine<sup>11,12</sup>. The occurrence of  $\delta$ -(aminosuccinyl)-ornithine in the hydrolysate of murein in *L. cellobiosus* indicates that aspartic acid is linked to the  $\delta$ -amino-group of ornithine and represents a cross-linking molecule like the pentaglycine in the murein of *Staphylococcus aureus*<sup>13</sup>.

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### Effect of Ionic Strength on the Apparent Anti-coagulant Activity of Bovine Heparin

THE preparation of a series of fractions<sup>1</sup> of commercial bovine heparin with varying molecular weight and biological activity by solvent (ethanol-water) elution and by Ecteola column chromatography gave us an opportunity to investigate the role of electrostatic interactions on the anti-coagulant activity of these fractions.

To examine the effects of molecular weight, inactivation and method of preparation on the sensitivity of anti-coagulant activity to ionic strength, the biological activity of various preparations of heparin was measured by a modified USP XVI method in the presence of increasing concentrations of sodium chloride. Preliminary measurements were made to determine the effect of high concentrations of sodium chloride on clotting time. At concentrations of sodium chloride greater than 1.25 molar, the clotting time is increased and the appearance of the final clot alters from opaque to transparent. At high concentrations of salt (3.0 molar) the polymerization of fibrinogen to fibrin is significantly retarded<sup>2</sup> and the configuration of the fibrin at the gel point was found to vary with the ionic strength<sup>3</sup>. The normal opaque clot was used as the end point in these experiments.

The sensitivity of the measured anti-coagulant activity to increasing concentrations of sodium chloride between 0.25 and 1.25 molar appears minimal for the fractions (Fig. 1) of heparin of high and low molecular weight and of high and low anti-coagulant activity prepared on

Ecteola. The anti-coagulant activity of unfractionated heparin is not sensitive to salt concentrations up to 0.5 molar sodium chloride. At higher salt concentrations there is a precipitous drop in activity, and a large decrease occurs between 0.5 and 1.0 molar sodium chloride. The fractions of high molecular weight prepared by alcohol elution behave in a similar manner. The fractions of low molecular weight are relatively less sensitive to increase in ionic strength. Partially inactivated heparin behaves like the unfractionated preparation, the largest decrease occurring above 0.75 molar. The anti-coagulant activity of inactivated heparin (prepared by incubating unfractionated heparin in hydrochloric acid at pH 2.0 and 38° C for 1–7 h) which retains only half its original activity, but without any significant change in molecular weight or viscosity, is relatively insensitive to increasing ionic strength. Inhibition of clotting by increasing salt concentration has been demonstrated<sup>2</sup>. Prolonged clotting time in the test for heparin activity would be recorded as an apparent high biological activity. Because no change or decrease in apparent biological activity was found below  $\mu = 1.25$ , the overall mode of action of sodium chloride in the presence of heparin is not in the fibrinogen-fibrin conversion.

Because the range of ionic strength in which the decrease in apparent activity is observed occurs above 0.5 molar, it

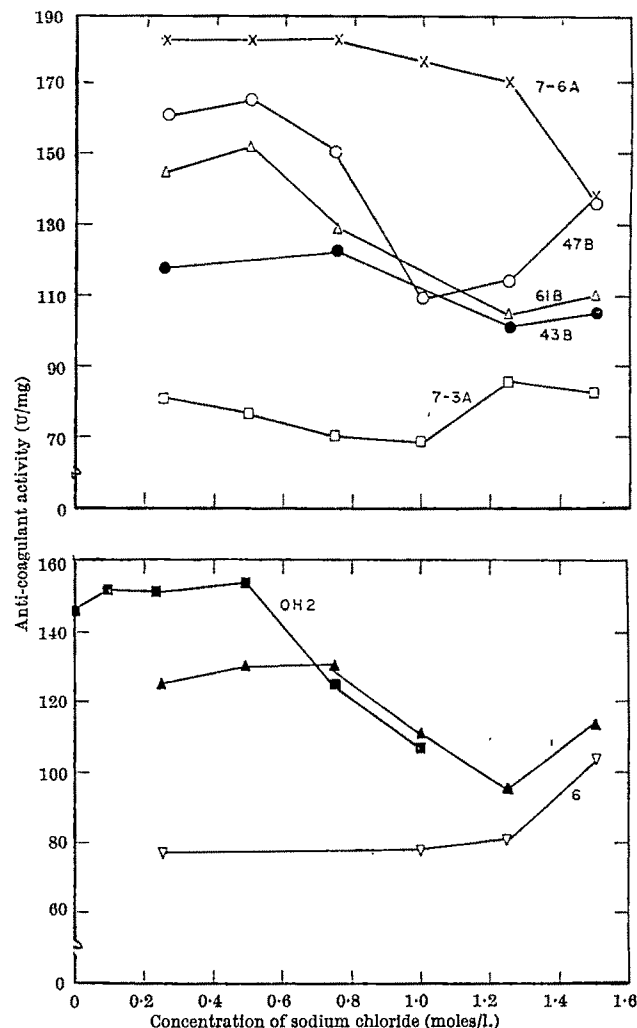


Fig. 1. Effect of ionic strength of sodium chloride on the anti-coagulant activity of heparin preparations. Ecteola fractions 7-6A and 7-3A have molecular weights of 15,680 and 8,460, respectively. Alcohol fractions have molecular weights of 11,940 (47B), 5,540 (61B) and 13,660 (43B). The unfractionated material had a molecular weight of 8,940. The molecular weights were determined at pH 2.5 in 0.5 molar sodium chloride using sedimentation equilibrium. x, Ecteola 7-6A; □, Ecteola 7-3A; ○, alcohol 47B; Δ, alcohol 61B; ●, alcohol 43B; OH2 ■, unfractionated; ▲, inactive 126 u/mg; ▽, inactive 77 u/mg.

is not expected that the ionic atmosphere of the polyelectrolyte or its configuration can play a major part in the effects observed. These effects have been observed for sodium desoxyribonucleate at lower ionic strengths, about 0.05<sup>4</sup>.

If the interaction of the polyanion and its substrate were electrostatic, an apparent decrease in biological activity would be observed with increasing ionic strength, as the anion competed with the polyanion for active sites. In parallel experiments Mora<sup>5</sup> found that the inhibition of ribonuclease activity on ribonucleic acid by polyglucose sulphate was strongly dependent on ionic strength. The inhibition was less efficient at high salt concentrations. The interaction of trypsin and polyglutamic acid was also found to depend on ionic strength, indicating an electrostatic interaction<sup>6</sup>.

Several workers have investigated the interaction of heparin with macromolecules. Scott<sup>7</sup> measured the sensitivity of the solubility of quaternary amine salts of aliphatic acids complexed with heparin to varying concentrations of sodium chloride, by determining the minimal concentration of potassium chloride necessary to solubilize the polymer complex. The change in solubility is sudden and occurs in a narrow range of electrolyte concentration. Heparin complex of low activity was most easily soluble while that of higher activity was solubilized at higher concentration of potassium chloride. Similarly, heparin of low molecular weight was solubilized at a lower ionic strength than the heparin of higher molecular weight. The ability to fractionate heparin into widely different molecular weights with the cellulose ion exchanger ECTEOLA is dependent, in part, on electrostatic interactions.

The interactions in the complex plasma test system involved in the evaluation of anti-coagulant activity have not been clarified, and the chemistry of the fibrinogen-fibrin conversion has itself not been elucidated<sup>8</sup>. Clot formation results when thrombin and fibrinogen interact to form the fibrin clot. Various anions, including chloride, *M*-benzene disulphonate and isopropyl sulphonate<sup>2</sup>, react either with thrombin or fibrinogen to retard clot formation, while isopropyl sulphonate, aurine, and *M*-benzene sulphonate are ineffective. Polyanions, including heparin<sup>9</sup>, ribonucleic acid<sup>10</sup> and polyglutamic acid<sup>11</sup>, have been known to inhibit the tryptic hydrolysis of proteins. The sensitivity of these inhibitors to ionic strength suggests that electrostatic interactions are of significance.

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### Shock-like Symptoms and Hyperplasia of the Adrenal Glands in Rats during Long Term Administration of Histamine

SOME tumours of "non-endocrine" tissue can produce hyperplasia of the adrenal cortex and Cushing's syndrome. These changes could be a result of adrenocorticotrophic hormone (ACTH)-like action, produced by these tumours<sup>1</sup>.

Such tumours are, however, frequently localized in organs able to produce relatively large quantities of histamine, for example the lungs.

Could the histamine play a definite and detectable part in the complex of the above mentioned pathological relations? We have investigated the influence of long term administration of histamine on the adrenal glands in rats.

Fifty adult male Sprague-Dawley rats ( $300 \pm 20$  g body weight) were used in standard conditions. Twenty rats were injected subcutaneously with 0.05 ml. of starch gel daily (group I, control). Thirty rats were injected subcutaneously with 2 mg of histamine-2HCl in 0.05 ml. of starch gel daily (group II). The behaviour of the animals was observed during the experimental period. All the preparation of starch gel, 1 g of amylium tritici plus 15 ml. of 0.9 per cent sodium chloride were heated to form a gel.

Ten control and ten animals treated with histamine were decapitated on day 37 of the experiment. The other animals were decapitated on day 67. Adrenal glands and other endocrine organs were weighed and fixed in 10 per cent formalin. Slices 5 $\mu$  thick were stained with haematoxylin and eosin.

During the first 30 days both the control and the experimental animals showed no noticeable behavioural changes. Later the animals which had been injected with histamine showed steadily intensifying symptoms of posthistaminic shock in the form of anxiety and of rales during respiration. During the following days the symptoms of posthistaminic shock were further intensified; the animals injected with histamine showed severe dyspnoea and forced respiration. All these symptoms appeared 3-5 min after histamine injection and lasted for the next 5 min. Then the animals were reassured, washed themselves, drank water and took food. The severity of such a posthistaminic shock was changeable in individual animals. One rat showed extremely severe shock after daily histamine injection. From about day 40 of the experiment the volume of the abdomen in that rat steadily increased. An autopsy made on this rat on day 67 revealed an enormous tumour (70 g in weight) on the left kidney. A striking enlargement of the adrenals was also found (left adrenal 80 mg; right adrenal 60 mg). Histological examination of the tumour revealed cancer of the kidney of a nephroblastoma type. The adrenals showed great enlargement of the fascicular layer and an increased number of clear cells in the medulla.

Table 1 shows the weights of the adrenal glands in the control and in the experimental animals. After 36 days of histamine administration the adrenals were heavier by about 9 per cent in comparison with the weight of those glands in the controls. The difference was not, however, statistically significant. The weight of the adrenals in rats treated with histamine for 66 days was statistically greater than that in the control animals. Microscopic examination of the adrenals of rats treated with histamine for 36 as well as for 66 days revealed enlargement of the fascicular layer (Fig. 1).

It was found that 0.05 ml. of starch gel used for injection could be absorbed from the subcutaneous site within 24 h. A solution of histamine in such a gel was made in order to prolong absorption and action of histamine in the system of the rat. The daily injection of 2 mg of histamine seemed to be rather a physiological dose, for it has been calculated<sup>2</sup> that an adult rat can produce in its body as much as 2 mg of histamine in a day. The mechanism by which histamine produced the described histo-

Table 1. WEIGHTS IN MG OF THE ADRENAL GLANDS IN CONTROL RATS AND RATS TREATED WITH HISTAMINE

36 days		66 days	
Histamine (10)	Control (10)	Histamine (19)	Control (10)
74	68	85	70
$\pm 8$	$\pm 4$	$\pm 7.1$	$\pm 7$
$t = 1.6$		$t = 5.3$	
$P > 0.05$		$P < 0.05$	

In parentheses, the numbers of animals. Weights are given in mg. Values are mean  $\pm$  S.D.

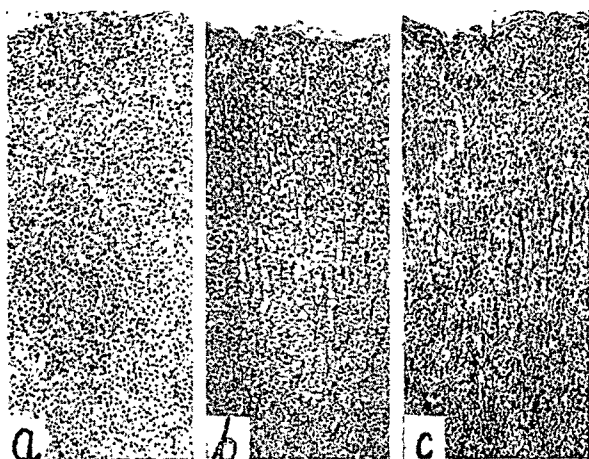


Fig. 1. The adrenal cortex (a) of control rat; (b) of rat treated with histamine for 36 days; (c) of rat treated with histamine for 66 days. Stain, haematoxylin and eosin. ( $\times 100$ .)

logical changes and the shock-like symptoms remains quite obscure. The development of shock-like symptoms seems to be an opposite process in comparison with the known phenomenon of adaptation to histamine<sup>3</sup>.

It is difficult to judge whether the effect of histamine on the adrenal cortex is direct or indirect, through the stimulation of ACTH production by the pituitary gland. The posthistaminic shock as a cause of adrenocortical hypertrophy may also be doubtful, because detectable histological changes had appeared in the adrenal cortex before initial development of shock symptoms. Previous investigations on rats injected daily with 1.0 mg of histamine-2HCl in 0.05 ml. of starch gel for 23 consecutive days revealed enlargement of the glomerular layer in the adrenal cortex but no detectable weight change of that organ<sup>4</sup>.

According to Kahlson the histamine forming capacity promotes both normal and malignant growths<sup>5</sup>. Extracellular histamine in the form of "long-acting histamine" (an oily suspension of histamine dipicrate), however, failed to promote wound healing or collagen formation<sup>6</sup>. The development of kidney cancer in one rat out of twenty treated with histamine for 66 days must also remain unexplained. The typical changes in the adrenals of the rat with kidney cancer were comparable with the changes in the adrenals of all the rats treated with histamine, and thus this seems to suggest that the tumour is not spontaneous. Further investigations are in progress.

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### Possible Synergic Influence of ACTH and FSH on the Adrenal Cortex in Hypophysectomized-Gonadectomized Rats

THE gonadotrophic hormones may be non-specific growth factors for the adrenocortical cells, chiefly in the so called intermediary zone<sup>1</sup>. These hormones, however, failed, unlike ACTH, to stimulate maturation and transformation of adrenocortical cells.

Purified preparations of ACTH can have a diminished ability to stimulate an increase in the adrenal weight, the incidence of mitotic activity and lipid depletion of the zona fasciculata<sup>2</sup>. It is suggested that purification discards a pituitary substance which potentiates the influence of ACTH on the adrenal cortex. An increased urinary 17-KS output was found to be caused by human chorionic gonadotrophin (HCG) in a case of congenital adrenal hyperplasia<sup>3</sup>. A suggestion has been passed on that HCG can stimulate 17-KS excretion when secretion of ACTH is normal or augmented.

The results of simultaneous influence of ACTH (ACTH 'Polfa') and of pituitary gonadotrophin ('Glanduantin', Richter) on the adrenal cortex in hypophysectomized-gonadectomized rats are presented in this report. Eight normal and twenty hypophysectomized-gonadectomized male Sprague-Dawley rats (body weight  $200 \pm 20$  g) were used in standard conditions. From the fifth post-operative day for 24 consecutive days the urine was collected from the groups of normal and of hypophysectomized-gonadectomized animals treated with hormones, as in Table 1.

Table 1

	I(8) Normals	II(6) Controls	III(4) FSH (v)	IV(4) ACTH (v)	V(6) FSH + ACTH (v)
5 days	—	—	—	—	—
10 "	—	gel	2.5	0.05	2.5 + 0.05
5 "	—	"	5.0	0.1	5.0 + 0.1
4 "	—	"	2.5	1.0	2.5 + 1.0

Numbers of animals in parentheses. FSH, follicle stimulating hormone.

The hormones were injected subcutaneously once daily in 0.05 ml. of starch gel (1 g of amylium tritici plus 15 ml. of 0.9 per cent sodium chloride heated until gel formation). Solutions of the hormones in such a gel were used to prolong absorption and action of hormones in the system of the rat. Control animals were injected with the starch gel only. Excretion of 17-KS (Zimmermann's method) and of acid mucopolysaccharides (carbazole and orcinol methods) was determined. In decapitated hypophysectomized-gonadectomized animals a macroscopic inspection did not reveal pituitary remnants. This report gives the results of histological examination of the adrenal cortex and of 17-KS determination.

Fig. 1 shows that the greatest and statistically significant increase of the adrenal weight was found in rats treated with FSH and ACTH in comparison with all the other groups of hypophysectomized-gonadectomized animals ( $P < 0.05$ ).

Adrenal cortex of hypophysectomized-gonadectomized control animals (Fig. 2, II) showed marked atrophy of the fascicular layer with weakly denoted cellular borders and a broad intermediary zone of small acidophilic cells. Administration of FSH caused marked enlargement of the intermediary zone and detectable enlargement of the

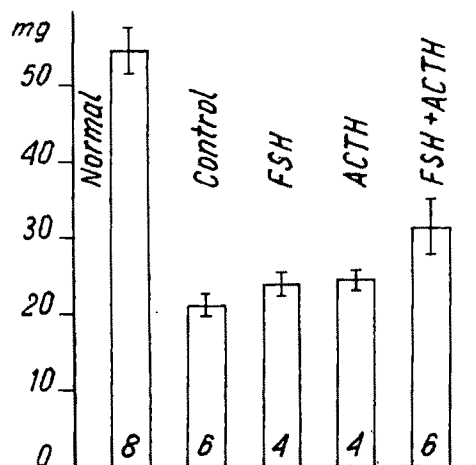


Fig. 1. The weight of the adrenal glands in normal and in experimental rats. The figures at the bottom of columns denote number of animals.



glomerular layer (Fig. 2, III). Administration of both ACTH alone (Fig. 2, IV) or ACTH in combination with FSH (Fig. 2, V) caused clear zonal reconstruction of the adrenal cortex. All the cortical layers were built of well demarcated but significantly smaller cells than in the adrenal cortex of normal animals (Fig. 2, I).

The circadian values of urinary 17-KS in normal rats were 20–26  $\mu\text{g}/\text{animal}$  or 65–90  $\mu\text{g}/\text{four animals}$ . Hypophysectomy–gonadectomy caused deep decrease of 17-KS excretion within the first few days after the operations. Steady recovery of adrenocortical function in respect to excretion of 17-KS was then observed and from days 5–10 after the operation all the animals showed quite normal but still changeable excretion of 17-KS (41–87  $\mu\text{g}/\text{four rats}$ ). The highest stimulation of excretion of 17-KS (of more than 100 per cent) was found within the first 2 days of administration of ACTH in a dose of 0.1  $\mu$  daily. ACTH in combination with FSH did not cause detectable changes in excretion of 17-KS in comparison with the values in the control animals.

The ACTH and gonadotrophin used were not highly purified hormone preparations. Quite comparable changes in the adrenal cortex, however, had been observed previously<sup>1</sup> in hypophysectomized–gonadectomized rats treated with 'Glanduantin' or FSH-NIH-S1. Similar changes caused by 'Glanduantin' were reproduced in this experiment. The most striking histological feature of the adrenal cortex subjected to the action of gonadotrophin was enlargement of the intermediary zone. Such a zone could be present in normal rats and it seems to be dependent on the dietetic regimen given to the animals. According to Chester-Jones *et al.*<sup>4,5</sup> the presence of the intermediary zone in the adrenal cortex could be a consequence of starvation of rats. Nevertheless, hypophysectomy produces a broad sudanophobic zone in the adrenal cortex. The same is true for the adrenal cortex in hypophysectomized–gonadectomized rats.

Administration of small doses of ACTH caused clear normalization of zonal structure of the adrenal cortex, but there was only slight increase of weight of the adrenals. Concomitant administration of ACTH and pituitary gonadotrophin ('Glanduantin') caused not only normalization of zonal structure of the adrenal cortex, but also a statistically significant weight increase of that gland.

It seems probable that pituitary gonadotrophin of FSH type may be a non-specific growth factor for the adrenal cortex, especially for the cells situated on the

border between the glomerular and the fascicular layers. ACTH is a potent factor stimulating transformation of the adrenocortical cells, Gotschau's theory. Concomitant influence of both hormones on the adrenal cortex may be synergic from the morphological, if not from the histo-functional, standpoint.

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### Early Effects of Hydrocortisone on Rapidly Labelled Rat Liver Ribonucleic Acids

ADMINISTRATION of single, large doses of hydrocortisone causes a marked increase in synthesis of rat liver RNA and the activity of several enzymes<sup>1</sup>. A direct gene action and enhanced synthesis of specific messenger RNAs has been suggested<sup>2</sup> and positive evidence for this hypothesis was presented<sup>3,4</sup>. In contrast, other experiments indicate that hydrocortisone provokes an unspecific stimulation of RNA synthesis<sup>5,6</sup>. In almost all the reported experiments, hydrocortisone was administered before injection of the labelled RNA precursors. In an attempt to elucidate the primary effects of hydrocortisone, we investigated the effects of administering this hormone after short term labelling of liver RNAs.

The experiments were carried out with male, albino rats of 100–150 g body weight. Carrier-free sodium phosphate labelled with phosphorus-32 (300  $\mu\text{C}/100 \text{ g}$ ) or orotic acid-6-<sup>14</sup>C (25  $\mu\text{C}/100 \text{ g}$ ; specific activity, 3.124  $\text{mc./mmole}$ ) was injected intraperitoneally, followed at different intervals by 10  $\text{mg}/100 \text{ g}$  of hydrocortisone. The animals were killed by decapitation and the livers were chilled in 0.14 molar sodium chloride. All subsequent operations were carried out at 4° C. A 10 per cent homogenate in 0.14 molar sodium chloride containing 0.05 per cent polyvinyl-sulphate (PVS) was prepared. The liver RNA fractions were obtained by the method of Georgiev and Mantiéva<sup>7</sup>, modified as follows: the homogenate was centrifuged at 600g for 5 min, the supernatant made 0.5 per cent with respect to sodium dodecyl sulphate (SDS) and deproteinized three times for 15 min, each time with phenol (saturated with 0.14 molar sodium chloride, pH, 5.0). RNA was precipitated from the last water phase with 2 volumes of ethanol containing 1 per cent potassium acetate. This fraction represented the bulk of the cellular RNA and is designated 'cytoplasmic RNA' (cRNA). The 600g sediment was extracted threefold with cold phenol: 0.14 molar sodium chloride (1 : 1) at 4° C and the resulting interphase layer was suspended in 0.14 molar sodium chloride containing 0.5 per cent SDS and 0.05 per cent PVS. An equal volume of phenol was added and the mixture was subjected to 40° C for 5 min. The cooled water phase was deproteinized twice and RNA was precipitated as already described. This fraction is designated 'nuclear R-RNA' (R-RNA). The interphase layer was extracted once with cold phenol: 0.14 molar sodium chloride at 4° C, then suspended in 0.14 molar sodium chloride (plus SDS and PVS) and treated with hot phenol at 65° C for 5 min. The RNA obtained from the water phase after three deproteinizations at 4° C is designated 'nuclear D-RNA' (D-RNA). The remaining interphase layer after this extraction contained only traces of RNA labelled with phosphorus-32. The different RNA fractions were washed with ethanol, dissolved in 0.01 molar sodium chloride and purified by gel filtration through 'Sephadex G-25' at 4° C (ref. 8). RNA was determined by its *O.D.*<sub>260</sub> and its radioactivity as cold 5 per cent trichloro-

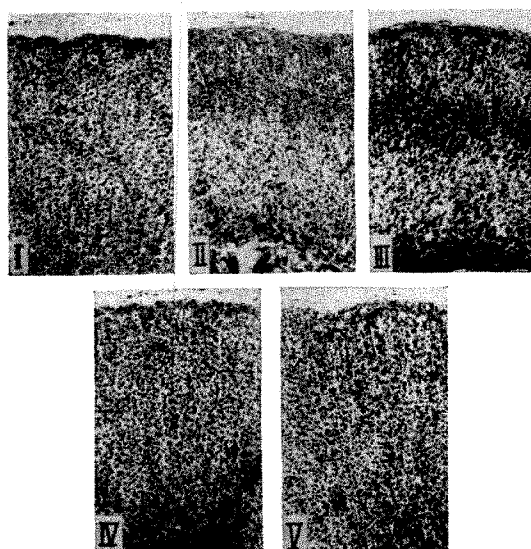


Fig. 2. The adrenal cortex (I) of normal rat, and of hypophysectomized-gonadectomized rats; (II) control; (III) treated with FSH; (IV) treated with ACTH; and (V) treated with FSH plus ACTH. Stained with haematoxylin and eosin. ( $\times 100$ ).

acetic acid insoluble counts. Agar gel electrophoresis and subsequent autoradiography were carried out by the method of Tsanev *et al.*<sup>9,10</sup>. The mononucleotide composition of RNA after 18 h of hydrolysis with 0.5 normal potassium hydroxide at 37° C was performed according to Katz and Comb<sup>10</sup>. The radioactivity of the nucleotides was determined directly in solution with a 'Vakutronic' VA-Z-430 counter.

In preliminary experiments it was found that incorporation of inorganic phosphate into all three RNA fractions proceeds almost linearly up to 4 h. Administration of hydrocortisone causes a marked increase in their labelling as followed for 2-4 h after administration of the hormone. The results obtained with shorter periods of hydrocortisone action are given in Table 1.

Two hours after administration of hydrocortisone the specific activity of all RNA fractions is greater than that of the controls, but 60 min after administration of hydrocortisone the specific activity of both nuclear RNA fractions is less than in control animals, while that of the cytoplasmic RNA is slightly greater.

Determination of the labelling of the four RNA nucleotides showed that the observed variations of RNA labelling are inherent to all constituent nucleotides. Table 2 shows the mononucleotide composition of the three RNA fractions (direct determination) and of their rapidly labelled components (distribution of phosphorus-32). It should be pointed out that at both times after application of hydrocortisone (60 or 120 min) the mononucleotide composition of RNA remained essentially the same as in control animals, and therefore only mean values are given. These results agree with previous findings on the mononucleotide composition of RNAs from different sub-cellular fractions<sup>11</sup>.

The results of our experiments and those of others<sup>5,6</sup> clearly show that hydrocortisone provokes an unspecific stimulation of RNA synthesis, thus making unlikely a direct gene action of this hormone. The decreased labelling of nuclear RNA fractions, 60 min after hydrocortisone, is of particular interest. This effect is an early one, for a high concentration of hydrocortisone into rat liver was de-

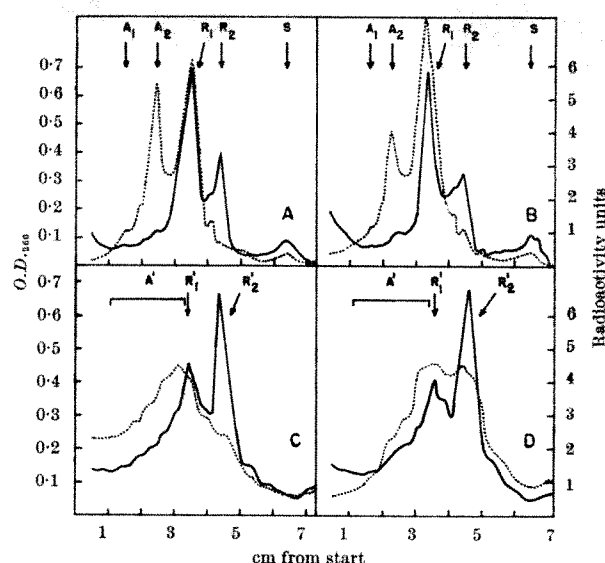


Fig. 1. Electrophoretic pattern of rat liver nuclear RNAs isolated from animals killed 120 min after *in vivo* administration of orotic acid-6-<sup>14</sup>C (25  $\mu$ Ci/100 g of body weight). Agar gel electrophoretic fractionation for 100 min at 9 V/cm. Solid line, O.D.<sub>250</sub>; dotted line, relative radioactivity units obtained from the autoradiogram recorded at 550 m $\mu$ . A and B, Nuclear R-RNA; C and D, nuclear D-RNA. A and C, Control animals; B and D, animals treated with 10 mg/100 g of hydrocortisone 60 min before death. For further details see text.

tected 30 min after its administration<sup>12</sup>. There are two possible interpretations: (1) that hydrocortisone increases the rate of nucleo-cytoplasmic transfer of newly synthesized RNA and (2) that hydrocortisone brings about an accelerated degradation of rapidly labelled nuclear RNA. No markedly increased ribonuclease activity of nuclear chromatin is observed after hydrocortisone<sup>13</sup>, and so the first possibility is more acceptable.

Agar gel electrophoresis and autoradiography of the three RNA fractions give more information. In these conditions, the two ribosomal RNA peaks in cRNA display the same specific activity and hydrocortisone induces a parallel increase in their labelling as early as 60 min after application. The results obtained with the two nuclear fractions are shown in Fig. 1. The two nuclear fractions are complementary to one another to give the picture obtained with total nuclear RNA by sucrose density gradient centrifugation<sup>14</sup> and by agar gel electrophoresis<sup>8</sup>. There are the following peculiarities. (1) R-RNA gives the two ribosomal RNA fractions R<sub>1</sub> and R<sub>2</sub> which correspond to 28S and 18S ribosomal RNA. In most preparations, the ratio R<sub>1</sub>/R<sub>2</sub> is greater than that observed with cytoplasmic RNA. In addition, two slow moving fractions, A<sub>1</sub> and A<sub>2</sub>, are detected by their high labelling. In kinetic experiments (30-90 min) these fractions are the first to become labelled, followed by the R<sub>1</sub> peak<sup>8</sup>. (2) D-RNA shows a predominant R'<sub>2</sub> peak with a mobility corresponding to that of 18S RNA, as well as several slow moving fractions, A', which in this case, too, are the first to be labelled. In our conditions of extraction, sharp and well delimited peaks are observed, which indicates that the broad size distribution observed by others<sup>15</sup>, for a similar DNA-like RNA fraction, reflects its enzyme or thermal degradation rather than the expected size heterogeneity of messenger RNA.

Hydrocortisone (60 min after application) caused the labelling of the slow moving A and A' fractions to be relatively decreased, while that of R<sub>1</sub> in R-RNA and of R'<sub>1</sub> and R'<sub>2</sub> in D-RNA are markedly increased (Fig. 1, B and D). There is evidence<sup>15,16</sup> that the "heavy" nuclear RNA fractions are precursors of the 28S and 18S nuclear RNAs, and so our results can be interpreted to show that hydrocortisone causes a more rapid conversion of the precursor RNA molecules into the corresponding product RNAs.

Hydrocortisone provokes an unspecific stimulation of RNA synthesis in liver cells. Our results also suggest that

Table 1. EFFECT OF HYDROCORTISONE ON THE SHORT TERM INCORPORATION OF LABELLED PHOSPHATE INTO RAT LIVER RIBONUCLEIC ACIDS

Experiment	Time after (min)	Specific activity in c.p.m./mg of RNA			
		Phosphorus-32	Hydrocortisone	cRNA	R-RNA
(1)	90	-	180	1,641	3,640
	240	-	510	2,400	5,790
	240	120	572	2,885	6,670
(2)	150	-	1,100	9,860	10,950
	210	-	1,720	12,900	19,950
	210	60	1,940	9,650	18,070
(3)	120	-	355	2,450	3,800
	180	-	428	3,280	6,100
	180	60	486	2,300	5,250
	300	-	774	4,800	11,000
	300	120	865	6,200	14,850

Three rats are included in every group; each is injected intraperitoneally with 300  $\mu$ Ci of labelled phosphate and 10 mg of hydrocortisone/100 g of body weight. In control animals, hydrocortisone is omitted.

Table 2. EFFECT OF HYDROCORTISONE ON THE MONONUCLEOTIDE COMPOSITION OF TOTAL AND RAPIDLY LABELLED RAT LIVER RIBONUCLEIC ACIDS

Fraction	No. of experiments	Direct determination					Distribution of phosphorus-32				
		Molar ratio				GC	Molar ratio				GC
		A	U	G	C	AU	A	U	G	C	AU
cRNA controls	8	16.9	21.7	32.9	28.5	1.59	18.2	26.0	25.7	30.1	1.27*
plus hydrocortisone	8	17.5	21.0	32.3	29.2	1.60	16.6	26.5	27.5	29.4	1.32*
R-RNA controls	6	17.0	21.5	34.7	26.8	1.60	17.0	19.3	37.4	26.3	1.75
plus hydrocortisone	6	16.2	21.9	32.7	29.2	1.62	16.4	19.6	36.1	27.9	1.80
D-RNA controls	8	21.3	25.2	27.3	26.2	1.15	28.0	24.2	26.5	21.3	0.92
plus hydrocortisone	8	20.3	25.8	27.3	26.6	1.16	27.0	24.0	26.5	22.5	0.96

Mean values for 150-240 min after labelled phosphate, and 60-120 min after hydrocortisone, are given.

A, Adenylic acid; G, guanylic acid; U, uridylic acid; C, cytidylic acid. \* These values are too large, because no correction for terminal addition of cytidylic acid to soluble RNA was made.

hydrocortisone causes a more rapid nucleo-cytoplasmic transfer of RNAs, as well as an accelerated precursor-product conversion of nuclear RNAs.

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### Relationship between Nuclear and Cytoplasmic RNA

Singh and Koppelman have subjected to mathematical analysis<sup>1,2</sup> some of the experiments carried out by my colleagues and myself on the relationship between nuclear and cytoplasmic RNA<sup>3,4</sup>. I have already demonstrated the inadequacy of the model presented by Singh and Koppelman in their first communication<sup>1</sup> by showing that a relationship defined by them as a constant ( $\lambda$  in my report) was in fact not a constant<sup>5</sup>. In their second communication<sup>2</sup>, Singh and Koppelman accept this point and present a modified version of the same exercise. As before, they seek to give the impression that they have taken the results of some of our experiments and shown that these results fit the particular mathematical model which they propose. Once again this is not so. In neither of the graphs produced by Singh and Koppelman have our results been accurately transcribed. In both instances, several points given in our original curves have been omitted from the graphs, and the curves have been truncated by deletion of early and late experimental points. This is well illustrated in the data for cytoplasmic RNA adenine shown in Fig. 1 of their communication. In our original report this curve is unequivocally sigmoid, but it appears in their communication as a straight line, an approximation which involves the omission of several

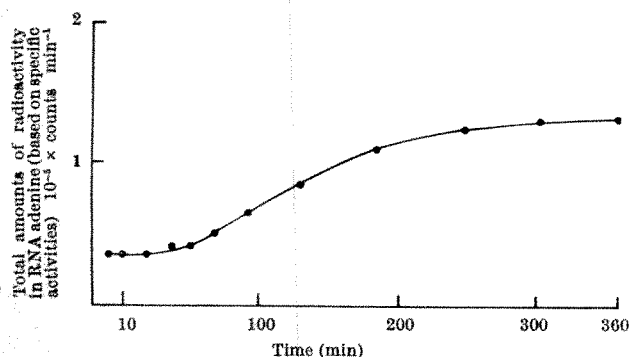


Fig. 1. Total amounts of radioactivity in cytoplasmic RNA adenine. The original graph from Harris *et al.* (ref. 4).

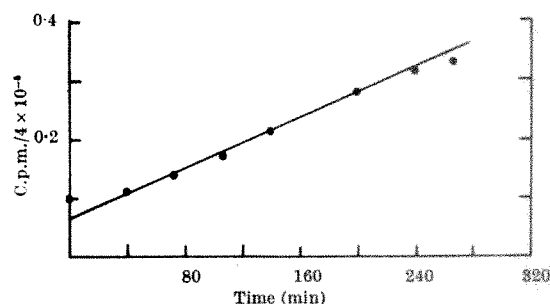


Fig. 2. The same graph as Fig. 1 re-plotted by Singh and Koppelman (ref. 2).

of our experimental points, the insertion of points which do not correspond to our experimental points and the deletion of the end of the curve. In order that readers may assess the extent of the transformation, I have reproduced in Fig. 1 the original experimental curve and in Fig. 2 the same curve as re-plotted by Singh and Koppelman.

It may have escaped the notice of some readers that the position initially adopted by Singh and Koppelman has undergone a change. The original point at issue between these authors and myself concerned the fate of the labelled nuclear RNA. My interpretation of our results was that much of this RNA underwent intracellular degradation. Singh and Koppelman took the view that this interpretation was incorrect. In their first communication<sup>1</sup> they stated: "Although the possibility of such degradation cannot be excluded, it appears from the analysis presented here that it does not occur to any significant extent". In a later communication, however, Singh<sup>6</sup> concluded that the nuclear RNA did contain a "labile fraction in high molecular weight RNA" and estimated that the "half-life time of the most labile component in nuclear RNA" was 20-30 min. In their second communication<sup>2</sup>, Singh and Koppelman state: "The deviation of experimental points from the straight line during the early period of incorporation is primarily due to the presence of low molecular weight RNA, which is known to undergo rapid turnover." (It should be noted that even our re-plotted curves fail to conform closely to their model; and no nuclear RNA was "known to undergo rapid turnover" until my colleagues and I revealed the fact<sup>7,8</sup>.) Again, later in the same letter: "Recent investigations (not yet published) suggest that such degradation is more prominent in non-growing cells." (With this I agree, and refer the authors to the papers by J. W. Watts and myself in 1959 in which the rapid turnover of nuclear RNA in a non-multiplying animal cell was described in some detail<sup>7,8</sup>.)

It thus appears that Singh and Koppelman do not any longer disagree with my view that much of the labelled nuclear RNA does undergo breakdown within the cell to end-products which are soluble in acid. That some nuclear RNA fractions might be precursors of stable cytoplasmic RNA is not, so far as I am aware, denied by anyone.

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Singh and Koppelman<sup>1,2</sup> fit a kinetic model to data of Harris and Watts<sup>3</sup>. Their model depends on the equation

$$\frac{d}{dt}(a_{sc}) = \lambda(a_{sn} - a_{sc})$$

where  $\lambda$  is constant. But the data insist that  $\lambda$  is not constant: it changes by a factor of 3.5 during the course of the experiment. Singh and Koppelman<sup>2</sup> try to overcome the difficulty by approximating an exponential as a straight line. They do not succeed. Kinetic models based on erroneous assumptions are unacceptable. Any model which can satisfactorily fit the data of Harris and Watts must be rather more sophisticated than that of Singh and Koppelman.

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## PATHOLOGY

### Premalignant Cells in Tumorigenesis induced by Plastic Film

PLASTIC material inserted under the skin of mice and certain other laboratory animals frequently leads to the formation of sarcomatous transplantable tumours<sup>1,2</sup>. This finding deserves consideration in view of the use of plastics in human surgery. It also attracts theoretical interest as an example of "physical" carcinogenesis. (The role of chemical cofactors is still under dispute although evidence is mounting against it<sup>3</sup>.)

Carcinogenic events are thought not to take place on the surface of the plastic insert but rather in connexion with the tissue capsule<sup>3</sup> which gradually builds up as a foreign body reaction. This communication presents evidence that 1-8 months before a tumour appears in mice, the premalignant cells are regularly found firmly attached to the insert itself where they seem to originate and mature. Premalignant cells could not be found in the capsule tissue except during the last 4 weeks before the appearance of palpable tumours.

Inbred mice (*CBA-T6*) at the age of 1.5-2 months received subcutaneous inserts in both flanks of double vinyl chloride acetate coverslips ('Dispo-slips') 15 × 22 mm in size. A control group (eighty animals) was left to itself. Tumours developed in 65 per cent of the male animals 9-12 months after operation, and in almost all the female animals after 7-12 months. In the experimental group, inserts and tissue capsules were cut in thirds after intervals of from 2 weeks to 12 months. One portion was left in the original animal. A second portion was used for histological, karyological and cultural examinations. The plastic and capsule pieces of the third portion were carefully separated and then individually transplanted into *CBA-H* recipient mice.

The results are shown in Table 1. No tumours developed in the original and the recipient animals when transplantation was carried out during the first 5 months after initial insertion of the plastic films. It is concluded that during this period premalignant cells either had not yet emerged or were unable to mature further outside the original film/capsule condition. The disturbance of the

primary processes at this time caused by removing two-thirds of the film and tissue capsule apparently stops tumorigenesis even in the original animal. (A separate control experiment has shown that pieces of plastic film one-third the size of the original inserts, that is, 7 × 15 mm, no longer cause tumours within 15 months; they are obviously below the threshold size critical for tumorigenesis.)

Tumours developed at the expected rate in the original and also in the recipient animals when the transplantation was carried out 6 or more months after the initial insertion of the plastic films. The tumours appeared simultaneously in the original and the corresponding recipient animals. The origin and identity of the tumours were established on the basis of the *T<sub>a</sub>*-marker chromosome. This indicates (1) that premalignant cells have emerged by the time of transplantation, (2) that premalignant cells are present in multiple foci, (3) that the population of premalignant cells is homogeneous with regard to the stage of development, and (4) that further maturation of premalignant cells towards the tumour occurs independently of the conditions of the film and capsule.

When the latent period (that is the time between transplantation and the appearance of the tumour) lasted 2-8 months, the tumours developed from transplanted film pieces only, not from capsule tissue. If the latent period happened to last 1 month or less, tumours developed in recipient animals from both the film piece and the capsule tissue. These results suggest that 2-8 months before the tumour appears the premalignant cells are firmly attached to the film and are not found in the surrounding capsule tissue. (It remains to be seen, however, whether premalignant cells in transplanted capsule tissue can be destroyed or returned to normality by the recipient animal in the absence of the piece of plastic film.) From the results available so far, it seems that tumorigenic cells detach from the plastic film and invade the capsule tissue only a few weeks before the growth of the tumour is recorded macroscopically. It can reasonably be assumed, therefore, that detachment of tumorigenic cells from the plastic film is actually one of the first manifestations of malignancy.

Apart from the experimental results described, it is suggested that this system allows the study of cell populations with a degree of premalignant maturation which can be determined accurately.

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Table 1. TUMOUR DEVELOPMENT FROM TRANSPLANTED PREMALIGNANT CELLS ON PLASTIC FILM AND IN CAPSULE TISSUE

Tumours simultaneous in original and recipient animals	Number and sex of animals	Insert carried in original animal (months)	Observed after transfer (months)
None	22 ♀ ♂ 6 ♀ 12 ♂	1/2-5 6-10	Up to 15 Up to 13
From transferred film pieces only	2 ♂ 1 ♀ 2 ♀ 2 ♀, 1 ♂ 2 ♀	7 8 6 6-12 8-9	6-8 5 4 3 2
From transferred film and capsule	4 ♀ 1 ♂	9-12 8	(<) 1 (<) 1

## BIOLOGY

### Probable Instance of Genetic Polymorphism in the Graptolites

A SPECIES, in the biological sense, is a community of individuals with a similar genetic structure, drawing on a common gene pool and having the ability to interbreed. Individual variation within such a community can be accounted for, in large part, by the segregation and recombination of genes according to Mendelian rules. This variation may be continuous and capable of being

expressed in terms of a unimodal distribution curve, or it may be discontinuous, with sharply contrasted differences which either do not overlap or else give rise to a bimodal (or multimodal) curve. Variation of the latter kind is polymorphism, and if the two or more distinct forms of a species co-exist in the same habitat, that is, if they are genetically determined rather than a product of geographical variation, this phenomenon can be called genetic polymorphism.

A genetically controlled variant may arise by the introduction of a new gene into the gene pool or through a new combination of existing genes. In either case, a relative and numerical increase of the variant in the community, brought about by a spread of the new gene or gene-combination by Mendelian inheritance, will be conditional on its conferring some selective advantage. If this requirement is fulfilled, the spread of the variant through the community will take place according to one of two patterns<sup>1</sup>. Polymorphism is said to be balanced when the spread of the advantageous character and the gene or genes controlling it is checked and held at some fixed level in the community due to environmental or genetic factors, or both. Transient polymorphism, on the other hand, is in operation only during the time taken for an advantageous gene to spread unhindered through the community; when the spread is complete, uniformity is reached once again, but of a new type from the old, and polymorphism is ended.

A probable case of transient polymorphism in the extinct planktonic graptolites (Class Graptolithina, Order Graptoloidea) is illustrated by the two "species" *Holmograptus lentus* (Törnquist) and *Nicholsonograptus fasciculatus* (Nicholson). The morphological resemblance between these two species is striking; indeed, the one important difference is the presence of two stipes in the rhabdosome of *H. lentus* (Figs. 4 and 5) as against a single stipe in *N. fasciculatus* (Figs. 1-3). Apart from this, there is similarity in thecal form, in the dimensions and form of the sicula (and, by implication, the siculozoid), in the position of the initial bud, and in the level at which stipe "1" (the only stipe in *N. fasciculatus*) leaves the sicula. Differences do exist, however, though these are probably not significant; thus the attitude of the proximal part of the single stipe relative to the sicula in *N. fasciculatus* varies beyond the limits observed in *H. lentus* and, moreover, the excessive distal recurving of the stipe in *N. fasciculatus* has never been recorded in *H. lentus*. These differences apart, the morphological resemblance between the two species is such that it is tempting

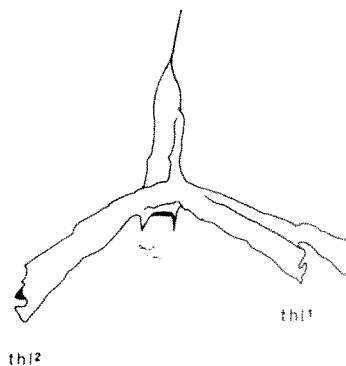


Fig. 4. *Holmograptus lentus* (Törnquist, 1911). Proximal end of rhabdosome. ( $\times 25$ .)



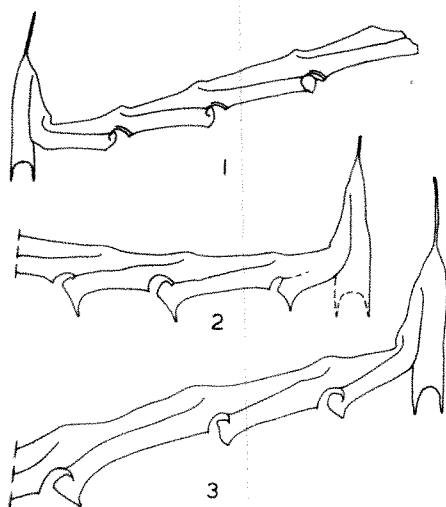
Fig. 5. *Holmograptus lentus* (Törnquist, 1911). Fragment of stipe. ( $\times 17$ .)

to attribute the alternative development of a bi- or unistiped rhabdosome to polymorphism.

In the graptolites, any variation in the number of stipes appears particularly abrupt because of the nature of the change involved; there is no intermediate stage between one stipe and two, even though one stipe may consist of but a single theca (as in *Peiragraptus* and *Parazygograptus*). In fact, in the case in point, the necessary change from two stipes to one could have been brought about by the suppression of a single bud. The graptolite colony is a kind of clone in which the first individual (the siculozoid) is thought to have been produced sexually while each subsequent member budded asexually from its predecessor; branching occurred when a pair of buds, instead of a single bud, was produced by one zooid. The mode of proximal end development in *H. lentus* accords with the simple *bifidus* type, involving a single crossing canal, and the non-appearance of stipe "2" would require merely a failure on the part of the first thecal zooid ( $th1^1$ ) to produce two buds, for if  $th1^1$  only gave rise to  $th2^1$ , and not to  $th1^2$  as well, stipe "2" could not develop. The resultant form would be that of *N. fasciculatus*. It seems reasonable to assume that not too remarkable an alteration in the genetic constitution of the siculozoid was necessary to effect the requisite change, particularly in view of the apparent ease with which the time of appearance of two buds and hence of two stipes could be varied in certain graptoloids.

Support for the suggested conspecificity of *H. lentus* and *N. fasciculatus* is provided by the extent of their common occurrence in space and time. In these respects, the well documented Swedish occurrences<sup>2,3</sup> are the most illuminating and suggest that polymorphism was of the transient kind, with ultimate replacement of the two-stiped by the one-stiped form. At Fågelsång, in Scania, *H. lentus* is confined to the zone of *Didymograptus bifidus* and it is stated to be of common occurrence throughout the zone<sup>2</sup>. On the other hand, *N. fasciculatus* (= *Azygograptus falciformis* Ekström) does not appear until some distance above the base of the *bifidus* zone and it occurs sparsely at first but then abundantly<sup>2</sup>; moreover, it has been shown that this form persists into the lower part of the *Didymograptus murchisoni* zone and thus into younger levels than *H. lentus*<sup>3</sup>. In Norway also, *N. fasciculatus* has been recorded (as *Azygograptus* sp.) from the lowermost part of the *murchisoni* zone<sup>4</sup>.

Elsewhere, the stratigraphical control is less precise, though in China *N. fasciculatus* occurs abundantly in the upper part of the zone of *Amplexograptus confertus*



Figs. 1-3. *Nicholsonograptus fasciculatus* (Nicholson, 1869). Three proximal ends showing the variable attitude of the stipe relative to the sicula (1,  $\times 15$ ; 2,  $\times 19$ ; 3,  $\times 18$ ).



or the highest of three sub-zones<sup>5</sup>. *Holmograptus lentus* (= *Tylograptus geniculiformis* Mu) appears earlier, probably in the highest part of the *Didymograptus hirundo* zone, and ranges through the *confertus* zone<sup>6</sup>.

The facts suggest that the uniramous variant was endowed with some particular selective advantage which enabled it to spread rapidly through the community, ultimately replacing what had been the normal variant. What the particular advantage attached to a single as against a two-stiped rhabdosome was is uncertain, particularly in view of the popularity of the bilaterally symmetrical rhabdosome among Ordovician graptoloids; however, it is possible that the uniramous condition was merely a secondary side-effect (in itself having no particular advantage) and the primary direct effect to which it was related may have been physiological.

With regard to taxonomy, both variants should be denoted by the same name—*fasciculatus*, because it has priority over *lentus*—if the relationship suggested here is the correct one. The recognition of two (morphological) species does, however, seem a necessary concession in this case, as the test of conspecificity is ability to interbreed, and in view of this requirement the suggested relationship must remain speculative. Separation of the two forms is also valuable from the stratigraphical standpoint. Moreover, if a distinction is to be drawn at the specific level it would seem advisable to adopt the same practice at the generic level, as the number of stipes in the rhabdosome is a primary taxobasis in the definition of graptoloid genera.

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## Ionic Balance in the Crustacea

THE internal medium of the Crustacea varies in inorganic composition from species to species, but keeps at least a general resemblance to sea water. In one sense the composition is entirely determined by the environment and regulatory mechanisms of the species, but these mechanisms have presumably evolved so as to maintain the balance of ions close to an optimum for each physiological process. Although some of these conditions of optimum balance may vary between species, those that do not should reveal themselves through correlations among the levels of various ions. The aim here is to consider correlations among sodium, potassium and magnesium. There is known to be an inverse relationship between concentration of magnesium and activity in marine species caused, perhaps, by the depressant action of magnesium at the neuromuscular junction<sup>1</sup>.

Data on thirty-one marine, terrestrial and freshwater species of Malacostraca are shown in Table 1 as either mmol/l. of blood or mmol/kg of water; the difference is not significant in the present context. Through natural variation (for example, in relation to the moulting cycle<sup>2</sup>) and differences in analytical techniques some of these figures may be unrepresentative, particularly when based on single determinations. Those for calcium vary so little that significant correlations with those for the other cations cannot be demonstrated and, in any case, only part of the calcium may be ionized<sup>3</sup>. Concentrations of potassium, which in marine forms may be greater or smaller than in sea water, show a marked correlation with those of mag-

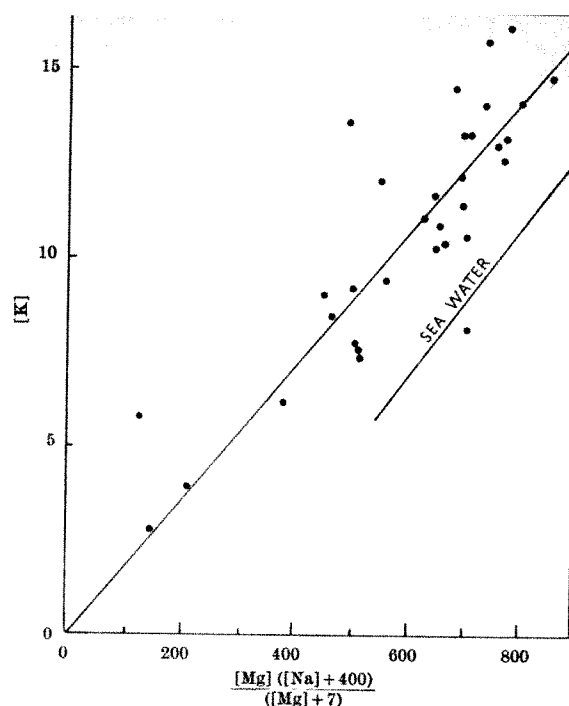


Fig. 1. Concentrations (mmoles) of potassium and values of the expression  $[Mg]([Na] + 400)/([Mg] + 7)$  for various Malacostraca and for sea water.

nesium (up to a concentration of magnesium of about 25 mmol/l) and also with sodium. These two relations are combined in Fig. 1 where values of [K] are shown plotted against those of the empirical expression  $[Mg]([Na] + 400)/([Mg] + 7)$ . The line through these points is such that  $[K] = \frac{0.0174 [Mg]([Na] + 400)}{([Mg] + 7)}$ . Another line corre-

sponding to sea water of salinities 2.0 per cent to 4.2 per cent is also shown and the points for Crustacea lie typically above it. This is, however, not achieved in the same way by each species, because concentrations of individual ions may be above or below those in sea water. Correction for variations in activity coefficients, particularly low for magnesium at the ionic strength of sea water, would

Table 1. CONCENTRATIONS OF CATIONS IN THE HAEMOLYMPH OF VARIOUS MALACOSTRACA

	Reference	Sodium	Potassium	Magnesium	Calcium
<i>Squilla mantis</i>	*	644	16.0	21.3	15.1
<i>Meiodotea entomon</i>	4	202	6.1	11.5	12.6
<i>Ligia oceanica</i>	5	586	14	21	36
<i>Palaemon serratus</i>	6	394	7.7	12.6	12.6
<i>Asiacus fluviatilis</i>	7	151	2.8	2.5	12
<i>Cambarus clarkii</i>	8	160	5.8	2.0	12.2
<i>Cambarus virilis</i>	9	146	3.9	4.3	8.1
<i>Homarus vulgaris</i>	*	507	8.4	7.5	15.0
<i>Homarus americanus</i>	10	464	9.0	7.7	16.1
<i>Nephrops norvegicus</i>	3	518	7.6	8.9	13.9
<i>Palinurus vulgaris</i>	3	543	10.3	16.6	13.5
<i>Panulirus interruptus</i>	8	531	12.0	10.3	19.5
<i>Coenobita perlatus</i>	11	465	10.5	30.9	14.7
<i>Eupagurus prideauxi</i>	*	478	15.7	39.4	15.3
<i>Eupagurus bernhardus</i>	*	497	13.2	27.2	16.7
<i>Galathea squamifera</i>	12	495	13.5	41	10
<i>Lithodes maja</i>	3	477	12.5	52.2	12.4
<i>Maia squinado</i>	*	491	13.1	46.3	14.4
<i>Hyas araneus</i>	*	468	12.9	49.5	11.9
<i>Callinectes hastatus</i>	10	460	13.5	9.5	19.7
<i>Cancer borealis</i>	10	460	10.2	21.9	11.5
<i>Cancer pagurus</i>	*	506	11.3	23.8	13.6
<i>Carcinus maenas</i>	2	503	10.8	19.0	12.5
<i>Gecarcinus lateralis</i>	11	459	9.4	13.5	20.9
<i>Portunus depurator</i>	*	485	13.2	25.1	12.2
<i>Portunus puber</i>	*	508	14.4	21.7	13.6
<i>Uca pugnax</i> + <i>U. pagulator</i>	13	328	11	46	16
<i>Pachygrapsus crassipes</i>	8	462	9.1	9.9	13.5
"	14	483	7.4	10.0	14.8
"	15	465	12.1	29.2	11.4
<i>Pachygrapsus marmoratus</i>	*	542	11.6	15.8	12.8
<i>Hemigrapsus oregonensis</i>	14	452	8.1	35.0	18.9
<i>Dromia vulgaris</i>	*	553	14.7	65.7	12.2

Concentrations were measured in mmol/l. of blood or mmol/kg of water.

\* Personal communication from J. D. Robertson.



change the constants in the equation but probably have little effect on the goodness of fit.

The interpretation of correlations is very difficult and, in this instance, can only accompany the recognition of some physiological process that is affected by cations in a manner reconcilable with these particular relations. A possible clue, however, is obtained when the above equation is rewritten in the following form:

$$\frac{(1 + 1/n)[K]}{([K] + 0.0174[Na]/n + 7/n)} = \frac{[Mg]}{([Mg] + 7/(n + 1))}$$

where  $n$  is any number. If, for example,  $n = 7$ , then

$$\frac{1.14[K]}{([K] + 0.0025[Na] + 1.00)} = \frac{[Mg]}{([Mg] + 0.875)}$$

Such an expression could apply if the optimum composition of the extracellular fluid were such that some surface adsorbed potassium and magnesium from it in definite proportions; the sites for the adsorption of the two ions on this view would differ, but sodium would be competing for the potassium sites. These potassium sites could conceivably be those on ATPase which is activated by magnesium and sodium and which is involved in the active transport of sodium and potassium across cell membranes<sup>16</sup>.

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### Association of *Xanthomonas sesami* with Two Types of Leaf Spots affecting Sesame

SESAME (*Sesamum orientale* L.) grown under rain-cultivation in the Sudan is severely affected by two types of leaf spotting. In one (Fig. 1) the spots are usually small, 2–4 mm in diameter, dark red-brown to black in colour, somewhat translucent, often angular with sharply defined margins, and may coalesce to form irregular lesions. The dead tissue of the spot later dries up and becomes brittle. In the other type (Fig. 2) the spots are large, 4–14 mm in diameter or more, greyish or light brown, usually opaque with somewhat diffuse margins, often irregular and frequently coalescing. Affected tissues become wrinkled before they dry. The first type is more often found towards the top of the plant while the second type is more frequently encountered towards the base.

Sabet and Dowson<sup>1</sup> attributed the first type of spots (dark brown spots) to a new species of *Xanthomonas*, namely, *X. sesami*. They considered the second type (light brown) to be a result of some physiological causes, possibly rain damage following waterlogging of the tissues. Rao<sup>2</sup> in India, dealing with what appears to be the light brown spot type, attributed the disease to a distinct strain of *X. sesami*. The cause of the light or grey brown spot type and its relation to the dark brown type are examined in the present communication.

The methods used for isolation, inoculation and characterization of the causal bacteria were those described by Sabet and Dowson<sup>1</sup>. Isolations were made from leaves which showed each of the two types of symptoms separ-

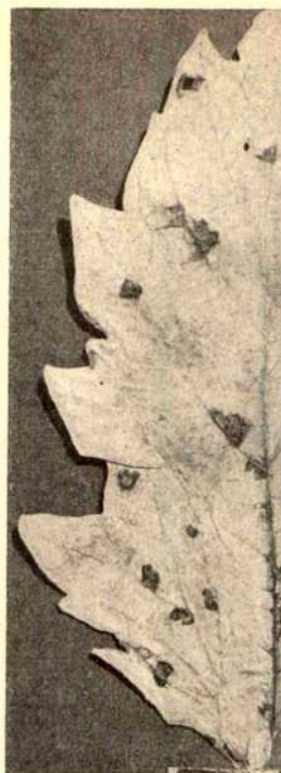


Fig. 1

Fig. 1. Sesame half-leaf showing dark brown spots ( $\times c. 1.3$ ).



Fig. 2

Fig. 2. Sesame half-leaf showing light brown spots ( $\times c. 1.3$ ).

ately. Two isolates of suspected *Xanthomonas* were obtained from each type. Bacterial suspensions for inoculation were prepared from 72 h old cultures grown on yeast extract-glucose agar. They were sprayed on field grown sesame planted in small well separated patches of about twenty plants each (potted sesame plants were somewhat chlorotic, stunted and unsuitable for inoculation). The plants of each patch were covered with polyethylene sacs and shaded for 72 h. Control plants were sprayed with tap water.

Two series of inoculation experiments were carried out during the periods December 1964–February 1965 and August–October 1965 inclusive. Three inoculation experiments were made in each series using plants 3–4, 6–8 and 10–12 weeks old, respectively. In each case two isolates (one from dark brown spots and the other from light brown spots) were used for inoculation.

The bacteria isolated from the two types of spots proved to be indistinguishable from each other in morphological, cultural and biochemical characters. They agreed in every respect with *Xanthomonas sesami* Sabet and Dowson<sup>1</sup> and are identified as such.

Under prevailing experimental conditions, the four isolates produced the first spot type, that is, dark brown, sharply defined, rather small spots. These spots developed readily on the younger leaves near the top of the plants. They also appeared on the capsules when these had already developed at the time of inoculation. The other type of spots (light brown) could not be reproduced by any of the isolates used, even those which were originally obtained from it.

Thus it is evident that the two types of sesame leaf spot described here are associated with infection by *X. sesami*. The dark brown type seems to be readily reproduced under a wide range of environmental conditions, at least those prevailing in Khartoum as well as in the central rainlands. The conditions which favour the development of the light brown spot type are not known.



These spots do not develop on the top leaves of the plant, yet it has not been possible to reproduce them, by repeated inoculations, on the lower leaves. This suggests that the effect of leaf position is an indirect one. It is possible that the high atmospheric humidity and high rainfall in the central rainlands favour the development of the grey-brown spot type, particularly on the lower leaves, which are more subjected to rain splashes. This spot type does not appear under the drier conditions of Khartoum. Sabet<sup>3</sup> has previously reported a similar instance in which mahogany leaf spots caused by *X. khayae* are small and dry in the Khartoum area, but large, with broad, dark green wet-shining margins in the central rainlands.

It is unlikely that the two spot types are produced by two distinct strains since isolates from the two types produce one type of spots under similar conditions. It is concluded that they are caused by one strain of *Xanthomonas sesami*. The dark brown type develops readily under a wide range of environmental conditions. Development of the light brown type requires a more exacting environment, possibly high atmospheric humidity and rainfall.

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### Selective Nitrogen Assimilation by *Poria weirii*

SOIL under a stand of *Alnus rubra* Bong. in mixture with conifers was found to contain markedly higher levels of nitrate nitrogen than soil under an adjacent stand of pure conifers<sup>1</sup>. This phenomenon has substantial implications for the ecology of root pathogens and their antagonists in forest soils. We were particularly interested in *Poria weirii* Murr., a severely damaging pathogen of conifer roots in western North America, and we have therefore investigated its ability to assimilate nitrate nitrogen.

Many fungi, including Basidiomycetes such as *Collybia tuberosa* (Fr.) Quél. and *Lentinus tigrinus* Fr., can use nitrate nitrogen<sup>2,3</sup> and therefore presumably produce nitrate reductase, the enzyme required for reduction of nitrate to usable ammonium. Other fungi, however, cannot use nitrate nitrogen, for example, *Armillaria mellea* (Vahl. ex Fr.) Kummer<sup>4</sup>, many other higher Basidiomycetes<sup>5-8</sup>, and members of the Saprolegniaceae<sup>9</sup> and Blastocladiaceae<sup>3</sup>. *Streptomyces* species, notable among the organisms likely to antagonize *P. weirii*, commonly thrive on nitrate as a nitrogen source<sup>10-13</sup>.

Experiments were designed to determine (a) the relative growth of *P. weirii* when supplied respectively with nitrogen equivalents in nitrate, ammonium, or amino forms; and (b) whether *P. weirii* produces nitrate reductase.

*Poria weirii* was grown on Jennison's<sup>7</sup> liquid medium with potassium nitrate, ammonium chloride, and asparagine, as respective sources of nitrogen. Each form of nitrogen was included at each of three concentrations, corresponding to 10, 100, and 1,000 p.p.m. nitrogen in the medium. In each case four replicate flasks were inoculated with 4 mm agar disks cut from margins of colonies of *P. weirii* growing on malt agar. Cultures were grown for 40 days at room temperature (24°-28° C), after which the mycelial mats were collected, dried overnight at 105° C and weighed.

The procedure for extraction of nitrate reductase was essentially that devised by Nason and Evans<sup>14</sup>; the enzyme activity of the cell-free extract was determined by the colorimetric test for nitrite. At the beginning of the experiment (zero time) 0.05 ml. enzyme extract was added to give a final volume of 0.5 ml. containing 0.1 ml. of 0.1 molar potassium nitrate, 0.05 ml. of 2.6 × 10<sup>-3</sup>

molar flavin adenine dinucleotide (FAD), 0.04 ml. of 2.0 × 10<sup>-3</sup> molar reduced diphosphopyridine nucleotide (DPNH) and 0.26 ml. of 0.2 molar pyrophosphate buffer, at pH 7.0. After incubation for 20 min at 26° C, 0.9 ml. of water and 0.5 ml. of sulphanilamide reagent were added to stop the reaction, followed by 0.5 ml. of *N*-(1-naphthyl) ethylenediamine reagent to develop the colour. After 20 min, the optical density was read on a colorimeter at 540 mμ. Control tubes lacking DPNH were used to correct for turbidity caused by the enzyme. A fungus known to produce nitrate reductase, *Neurospora crassa*, was used to check the technique.

Table 1. MEAN DRY WEIGHT OF MYCELIUM OF *Poria weirii* GROWN IN SYNTHETIC MEDIA CONTAINING VARIOUS SOURCES OF NITROGEN\*

Nitrogen (ppm)	Potassium nitrate (mg)	Source Ammonium chloride (mg)	Asparagine (mg)
10	2.55	17.75	20.33
100	2.00	32.00	32.48
1,000	1.28	37.23	40.08

\* Mean of four replicates.

Table 2. NITRATE REDUCTASE ACTIVITY OF *Neurospora crassa* AND *Poria weirii*

Reagent	(ml.)	Addition (ml.)	(ml.)	(ml.)
0.1 molar KNO <sub>3</sub>	0.10	0.10	0.10	0.10
2.6 × 10 <sup>-3</sup> molar FAD	0.05	0.05	0.05	0.05
2.0 × 10 <sup>-3</sup> molar DPNH	0.04	0.04	0.04	0.04
0.2 molar pyrophosphate, pH 7.0	0.26	0.26	0.26	0.26
Enzyme extract	0.05	0.10	0.15	0.20
Water	0.90	0.85	0.80	0.75
<i>O.D.</i> at 540 mμ for <i>N. crassa</i>	0.01	0.03	0.05	0.06
Nitrite formed for <i>N. crassa</i>	1 × 10 <sup>-3</sup> μmole	2.5 × 10 <sup>-3</sup> μmole	3.0 × 10 <sup>-3</sup> μmole	4.5 × 10 <sup>-3</sup> μmole
<i>O.D.</i> at 540 mμ for <i>P. weirii</i>	0	0	0	0
Nitrite formed for <i>P. weirii</i>	0	0	0	0

*Poria weirii* did not use nitrate as a nitrogen source but grew well with ammonium or amino nitrogen (Table 1). Its behaviour in culture was markedly similar to that reported for *Armillaria mellea*<sup>4</sup>, another serious destroyer of tree roots. Moreover, the cell-free extracts of *P. weirii* completely lacked nitrate reductase activity (Table 2).

The high nitrate content of the soils under the stand with *Alnus rubra* appears disadvantageous to *P. weirii*: it cannot use nitrate nitrogen but antagonists such as *Streptomyces* spp. can. On the basis of these results and those of other investigations of this biological complex which are now in progress, we tentatively conclude that *Alnus rubra* mixed with conifers has a potential in the biological control of *P. weirii* and probably other pathogens on many sites of the Douglas fir region.

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## GENETICS

## Criminal Behaviour and the XYY Male

IN 1965, Jacobs *et al.* published their preliminary findings of a chromosome survey conducted at a maximum security hospital, The State Hospital, Lanarkshire, Scotland<sup>1</sup>. The most remarkable finding in the completed survey was the discovery among 315 men of nine patients with an XYY sex chromosome constitution. Their behaviour, together with their pattern of crime, has now been closely studied. The full clinical details of this investigation will be published elsewhere by us, and this communication directs attention to the ways in which the XYY males differ from males with an XY sex chromosome complement at the same hospital.

All the patients admitted to this hospital have severely disordered personalities and they have been classified according to whether the cause is known or not. For example, some have brain damage which followed infections, others are epileptics, and others suffer from a psychosis. The largest group of patients have no known cause for their personality disorders. All the men with an XYY complement were classified in this category and eighteen other men have been randomly selected from this group for comparison with the nine XYY males. Seventeen of the eighteen control males were known to have an XY sex chromosome complement, the remaining being one of twenty-seven who had not been willing to be investigated when the chromosome survey was carried out.

There are three ways in which the XYY males differed importantly from the controls. First, although the patients in the two groups have penal records of comparable length, those of the XYY males include considerably fewer crimes of violence against persons. Thus, the nine XYY males had been convicted on a total of ninety-two occasions, but only eight of these convictions (8.7 per cent) had been for crimes against the person, while eighty-one (88.0 per cent) had been for crimes against property. In contrast, the eighteen control males had been convicted on 210 occasions, and forty-six of these (21.9 per cent) had been for crimes against the person while 132 (62.9 per cent) had been for crimes against property. Second, the disturbed behaviour of the XYY patients showed itself at an earlier age. This is reflected in a mean age at first conviction of 13.1 yr, compared with a mean age of 18 yr for the control patients, a difference which is significant at the 5 per cent level. Third, in the families of these patients the incidence of crime among the siblings of the XYY patients is significantly less than among those of the control patients. Thus, only one conviction is recorded among thirty-one sibs of the XYY patients while no less than 139 convictions are recorded for twelve of sixty-three sibs of the control patients.

The distribution of intelligence quotient among the XYY males probably reflected the distribution among the patients of the hospital as a whole. Seven were considered to be mentally sub-normal, but it is worth noting that the pattern of behaviour among the two whose intelligence quotients were not unusually low conformed with those of the other seven.

The picture of the XYY males that emerges from examination of those detained at the State Hospital is of highly irresponsible and immature individuals whose waywardness causes concern at a very early age. It is generally evident that the family background is not responsible for their behaviour. They soon come into conflict with the law, their criminal activities being aimed mainly against property, although they are capable of violence against persons if frustrated or antagonized. Their failure to respond to corrective measures leads to a sentence of prolonged detention in safe custody at an earlier age than is usual for offences of this kind. All nine men with an

XYY chromosome complement conform fairly closely to this broad description and it seems reasonable to suggest that their antisocial behaviour is due to the extra Y chromosome.

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### Microgeographical and Ecological Distribution of Colour Morphs of *Botryllus schlosseri* (Asciidiacea)

*Botryllus schlosseri* (Pallas), a compound ascidian widely distributed on European and western Atlantic coasts, is well known for its colour polymorphism, which has been shown to be under genetic control<sup>1-4</sup>. In the Venetian Lagoon *Botryllus* is found chiefly in two typical biotopes: the piles marking the navigable canals which cross the Lagoon, and the beds of *Zostera* which cover its bottom.

A colony of *Botryllus*, founded by a larva, grows by budding up to hundreds and thousands of zooids. Its life cycle consists of a long series of blastogenic generations which at a temperature of 18° C succeed each other at a rate of one a week<sup>5</sup>. Sexual reproduction in the Lagoon starts in April and continues until November. The new colonies in their turn reach sexual maturity in 1 or 2 months, and so several generations co-exist, reproducing at the same time. Like most other ascidians *Botryllus* is hermaphrodite, but, because the ripening of spermatozoa is somewhat delayed in comparison with the ripening of eggs<sup>6</sup>, selfing does not occur in the presence of spermatozoa from other colonies. Fertilization of eggs and development take place inside the zooids, and free-swimming larvae are liberated; these settle down and metamorphose within a few hours.

We collected colonies of *Botryllus* in two areas of the Lagoon, Venice and Chioggia, about 20 km apart, from two piles stations in Venice and from a single piles station and a *Zostera* station in Chioggia. The two stations in each area were about 1,300 m apart. The colonies on the beds of *Zostera* were collected at an average depth of 2.5 m and those on the piles from this same depth up to the surface. Samples from each station were collected in March and July 1964, the March samples accounting for the composition of the population after wintering and those of July including also the first generations of the new year.

The colonies were classified in the laboratory according to the presence or the absence in the zooids of (a) orange pigment and (b) an intersiphonal double band. These are genetic characters controlled by two independent genes<sup>1-4</sup>, both with an allele for the presence of the character dominating over an allele for the absence. A total of 2,587 colonies were examined; their distribution in the samples taken from the different stations and the frequencies of one of the phenotypes related to either gene considered are reported in Table 1.

The  $\chi^2$  test by the Brandt and Snedecor method was used for the statistical analysis of the samples. The results, which are given in Table 2, may be summarized as follows: (1) the two samples from each station do not differ significantly in either character; (2) the same is true of the animals taken from the two piles stations in Venice, whereas those sampled from the two stations, the

Table 1. COLONIES OF *B. schlosseri* SAMPLED IN TWO BIOTOPES FROM TWO AREAS OF THE VENETIAN LAGOON

Stations	March			July			March with July		
	Total No.	No. of colonies and per cent* Without orange pigment	With double band	Total No.	No. of colonies and per cent* Without orange pigment	With double band	Total No.	No. of colonies and per cent* Without orange pigment	With double band
<i>Piles-Venice</i>									
Station 1	444	48 10.81	17 3.82	431	61 14.15	20 4.64	875	109 12.45	37 4.22
Station 2	425	46 10.82	12 2.82	118	11 9.32	3 2.54	543	57 10.49	15 2.76
<i>Piles-Chioggia</i>									
Station 3	391	75 19.18	21 5.37	492	112 22.76	19 3.86	883	187 21.17	40 4.53
<i>Zostera-Chioggia</i>									
Station 4	144	125 86.80	— 0.00	142	126 88.73	2 1.40	286	251 87.76	2 0.69

\* Percentages in italics.

Table 2.  $\chi^2$  ANALYSIS OF THE SAMPLES

Samples compared	Orange pigment			Double band		
	$\chi^2$	d.f.	P	$\chi^2$	d.f.	P
<i>Piles-Venice</i>						
Station 1 March	0.11167	1	> 0.70	0.35564	1	> 0.50
Station 2 March	0.22043	1	> 0.50	0.02681	1	> 0.80
<i>Piles-Chioggia</i>						
Station 3 March	1.68008	1	> 0.10	1.13991	1	> 0.20
<i>Zostera-Chioggia</i>						
Station 4 March	0.24162	1	> 0.50	—	—	—
<i>Venice</i>						
Station 1	1.25263	1	> 0.20	2.03340	1	> 0.10
<i>Chioggia</i>						
Station 3	408.80169	1	< 0.001	9.16637	1	< 0.01
<i>Venice and Chioggia</i>						
Station 1, 2	273.82166	1	< 0.001	1.05992	1	> 0.30

piles station and the *Zostera* station, in Chioggia, show highly significant differences; (3) the two groups of animals sampled from the piles in Venice and Chioggia respectively differ significantly in the frequency of the "orange pigment" character.

The conclusion may be drawn that the population of *Botryllus schlosseri* in the Venetian Lagoon is subdivided into sub-populations significantly different in the frequencies of genetically controlled colour morphs. Evidence has been given of the presence of microgeographical sub-populations living on the same biotope, the piles, in two areas about 20 km apart and of ecological sub-populations living in the same area on two different biotopes, the piles and the *Zostera* beds. At least as far as the sub-populations of the same area are concerned, their differences are likely to depend on the characters, which we have considered, exhibiting different adaptive values in the two biotopes.

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## HAEMATOLOGY

### Evidence for Facilitated Diffusion of Anion in Erythrocytes

THE suggestion has been made by various authors<sup>1</sup> that anions as well as various non-electrolytes cross the membrane of erythrocytes by a special mechanism. An investigation of the permeability of human and rabbit

erythrocytes to ammonium chloride was carried out which suggested that the anion exchange in the erythrocyte of these two species involved facilitated diffusion (Hunter, F. R., and Ospina, B., unpublished results). The disadvantage of using ammonium chloride was that in addition to the movement of anions, ammonia also crosses the membrane<sup>2</sup>. Consequently, a less complicated system which involved only anion exchange was sought to test the conclusions reached with the ammonium chloride system.

There are several reports in the literature of volume changes of erythrocytes which result from the exchange of a monovalent for a divalent anion<sup>3-5</sup>. In the physiological exchange of chloride ion for bicarbonate ion across the erythrocyte membrane slight volume changes are expected as a result of a shift in the amount of base bound by haemoglobin with the associated slight change in pH (refs. 5 and 6). Much larger volume changes, however, would be expected if erythrocytes are placed, for example, in a solution of sodium sulphate. One sulphate ion will enter the cell for each two chloride ions that leave. Such an exchange should decrease the internal osmotic pressure of the cells and cause shrinkage<sup>4</sup>.

Parpart<sup>3,4</sup> mentioned that various substances, including butyl alcohol, decrease the rate of anion exchange. It has also been shown that tannic acid greatly increases the rate of anion exchange<sup>7,8</sup>. According to the interpretation of Hunter *et al.*<sup>9</sup> such a decrease in permeability in the presence of butanol and tannic acid indicates that facilitated diffusion is involved. Omachi<sup>10</sup> studied the effect of various metabolic inhibitors on the permeability of human erythrocytes to the sulphate ion and found a decrease in the rate at which this ion left these cells. He concluded, however, that his data could be reconciled with the suggestion of Passow<sup>11</sup> that the sulphate ion is "passively transported across the mammalian red cell membrane". A subsequent report<sup>12</sup> suggested that the regulation of anion permeability may depend on "biochemical processes resembling partial reactions in mitochondrial oxidative phosphorylation". For the foregoing reasons, a re-investigation of the exchange of sulphate for chloride ion across the erythrocyte membrane seemed of interest.

Human blood was obtained by venipuncture and citrated. Rabbit blood was obtained by cardiac puncture and heparinized. Both types of blood were washed three times with an isotonic sodium chloride solution buffered with phosphate to pH - 7.1. The cells were used immediately in most experiments although similar results were obtained with cells that were refrigerated overnight. Volume changes of the cells were measured at 30° C using a densimeter<sup>13</sup>. An aliquot (0.05 ml.) of washed cells was added to 10 ml. of 1/9 molar sodium sulphate buffered with tris buffer—pH - 7.5 in the chamber of the densimeter and a record of the shrinking of the cells was obtained. The effect of butanol and tannic acid on the rate of this volume change was studied. A 1 molar butanol solution in sodium sulphate solution was used throughout all the experiments. A fresh 100 mg tannic



acid in sodium sulphate solution was prepared each day. The time for half the total deflexion of each shrinking curve was measured. Experimental half-times were divided by control half-times to give relative times. Typical curves are shown in Fig. 1. A comparison of curves 1 and 2 and of 4 and 5 shows the decrease in the rate of shrinking with both types of erythrocytes in the presence of 0.1 molar butanol. The very marked inhibition in the presence of a low concentration of tannic acid is obvious from curves 3 and 6. All the data are summarized in Fig. 2. With human cells increasing concentrations of both butanol and tannic acid increase the relative times for shrinking which results from the exchange of sulphate for chloride ions. The tannic acid effect is about five times greater than that of butanol with the concentrations used. The inhibition in the presence of a single concentration of butanol and of tannic acid with rabbit cells can also be seen in the figure.

The rate of exchange of sulphate ion for chloride ion across the membrane of both human and rabbit erythrocytes is inhibited by butanol and markedly inhibited by

tannic acid. It has previously been postulated that such changes indicate facilitated diffusion<sup>9</sup>. Thus it is suggested that at least certain anions cross the membrane of the erythrocytes of some species by this mechanism.

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### Inhibitor of Glucose-6-phosphate Dehydrogenase Activity in the Erythrocytes of *Macaca nemestrina* Monkeys

DATA are available on the activity of G-6-P dehydrogenase in the erythrocytes of different species of monkeys<sup>1</sup>, using the dye decoloration method of Motulsky and Campbell<sup>2</sup>. In *M. nemestrina* the test did not clear after 5 h while in four other species the decoloration time was normal. We have further examined this phenomenon. Blood of seven individuals of *M. nemestrina*, seven *M. mulatta* and three *Presbytis entellus* in acid citrate dextrose inosine (ACDI) was examined. In agreement with earlier findings, *M. nemestrina* is the only species in this group which gave abnormal results. When fresh haemolysate ( $\pm 14$  g per cent) of *M. nemestrina* was used in the test instead of erythrocytes, some haemolysates cleared within normal time, others did not. In contrast, hundreds of normal human haemolysates gave normal results. Starch gel electrophoresis of the G-6-P dehydrogenase<sup>3</sup> showed a large amount of enzyme in all the monkey haemolysates, including those of *M. nemestrina* (Fig. 1). The mobility of the enzyme is similar to the fast-moving A type of human G-6-P dehydrogenase. Some factor must therefore have been present which prevented reduction of the dye in the test system, either by inhibition of the activity of G-6-P dehydrogenase or by continuous oxidation of the brilliant cresyl blue.

Origin  
— +

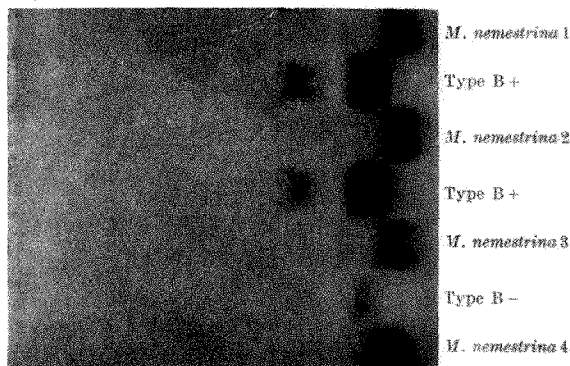


Fig. 1. Starch gel electrophoresis patterns of erythrocytic G-6-P dehydrogenase<sup>3</sup>. *M. nemestrina* G-6-P dehydrogenase compared with human B+ and B- types.

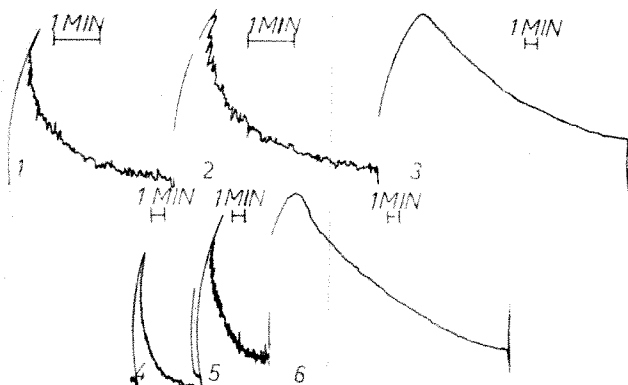


Fig. 1. Shrinking of erythrocytes in an isotonic sodium sulphate solution. 1-3, Human; 4-6, rabbit; 1 and 4, control; 2 and 5, 0.1 molar butanol; 3 and 6,  $50 \times 10^{-4}$  per cent tannic acid.

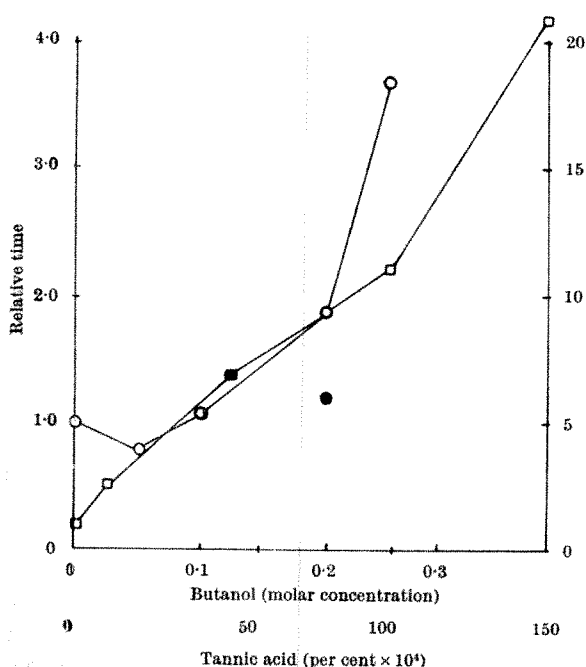


Fig. 2. Average curves showing the effect of butanol and tannic acid on the rate of shrinking of erythrocytes in isotonic sodium sulphate. Abscissae, concentration of butanol and tannic acid. Ordinates, relative times for one-half the total deflexion. Left hand, butanol. Right hand, tannic acid.  $\circ$ , Human cells, butanol;  $\square$ , human cells, tannic acid;  $\bullet$ , rabbit cells, butanol;  $\blacksquare$ , rabbit cells, tannic acid.

Table 1. RESULTS OF BRILLIANT CRESYL BLUE DYE-DECOLORATION TEST\*

Test No.	Material tested	No. of tests performed	Clearance time (brilliant cresyl blue)
(1)	0.02 ml. of normal human whole blood in ACDI	15	All cleared within 1 h
(2)	0.02 ml. of normal human haemolysate	15	All cleared within 1 h
(3)	0.02 ml. of <i>M. nemestrina</i> whole blood in ACDI	7	All did not clear after 5 h
(4)	0.02 ml. of <i>M. nemestrina</i> haemolysate	7	Five cleared within 1 h and remained clear; one cleared within 1 h but turned blue again; one did not clear after 5 h
(5)	0.02 ml. of normal human whole blood in ACDI + 0.02 ml. of <i>M. nemestrina</i> whole blood in ACDI	10	All did not clear after 5 h
(6)	0.02 ml. of normal human haemolysate + 0.02 ml. of <i>M. nemestrina</i> whole blood in ACDI	7	All did not clear after 5 h
(7)	0.02 ml. of normal human haemolysate + 0.02 ml. of human whole blood in ACDI	4	All cleared within 1 h
(8)	0.02 ml. of normal human whole blood in ACDI + 0.02 ml. of <i>M. nemestrina</i> haemolysate	8	All cleared within 1 h, several turned blue again after 2-3 h

The test of Motulsky and Campbell was performed on material listed in Table 1. The right-hand column of Table 1 shows the decoloration time of brilliant cresyl blue. Tests 1 and 2 showed that the human whole blood and the human haemolysates had normal amounts of active G-6-P dehydrogenase; tests 5 and 6 showed that, although enough active G-6-P dehydrogenase was present in the test, the addition of blood of *M. nemestrina* prevented the decoloration of the dye, indicating the presence of an inhibitor in the monkey blood. When the same amount of *M. nemestrina* haemolysate was used rather than whole *M. nemestrina* blood, some samples cleared, others did not. Doubling the amount of substrate G-6-P in test 4 involving *M. nemestrina* haemolysate led to clearance of all tests within 1 h. This did not occur in tests with *M. nemestrina* whole blood. When *M. nemestrina* blood cells were separated from the plasma in ACDI, only the cells gave inhibition. The erythrocytes, further separated from the leucocytes and platelets, gave strong inhibition. Haemolysates did not always give inhibition; the inhibiting factor apparently is not in the haemolysate, but it can contaminate some haemolysates and is probably present in the erythrocyte membrane. Packed cells of *M. nemestrina* were washed, lysed with water and toluene and centrifuged; the clear haemolysate was carefully separated from the grey-white layer containing red cell membrane, leucocytes and platelets. This debris layer was washed in saline and dissolved in water. The haemolysate showed normal enzyme activity, whereas in those tests in which a small amount of debris was added, the test had not cleared after 5 h, showing that the inhibiting factor is in the debris. The same results were obtained when 0.2 ml. of membrane solution obtained by gradual haemolysis with cold water<sup>4</sup> (without the use of toluene) was added. This membrane solution contained about 30 mg dry weight/ml. Toluene in itself did not give inhibition.

To show that the failure of brilliant cresyl blue to clear was not caused by specific direct action of the factor on the dye, a test devised by Beutler<sup>5</sup> was used, in which an indicator is not added, and reduced triphosphopyridine nucleotide (TPNH) is demonstrated directly. In this test G-6-P is oxidized to 6-phosphogluconate and TPNH is reduced to TPNH. When activated with ultra-violet light, the reduced TPNH fluoresces brightly. Normal human whole blood or haemolysates gave bright fluorescence, and those of deficient individuals did not. Whole blood of *M. nemestrina* did not produce fluorescence in this test, but haemolysates carefully prepared to prevent contamination with membrane debris of red cells gave bright fluorescence. These results again indicate the presence of an inhibitor in whole blood of *M. nemestrina* not present in haemolysates. When erythrocyte mem-

brane solution was added to the reagent mixture before normal human blood was introduced, there was no fluorescence. When the membrane solution was introduced after normal human blood was added and after incubation, however, fluorescence did occur, showing that the inhibitor does not immediately eliminate the TPNH formed.

Although both the Motulsky and Campbell and the Beutler tests do not permit an accurate estimation of the inhibition of enzyme activity, we have shown that a strong inhibitor in the erythrocyte membrane of *M. nemestrina* monkeys was the cause of the abnormality of the dye decoloration test in this species. Some depressing action on G-6-P dehydrogenase of general nature in normal human red cell stroma was described by Carson *et al.*<sup>6</sup>

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### Eosinophil Granulocytes and Hypoxia

ALTHOUGH the response of erythrocytes and their precursors to hypoxia has been extensively studied, much less is known of the changes in the leucocytes and their precursors. The presence of increased numbers of eosinophil granulocytes in the circulation of human subjects following exposure to hypoxia, however, has been noted<sup>1</sup>.

In the course of a quantitative study of blood and bone marrow in guinea-pigs exposed to hypoxia, striking changes in the numbers of eosinophil granulocytes were previously found in routine counts<sup>2</sup>. Results based on the small numbers of eosinophils encountered in routine differential counts of blood and bone marrow smears must, of course, be treated with a good deal of caution, and it seemed advisable to carry out further observations using a more accurate method. For this purpose three groups of guinea-pigs were studied, in which both the blood and bone marrow eosinophil levels were determined by direct haemocytometric methods after dilution of the marrow suspension or blood with a modification of Randolph's fluid; 150-200 eosinophils were counted on each occasion<sup>3</sup>. One group of six male guinea-pigs weighing 400 g each was placed in the decompression chamber and kept at general atmospheric pressure for 48 h. A similar group of six animals was exposed to a simulated altitude of 10,000 ft. for 48 h, while a third group of seven animals was exposed to a simulated altitude of 20,000 ft. for a similar period.

The results are summarized in Table 1. Placing the animals in the decompression chamber for a period of 48 h

Table 1. SUMMARY OF EOSINOPHIL COUNTS

	Control Group (A) (per mm <sup>3</sup> )	10,000 ft. Group (B) (per mm <sup>3</sup> )	20,000 ft. Group (C) (per mm <sup>3</sup> )	A v. B t (P)	A v. C t (P)
Blood	88 ± 44	59 ± 22	994 ± 516	1.4 (> 0.1)	4.3 (< 0.01)
Marrow	79,500 ± 16,000	86,700 ± 12,400	52,100 ± 6,300	0.9 (> 0.3)	4.2 (< 0.01)

±, S.D.

The *t* values are those of the standard error test for small samples, and *P* represents the probability of the value being exceeded in random sampling.

produced no apparent change in the numbers of blood and bone marrow eosinophils, the average values for these animals (column A) being within the normal limits<sup>4</sup>. There was also no significant change in eosinophil levels after exposure to hypoxia at an altitude-equivalent of 10,000 ft. At "20,000 ft.", however, there were highly significant changes both in blood and bone marrow eosinophils, the counts of the former being raised, those of the latter being lowered. It is tempting to speculate that these changes may be caused by crowding out of the eosinophil series from the bone marrow as a result of erythroid proliferation.

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### Electrophoretic Haemoglobin Patterns in One Random Bred and Two Inbred Strains of Laboratory Rats

It has previously been shown by paper electrophoresis that two distinct electrophoretic types of haemoglobin, "single-spot" and "diffuse", occur in mice of several inbred strains and these have been proved to be under genetic control<sup>1,2</sup>. Rosa *et al.*<sup>3</sup> classified seven strains of mice into four different groups according to their haemoglobin patterns obtained by starch-gel electrophoresis. The largest number of bands observed was five<sup>3</sup>. Using starch-gel electrophoresis in a discontinuous buffer system, Morton<sup>4</sup> showed that the "diffuse" type of mouse haemoglobin separates into five bands. As far as electrophoretic investigations of haemoglobin in rats are concerned, we have not been able to find other published data except those by Giri and Pillai<sup>5</sup>. Using agar-gel electrophoresis they found that in the rat blood haemolysates two minor components of proteins were present in addition to one major component of haemoglobin.

The present experiments were conducted to determine whether or not the electrophoretic pattern of haemoglobin from rats of our stocks shows differences within and/or between the strains.

Haemoglobin from random bred albino, inbred Wistar and inbred black male and female adults was examined by starch-gel electrophoresis in a discontinuous buffer system. No animal studied was less than 3 months old. The random bred albino rats belonged to a local stock freely interbred for 35 yr and with no fresh blood introduced for the past 15 yr. The inbred Wistar rats belonged to the sixteenth filial generation of brother with sister matings starting from a pair of rats of the Wistar strain obtained from the Medical Faculty of the Zagreb University. The black rats belonged to the thirteenth filial generation of an accidental cross between a Wild-type

male and an albino female subsequently bred in our laboratory.

Blood samples were obtained by cardiac puncture and collected in tubes which contained heparin. The erythrocytes were washed seven times with isotonic saline at 4° C and lysed with 1 volume of ice-cold distilled water and a 0.2 volume of cold toluene. Stroma was precipitated by centrifugation in a Servall centrifuge for 30 min at 10,000 r.p.m., and the clear haemoglobin solution decanted and adjusted to the same concentration in all samples. Haemoglobin solution was kept at 4° C and used for analysis within one week after bleeding the animals. Samples were run as oxyhaemoglobin. The starch gels were prepared according to Smithies<sup>6</sup> and the discontinuous buffer system according to Morton<sup>4</sup> (tris-disodium-ethylenediamine tetraacetate dihydrate-citric acid and veronal buffer, pH 8.6, i.s. 0.05). Samples were run at 4° C for 16–18 h at 3 V/cm and 1 m.amp/cm. Gels were sliced and stained with benzidine.

The animals from the three strains used in the experiments could be classified into three different groups according to their haemoglobin pattern. Fig. 1 shows the separation of haemoglobin from animals of each strain. Haemoglobins from rats of the inbred Wistar and black strain were shown to be electrophoretically homogeneous within the populations, but they differed between themselves in respect to the mobility of bands. Within the random bred population of albino rats three types of electrophoretic haemoglobin pattern occurred, two of which proved to be identical with those of the Wistar and the black strain, respectively, and a third one which differed from both in the number of bands.

The three different haemoglobin patterns that we observed are presented schematically in Fig. 2. They are denoted as types I, II and III, and the bands marked 1–5. Two out of the three different patterns (types I and III) possess four bands each, three of which have equal mobility (bands 2, 4 and 5), whereas the fourth is located at position 3 in type I and at position 1 in type III. Electrophoretic type II contains five bands. In addition to the three bands which are common for types I and III (bands 2, 4 and 5), type II also shows the presence of one band absent in type I (band 1) and another one absent in type III (band 3). Table 1 is a summary of our results.

The results of the electrophoretic analyses of the same sample and of each individual pattern are reproducible.

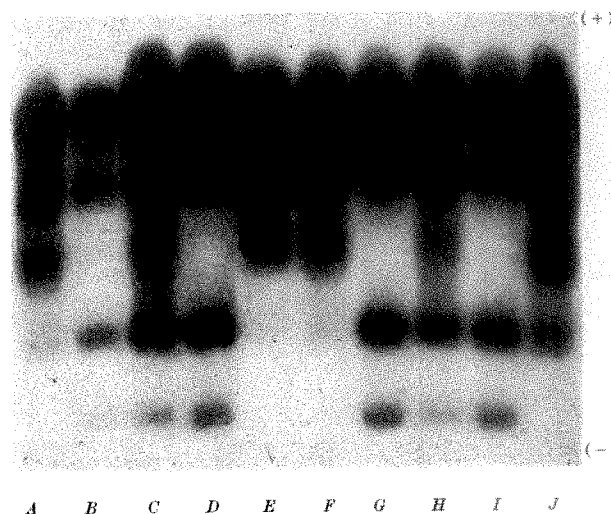


Fig. 1. Starch-gel electrophoresis (Morton's discontinuous buffer system) of oxyhaemoglobins prepared from 4-month-old rats of random bred albino, inbred Wistar, and inbred black strain. A—Wistar male; B—black male; C, D, E—albino males; F—Wistar female; G—black female; H, I, J—albino females.

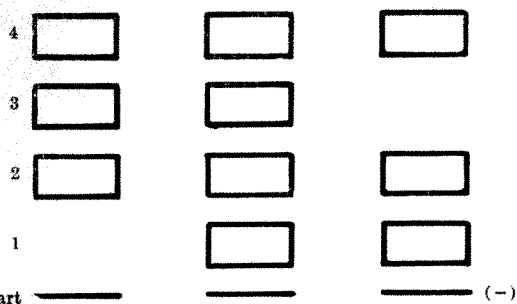


Fig. 2. Three types of the electrophoretic haemoglobin pattern observed in three strains of laboratory rat.

Table 1. ELECTROPHORETIC TYPES OF HAEMOGLOBIN IN THREE STRAINS OF LABORATORY RAT

Strain		No. of animals studied	Electrophoretic haemoglobin pattern		
			Type I	Type II	Type III
Albino random bred	Males	71	29	29	13
	Females	53	19	28	6
	Total	124	48	57	19
Wistar inbred	Males	8	8	0	0
	Females	15	15	0	0
	Total	23	23	0	0
Black inbred	Males	10	0	0	10
	Females	13	0	0	13
	Total	23	0	0	23

We believe that type II is a combination product of the other two.

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## IMMUNOLOGY

### Coral Snake Venom: Antibody Response in Rabbits

CORAL snake venom is one of the most toxic venoms of American snakes<sup>1</sup>. Reports of coral snake bites in humans<sup>2,3</sup> suggest the venom acts as a neurotoxin causing paralysis of the respiratory muscles. Experiments in our laboratory indicate that coral snake venom produces neurotoxic effects in various species of animals.

Gitter *et al.*<sup>4,5</sup> found the neurotoxic components of viper venom poorly antigenic in the horse. Although the haemolytic components in large doses of venom were neutralized by horse antiserum produced against the venom, lethal concentrations of neurotoxins in the same venom doses were not neutralized by even the largest amounts of antiserum.

We now report a method for producing rabbit antiserum of high potency against the venom of the Eastern coral snake and an analysis of the venom antigens with antiserum by gel diffusion.

The same lot (1.6 g) of freeze-dried venom from the Eastern coral snake (*Micrurus fulvius fulvius*) (obtained from W. E. Haast, Miami Serpentarium Laboratories) was used in all experiments.

Zealand rabbits, weighing 2.4 kg each, were injected. The venom was dissolved in physiological saline and 1.0 ml. injected subcutaneously. Rabbits receiving up to 0.6 mg of venom remained free of overt symptoms. A dose of 1.0 mg produced flaccid paralysis. The condition of the paralysed rabbits did not improve in 48 h, so they were killed. Rabbits which received more than 1.0 mg of venom died after 16–20 h.

The  $LD_{50}$  for the venom in mice was determined by the method of Reed and Muench<sup>6</sup>. Six male albino mice which weighed 16–18 g were injected with 0.5 ml. intraperitoneally at each concentration of venom. Tests were considered completed in 48 h. The dose-response curve for the venom in mice is shown in Fig. 1. The  $LD_{50}$  is 13  $\mu$ g.

Six female New Zealand rabbits, weighing approximately 2.5 kg, were immunized according to the schedule shown in Fig. 2. The venom was dissolved in saline and filtered through a 'Millipore' type HA (0.45  $\mu$ ) filter. Equal volumes of the venom and 'Amphojel' (aluminium hydroxide gel) were mixed and 1.0 ml. of the mixture was injected subcutaneously. Each rabbit was bled weekly for 10 ml. from the marginal ear vein and the serum was recovered.

The rabbit sera were pooled and tested for potency by determining the number of mouse  $LD_{50}$  of venom the serum would neutralize. The method used was based on that of Keegan *et al.*<sup>7</sup>. Venom was diluted in saline to a concentration of 100  $\mu$ g/ml. (7.7 mouse  $LD_{50}$ ). Two-fold serum dilutions were made in saline. Equal volumes of diluted serum and venom were mixed and incubated for 1 h at 37° C. Precipitates which formed were removed by centrifugation; mice were injected intraperitoneally with 0.5 ml. of the supernatant fluids; and the number of  $LD_{50}$  neutralized by the antiserum was calculated.

The production of neutralizing antibody in rabbits is shown in Fig. 2. Neutralizing antibody was initially detected between the third and fourth week after the first injection of venom. The total amount of venom given over this period was 1.5 mg. The maximum amount of neutralizing antibody serum (38 mouse  $LD_{50}$  or 494  $\mu$ g of venom neutralized/ml.) was detected during the tenth week, 1 week after a booster injection of 1.0 mg. Although the booster injection produced a rapid increase in the amount of neutralizing antibody, the level reached was

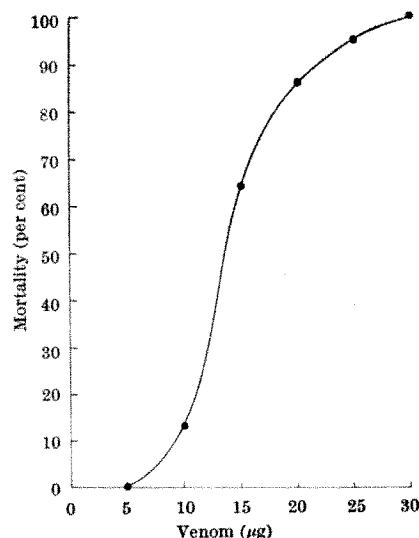


Fig. 1. Dose-response relationship for Eastern coral snake venom in mice.

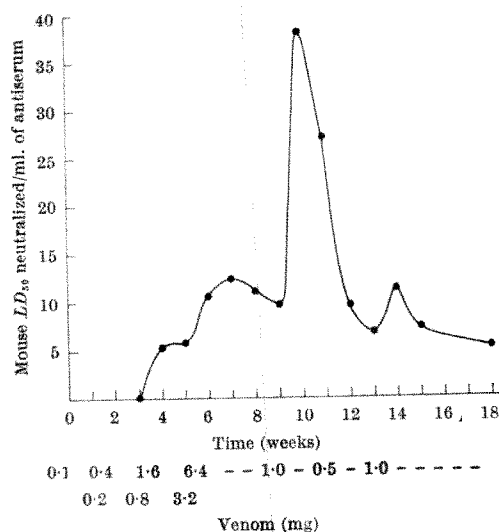


Fig. 2. Production of neutralizing antibody in rabbits immunized with Eastern coral snake venom.

not maintained for any significant period of time. The titre dropped abruptly after reaching the maximum concentration. We were unable to boost the level of antibody back to the maximum level once the titre dropped. Different combinations of rest periods and venom doses were tried, but only slight rises in the amount of neutralizing antibody were measured.

The maximum single dose of venom given to the rabbits during the course of immunization was 6.4 mg. The rabbit antiserum could neutralize at least 10 mouse  $LD_{50}$  of venom when the rabbits received this dose. Because the lethal dose for a 2.5 kg rabbit is approximately 1.0 mg, they tolerated a minimum of 6.4 rabbit  $LD$  at that time.

The venom was analysed for precipitating antigens by reacting it with the individual antisera from the six immunized rabbits. The sera were tested at the fourth, seventh and tenth weeks of immunization. This permitted an estimation of the time during the course of immunization when antibodies to different venom components were produced.

Gel-diffusion reactions were carried out in small Petri dishes. 10 ml. of 'Ion Agar No. 2' prepared in saline to a concentration of 1 per cent were dispensed per plate. The agar contained merthiolate in a final concentration of 1:10,000. A Feinberg gel cutter, No. 1801, was used to cut a pattern in the agar and the six rabbit sera were placed in the outside wells to react with the venom (100  $\mu$ g/ml.) in the centre well. Diffusion took place at 25° C for one week.

Photographs of the gel-diffusion reactions are shown in Fig. 3; a summary of the number of precipitin lines that formed is presented in Table 1. The number of precipitin lines ranged from one to four in the individual sera obtained from a specific bleeding. This demonstrates significant differences in the response of the individual rabbits to the components in the venom. There also were differences in the number of lines that formed when the three bleedings from the same rabbit were compared. For example, the initial serum sample (fourth week) from rabbit *F* produced three lines; however, serum from

the latter two bleedings (seventh and tenth weeks) produced a single line. Conversely, the initial serum sample from rabbit *A* produced only one line whereas three lines formed with serum from the latter two bleedings.

The possibility that the intense single lines produced by rabbit *F* antisera (seventh and tenth weeks) obscured the formation of additional weaker lines was examined by preparing two-fold dilutions of the antisera (to 1:16) and reacting them against the venom. Single lines were also obtained with diluted antisera. The lines, however, were more diffuse and not as intense as reactions with undiluted antisera.

The development of additional precipitin lines in reactions with sera from the latter stages of immunization may indicate that the rabbits are responding to antigens present in the venom in small amounts; to components that are poor antigens; or to the presence of antibodies synthesized during later stages of the immune response. On the other hand, several rabbit sera taken during the

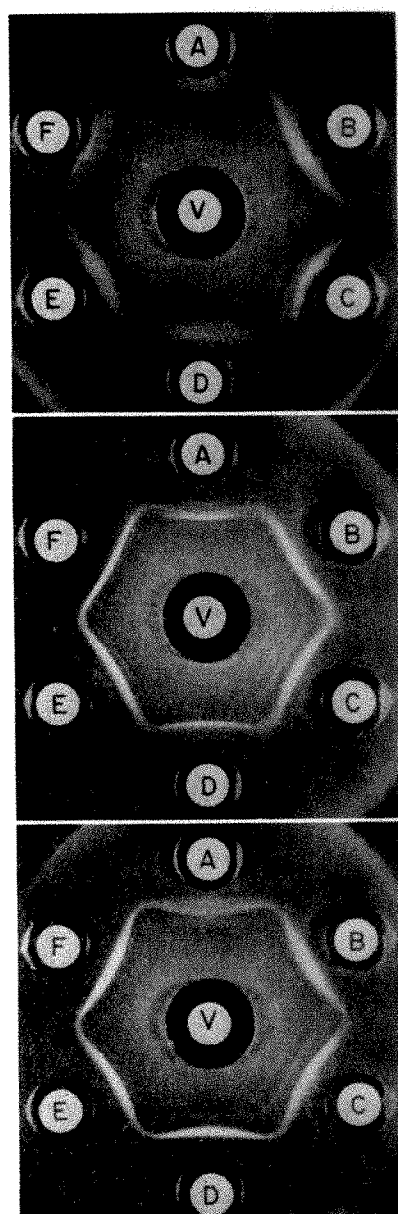


Fig. 3. Gel-diffusion reactions of individual rabbit antisera A-F (outside wells) and Eastern coral snake venom (V), 100  $\mu$ g/ml. (centre well). a, Sera taken 4 weeks after start of immunization; b, 7 weeks; c, 10 weeks.

Table 1. NUMBER OF PRECIPITIN LINES PRODUCED BY EACH RABBIT ANTISERUM AT THREE STAGES OF IMMUNIZATION

Rabbit antiserum	Week of immunization		
	4th	7th	10th
A	1	3	3
B	2	1	2
C	2	4	3
D	3	2	2
E	3	2	2
F	3	1	1



precipitin lines than did sera from the fourth week. The work of Richter *et al.*<sup>8</sup> may provide an explanation for this observation. They found that antibodies in rabbit antisera tested during early stages of immunization (4 weeks) could precipitate with serum antigens and agglutinate sensitized red cells, whereas those present in late antisera (12–17 weeks) exhibited only the latter property. Some of our rabbit antisera may have had, qualitatively, relatively small amounts of precipitating antibody in the later bleedings which resulted in fewer precipitin lines.

A comparison of our data with those of Kochwa *et al.*<sup>5</sup> suggests that the neurotoxins in venoms of different snakes may have different antigenic properties, particularly in their capacity to elicit neutralizing antibodies. In their experiments both horse and rabbit antisera failed to protect mice against the neurotoxic effects of viper venom. When rabbits initially immunized with whole venom were subsequently immunized with only the neurotoxic fractions, however (a superimposed immunization), full protection by the antiserum against the effects of the venom was obtained. We used only whole coral snake venom but achieved complete protection against the neurotoxic effects of the venom.

Besides possible differences in the antigenicity of neurotoxins in different venoms, the production of neutralizing antibodies may depend on the route of injection of the venom. Staab *et al.*<sup>9</sup> showed that normal rabbits are very resistant to venom given intradermally and intraperitoneally. They suggested that this resistance, in the case of intraperitoneal injection, may be caused by an active detoxifying process. Kochwa *et al.*<sup>5</sup> used both the intravenous and intraperitoneal routes in their immunizations. Their use of the latter route may partially explain the lack of antibodies against neurotoxins in rabbit antiserum produced against whole viper venom. We used the subcutaneous route to immunize rabbits, and antiserum produced in this manner protected mice against the neurotoxic effects of the venom.

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## Novel Alterations in Sera from Pseudopregnant and Deciduoma-bearing Rats

CONSIDERABLE attention has recently been focused on a new serum component of rats which was described by Darcy as being associated with rapid growth of tissue. Although it was not found originally in electrophoretically partitioned sera of healthy adult males or non-pregnant females, the protein was found in sera of foetal, neonatal, tumour-bearing, liver-regenerating, lactating, and pregnant rats<sup>1–5</sup>. Identified as an alpha-1-glycoprotein, it has been shown to be synthesized by the liver during the acute phase of an inflammatory response<sup>6</sup>. An apparently

mentally poisoned by cadmium and other metals<sup>7</sup> and in rats given single doses of *Escherichia coli* endotoxin<sup>8</sup>. A new protein migrating in the region of the alpha globulins has also been described in mice treated with a variety of toxic agents<sup>9</sup>.

Because there is rapid growth of tissue during the development of deciduoma in the uterus of the pseudopregnant rat (the uterine horns increase eight- to ten-fold in weight within four days), it was of interest to see whether the Darcy component was present in sera of decidua-bearing female rats. Because deciduoma formation is initiated by stimulating the uteri of pregnant or pseudopregnant animals (pseudopregnancy can be induced by various stimuli which cause an alteration of the normal 4-day oestrous cycle, a development of functional corpora lutea and an appearance of leucocytes in vaginal smears; this condition of pseudopregnancy persists for 11–14 days before oestrus is again experienced<sup>10,11</sup>), the sera of pseudopregnant animals were also examined and these compared with the sera of newborn and pregnant rats. The present communication is concerned with the novel alterations observed among electrophoretically separated components of sera from pseudopregnant, deciduoma-bearing rats.

Pseudopregnancy was induced by electrical stimulation applied to the cervix of the female rat on the day of oestrus (as determined by microscopic examination of daily vaginal scrapings) and on the following day<sup>11</sup>. Blood samples were taken from animals (*B.I.* strain, colony-bred albino rats originally of Wistar stock maintained in this department) before and at various intervals during pseudopregnancy but most often at 2, 8 and 10 days after the second electrical stimulation, (designated as leucocytic days of pseudopregnancy  $L_2$ ,  $L_8$  and  $L_{10}$ ).

Pre- and post-treatment sera from each animal were placed in adjoining troughs of starch-gels and electrophoresis was conducted at room temperature by the vertical technique of Smithies<sup>12</sup>; staining was with amido-black.

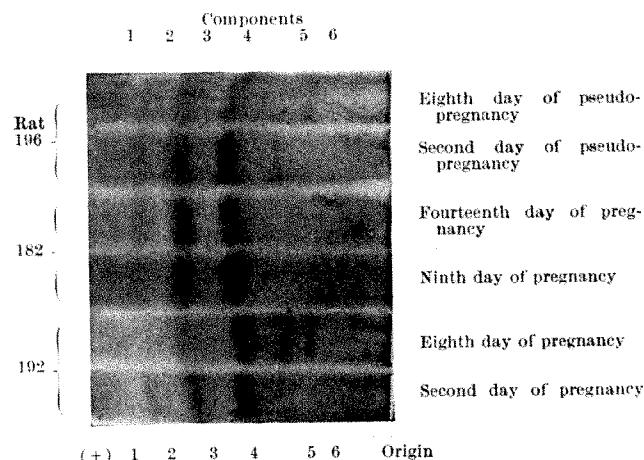


Fig. 1. Starch-gel electrophoretic separation patterns of two serum samples from each of three rats are presented showing, in particular, the slow alpha globulin zone. A new serum component, at site 6, may be seen in the sera of two of the rats, 196 above and 192 below, taken late in pseudopregnancy. The new serum component present on the eighth day but not the second day of pseudopregnancy was not seen in the sera of newborn or pregnant rats (compare pregnant rat 182, centre).

Sera from rats in late pseudopregnancy ( $L_8$  and  $L_{10}$ ) differed markedly from those taken before and in early pseudopregnancy ( $L_2$ ). A new component (position 6, Fig. 1, animals 196 and 192) was seen in four of thirteen sera obtained on  $L_8$  but not in any of those taken on  $L_2$  of pseudopregnancy. In addition, smaller amounts of components 1, 2 and 3 were present in sera on  $L_8$  than were seen in those on  $L_2$ . Sera taken before electrical stimulation of these animals appeared much the same as sera taken on  $L_2$  of pseudopregnancy (for example, see the pre-bleedings of the five rats in Figs. 2 and 3).

Attempts were made to see whether component 6 also existed in pregnant or neonatal rats. None of the sera from eleven pregnant rats appeared to have component 6 despite the fact that they were taken at various times during pregnancy (for example, see animal 182, Fig. 1), nor was component 6 present in sera of neonates (eight in two pools of four). The alpha-1-glycoprotein described as present in pregnant and neonatal rats, however, was not seen in the sera of rats from this colony. As an added precaution, sera from all neonates and from four of the pregnant animals were derived from decapitated animals as was described by Beaton *et al.*<sup>2</sup> and not according to our usual procedure, that is, not from heart blood taken from etherized animals.

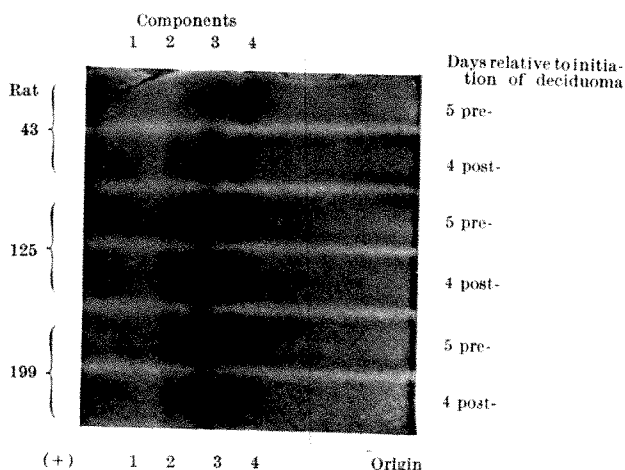


Fig. 2. Electrophoretic patterns are shown of serum samples taken before and after initiation of decidual formation. In sera taken four days after start of decidual formation, the slow alpha globulin (component 4 of animals 43 and 125) is seen decreased, while components 2 and 3 are markedly increased. These changes are barely visible, if at all, in sera of a third animal (199) that formed a small decidualoma.

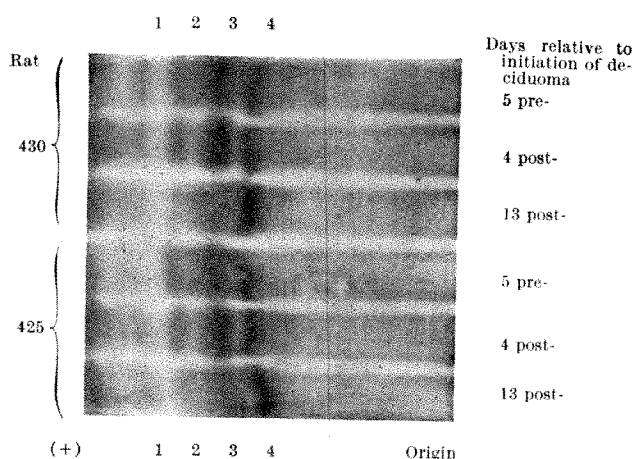


Fig. 3. Serum changes are shown in two rats experiencing formation and regression of decidualoma. The increases in components 2 and 3, apparent in sera taken four days after initiation of decidualoma, are no longer visible on the thirteenth day. By the thirteenth day, when the serum patterns have returned to normal, the decidualoma have also regressed causing uteri to look normal.

To examine sera of animals possessing decidualoma, pre-bled adult females which weighed 140–210 g were made pseudopregnant by electrical stimulation of the cervix. On  $L_4$  of pseudopregnancy, as judged from the persistence of leucocytic-type vaginal smears, laparotomy was performed under ether anaesthesia and both horns of the uterus were stimulated to produce decidualoma by intraluminal scratch. This trauma serves to initiate decidualoma formation in the sensitized pseudopregnant rat<sup>10,11,13</sup>. Four days later ( $L_8$ ), the animals were re-bled and either autopsied to confirm the presence of decidualoma

or their uteri were examined by laparotomy. These rats were then sutured and re-bled on  $L_{17}$  when decidual regression had occurred. Thus, small samples of serum taken before, during, and following development of decidualoma were available for investigation.

Electrophoretic separation of sera from sixteen animals bearing decidualoma of various sizes showed ten with a regular alteration which involved more than one component in the slow alpha globulin zone. Typically, the slow alpha globulin was seen to be a narrower less intensely staining band in sera taken four days after start of decidualoma formation ( $L_8$ ) than in sera taken 5 days before decidualoma initiation (Fig. 2, component 4 of animals 43 and 125). At the same time, there was a marked increase in components 2 and 3.

A direct correlation was observed between the degree of the decidual response and the degree of change in serum components. When decidual responses were small, the serum changes were not dramatic (Fig. 2, rat 199). The sera from four control rats the uteri of which had been traumatized along with experimental animals showed no visible changes; these animals were not pseudopregnant and thus were incapable of developing decidualoma.

To ascertain the duration of these changes in the sera of decidualoma-bearing animals, sera taken on  $L_{17}$ , 13 days after initiation of decidualoma, were examined. At this time uteri generally return to normal and animals experience oestrus once more. As may be seen from Fig. 3, the serum components also reverted to their former patterns, that is, after first showing an increase in concentration, components 2 and 3 were seen to be diminished once more while component 4 was first decreased and then later replenished or even slightly augmented.

Whether formation of decidualoma triggers an increased production of serum components 2 and 3 at the expense of a slow alpha globulin (component 4) or simply blocks utilization or catabolism of 2 and 3, while hastening circulatory removal of component 4, remains undetermined. Reversion of each of the three components to normal concentration after decidual regression points to an apparent interdependence among these serum components.

The source of components 2 and 3 is also unknown. Because they are normally present in sera, but in small amounts when decidual tissue is not present, it may be possible that pre-decidual cells in the uterus produce a small amount of each component under ordinary circumstances but much more as the cells develop into the decidual. Alternatively, components 2 and 3 may be manufactured in the liver in support of tissue growth.

A comparison on the same starch-gel of component 6, seen here in pseudopregnant females, with the alpha-1-glycoprotein of Darcy could not be made because neither of these components was present in sera from pregnant or neonatal animals of this colony. A comparison of photographs of the two components, however (that is, Fig. 1 here with Fig. 1 in refs. 2, 4 and 7), reveals them to be in distinctly separate positions and therefore not identical.

A further difference between the two serum components may be the manner in which they arise; whereas the alpha-1-glycoprotein is thought not to appear at the expense of other serum components<sup>4</sup>, the appearance of component 6 coincides with a diminution in concentration of components 1, 2 and 3. The interrelationship that apparently exists among components 1–6 suggests they may be members of a common family of alpha globulins.

That only one-fourth of the pseudopregnant animals and two-thirds of the decidual animals were capable of expressing the serum differences may be referable to genetic differences. Perhaps genetic differences will also explain the inability of pregnant and neonatal rats of this colony to produce the reproduction associated protein.

Quantitative aspects of the changes in sera would be essential to affirm the visual evidence reported here for rats with decidualoma and those experiencing pseudopregnancy.

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### Recognition of Altered Autologous Constituents as Foreign by Phagocytic Cells

THE passage of antigen into an organism and its subsequent uptake by specialized cells are essential preliminary events in any process in the synthesis of antibody.

It has been shown that these "specialized" cells (for example, circulating leucocytes and macrophages of the reticulo-endothelial system) engulf particulate antigens (for example, from microbes, protozoa and red blood and other cells) by means of phagocytosis<sup>1,2</sup>. They will also take up isolated protein macromolecules<sup>3-7</sup> or complex bacterial endotoxins (both thermostable and thermolabile) and some exotoxins by means of pinocytosis<sup>7,8</sup>.

These well known mechanisms may or may not be necessary antecedents of activation of the antibody production system. Moreover, it is not known whether any degree of recognition of self is possessed by the phagocytic cell series. In order to explore this point, we have investigated the rates of pinocytosis and phagocytosis of autologous proteins (for example, gamma-globulins) and cells (for example, red cells) using both untreated cells and cells structurally modified by means of physical or chemical treatment.

Pinocytosis of gamma-globulins was investigated by observing under a fluorescent microscope a mixture which had been incubated at 37° C for 30 min and which consisted of 4.5 ml. of a suspension of leucocytes taken from guinea-pig peritoneal exudate (90,000 cells/mm<sup>3</sup>) in Alsever solution and 0.5 ml. of a 1 per cent suspension of fluorescent autologous gamma-globulins in saline. The exudate was produced in guinea-pigs by means of two successive daily intraperitoneal injections, each with 30 ml. of 5 per cent polyvinylpyrrolidone at pH 7. Ninety per cent of the phagocytic cells obtained in this way were polymorphonuclear leucocytes, while the other 10 per cent were monocytes. Autologous gamma-globulins were isolated by chromatography on 'Sephadex' DEAE and later conjugated with fluorescein isothiocyanate<sup>9</sup>. Before conjugation, each sample was divided in two equal parts, one of which (the control) was not treated; the other portion was heated for 10 min at 80° C (Fig. 1).

Results are given in Table 1 as the percentage of phagocytes showing pinocytosis (five hundred elements were counted in each sample) and quantitative data of this phenomenon are expressed in terms of the number of pinocytic vacuoles per leucocyte.

Phagocytosis of red cells was investigated in a similar way, but light microscopy was used after staining with May-Grünwald-Giemsa. The exudate was produced in

Table 1. PINOCYTOSIS OF AUTOLOGOUS GAMMA-GLOBULINS

	Mean rate of pinocytosis (per cent)	No. of pinocytic vacuoles/leucocyte
(A) Controls (non-treated gamma-globulins)	13.7 ± 1.87	10.09 ± 0.6
(B) Altered gamma-globulins	65.87 ± 2.8	10.04 ± 0.73

The difference between (A) and (B) is highly significant.

guinea-pigs by means of two intraperitoneal injections: the first consisted of 10 ml. of 5 per cent glycogen in Ringer solution, and the second (48 h later) of 30 ml. of 5 per cent polyvinylpyrrolidone.

Autologous red cells obtained by cardiac puncture were washed three times and finally resuspended in saline (1.4 × 10<sup>6</sup> cells/mm<sup>3</sup>). Each sample was later divided into four equal parts each of 0.5 ml. The parts were treated as follows: (1) one part (the control) was maintained as such; (2) another part was incubated at 37° C for 30 min with an equal amount of 1 per cent periodic acid; (3) another was incubated at 37° C for 90 min with an equal amount of cathepsin solution (that is, 10 mg cathepsin NBC/ml., pH 5 acetate veronal buffer, containing 0.025 molar cysteine hydrochloric acid); the final part was incubated at 37° C for 90 min with an equal amount of a solution containing 0.5 mg *Clostridium perfringens* lecithinase/ml.).

Each sub-sample of the suspension of red blood cells was later mixed in a silicon-coated tube with the corresponding leucocyte suspension (the numerical ratio of red cells to leucocytes was 15:1 in each mixture) and immediately incubated at 37° C for 90 min. Results are given in Table 2, in terms of the percentage of leucocytes showing phagocytosis (in every mixture 500 elements were counted), and quantitative data are expressed in terms of the number of erythrocytes phagocytosed by one cell. In some instances, the leucocytes were washed in order to exclude the action of any "specific opsonization" as a result of serum components (Table 2, D').

Table 2. PHAGOCYTOSIS OF AUTOLOGOUS RED CELLS

	Mean rate of phagocytosis (per cent)	No. of erythrocytes phagocytosed/leucocyte
(A) Controls (non-treated cells)	0.3 ± 0.1	one erythrocyte/leucocyte
(B) Cells following treatment with cathepsin	0.93 ± 0.2	one erythrocyte/leucocyte
(C) Cells following treatment with periodic acid	21.46 ± 8.45	one erythrocyte/leucocyte
(D') Cells following treatment with lecithinase	22.1 ± 1.61	one erythrocyte/98 per cent of the leucocytes, and two erythrocytes/2 per cent of the leucocytes
(D'') As D' but with washed leucocytes	20.26 ± 1.56	

Supplementary control in order to eliminate any possible specific opsonization due to serum components.

Differences between (A) and (B), (A) and (C) and between (A) and (D') are highly significant. The difference between (D') and (D'') is not statistically significant.

The results clearly show that:

(1) The rate of uptake of autologous unaltered red cells is negligible (0.2 per cent, see Table 2). Previously, we

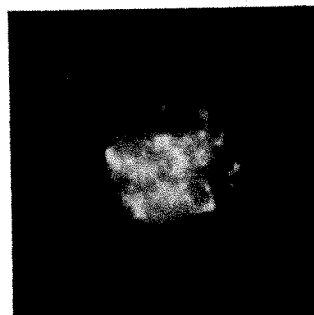


Fig. 1. Pinocytosis of thermally altered autologous gamma-globulins. (Fluorescent microscopy, × 1,250.)

have not been able to determine whether pinocytosis of autologous non-treated gamma-globulins (observed in 15 per cent of the leucocytes counted, Table 1) results from some "artefactual" alteration of gamma-globulins, during manipulation for reparation and conjugation, or can be attributed to some minimal basic rate of physiological pinocytosis. The former seems to be the most likely cause.

(2) Certain artificially induced structural alterations of autologous components (either macromolecules or cells) occur, which result in an increase of their pinocytic or phagocytic rates.

(3) At the phagocytic level, discrimination between autologous and non-autologous material occurs independently from humoral specific factors, even in chordates. When leucocytes were washed the differences between the phagocytic rates of altered and unaltered red cells (Table 2, D") and pinocytosis of altered and unaltered gamma-globulins were not diminished. These data are in agreement with the observations showing that opsonins are not necessarily involved in the process of phagocytosis of aged or damaged erythrocytes, by leucocytes<sup>10</sup>.

(4) Not all the components of a cell are equally relevant in the recognition process at the phagocytic level, but the superficial ones tend to be prominently displayed. (a) Cathepsin, which affects haemoglobin (see Table 2, B), increases only slightly (however, statistically significantly) the rate of erythrophagocytosis. This is probably because haemoglobin is "hidden" within the cell and virtually unable to interact with the phagocytic cell wall during the very moment of realization of phagocytosis (that is, haemoglobin structure is scarcely recognizable in the erythrophagocytosis process). (b) Lecithinase, which results in hydrolysis of lecithin—a component of the membrane of red cells (and, as such, "displayed" for interaction with the phagocytic cell membrane)—greatly increases the rate of erythrophagocytosis (Fig. 2). (c) Periodic acid, which affects the hydroxy groups of carbohydrate components and the alpha-amino alcohol functions of proteins in the cell wall of red blood cells<sup>11-13</sup>, also considerably increases the rate of phagocytosis (Fig. 3).

This interpretation is in close agreement with the now unanimously accepted idea that the recognition of "foreign" red cells as antigens is a consequence of the ability of the immunological defence mechanism of chordates to discriminate even fine natural differences of structure (when displayed for interaction) between functionally similar macromolecules of cells. The gradual increase of phagocytosis, paralleling the phylogenetic distance between the "donor" and the "host" species<sup>12</sup>, seems, in fact, to mirror the more easy recognition of



Fig. 2. Phagocytosis of autologous red cells following treatment with lecithinase. May-Grünwald-Giemsa staining ( $\times 1,250$ ).



Fig. 3. Phagocytosis of autologous red cells following treatment with periodic acid. May-Grünwald-Giemsa staining ( $\times 1,250$ ).

larger structural differences in physiological conditions. From the point of view of "immunological recognition", "natural" (that is, genetically controlled) and "artificially induced" differences in structure between macromolecules or cells are both recognizable to the same degree, provided that they are "adequately displayed" for interaction with the recognition mechanism of the corresponding defence level. At the level of the phagocytic defence mechanism, "adequately displayed" means situated on the surface (that is, on the membrane or cell wall).

The ability to discriminate such differences is an attribute of leucocytes and macrophages, at the same time and before discrimination by antibody synthesizing lymph-plasmacyte clones<sup>14,15</sup>.

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### Solid Phase Radioimmunoassay

ALL forms of radioimmunoassay used at present include a procedure to separate free and bound tracer antigen when equilibrium is reached in the incubation mixture. This separation may involve precipitation by a second antibody<sup>1,2</sup>, electrophoresis<sup>3,4</sup>, chromatoelectrophoresis<sup>5</sup>, ion exchange<sup>6</sup>, solvent fractionation<sup>7</sup>, gel filtration<sup>8,9</sup>, salt precipitation<sup>10</sup>, or adsorption to charcoal<sup>11</sup>. The need to

use such formal isolation procedures increases the length and complexity of the assay and introduces a number of possible sources of error.

For these reasons we felt that a system in which both the antigen-antibody reaction and the separation of bound and free tracer antigen could be achieved in a single step would be a valuable innovation. The application of substituted polymers conjugated to the antigen for the isolation of antibody<sup>12,13</sup> suggested the possibility of using this type of procedure in reverse to achieve this aim. In the presence of antibody coupled to an insoluble polymer, antigen which becomes bound to antibody will be attached simultaneously to a solid supporting medium. Then, if radioactive tracer antigen is present in the system, the solid phase has simply to be washed and counted to quantitate the degree of antibody binding. This would result not only in a simple and rapid radioimmunoassay, but also in the elimination of a variable number of handling and other errors which are inherent in the separation techniques mentioned.

The feasibility of this method was tested with a solid phase antibody consisting of diazo-polystyrene coupled to rabbit antiserum to human growth hormone, and human growth hormone labelled with iodine-131 as tracer antigen. This initial experiment showed that the binding of labelled human growth hormone to the solid phase was reduced by the presence of unlabelled human growth hormone over the range 1–10  $\mu\text{g}$ .

This demonstration of the suitability of the solid phase antibody as the basis of a radioimmunoassay led us to extend the sensitivity of the method to the physiological range. This aim was facilitated by the use of a solid phase support consisting of the isothiocyanate derivative of a graft copolymer of poly(tetrafluoroethylene) ('Fluon G4') and styrene. This copolymer<sup>14</sup>, which was developed for use in analytical, synthetic and immunochemical procedures, allows a variety of reactive substituents to be attached to the aromatic rings on the surface of a dense and inert core of poly(tetrafluoroethylene).

An example of the use of this polymer, coupled to anti-human growth hormone rabbit gamma globulin, for the radioimmunoassay of human growth hormone, is shown in Fig. 1. In this experiment, 5 mg of solid phase-antibody was incubated for 12 h with 0.2  $\mu\text{g}$  of labelled human

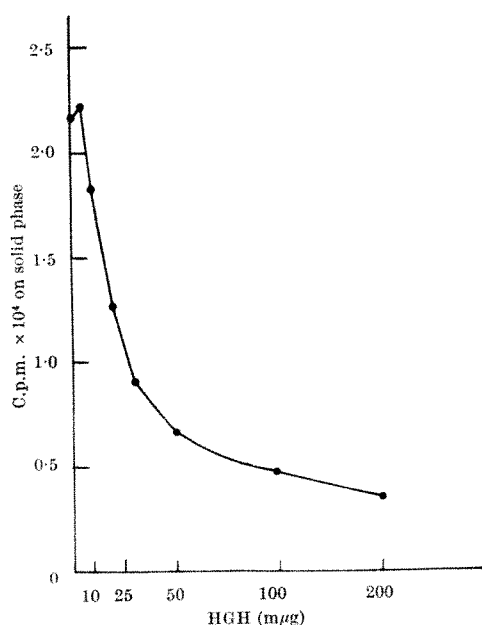


Fig. 1. The effect of adding 5–200  $\mu\text{g}$  of human growth hormone (HGH) on the binding of tracer amounts of human growth hormone labelled with iodine-131 by solid phase-antibody.

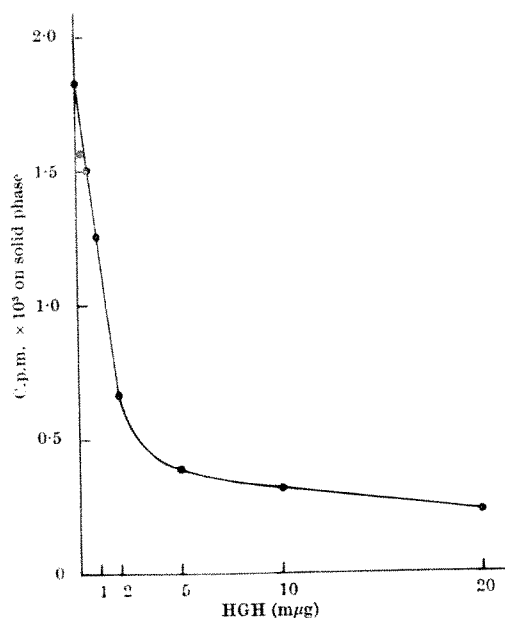


Fig. 2. The effect of adding 0.2–20  $\mu\text{g}$  human growth hormone (HGH) on the binding of tracer amounts of human growth hormone labelled with iodine-131 to solid phase-antibody. The concentration of antibody in this solid phase preparation was approximately a tenth of that used to achieve the result shown in Fig. 1.

growth hormone in the presence of increasing quantities of unlabelled human growth hormone. The binding of tracer human growth hormone to the solid phase was sharply reduced by the addition of as little as 10  $\mu\text{g}$  of unlabelled growth hormone. The use of a solid phase more lightly coated with antiserum, and an incubation period of 2 days, enabled 0.2  $\mu\text{g}$  of human growth hormone to be detected giving a standard curve over the range 0–20.0  $\mu\text{g}$  (Fig. 2). The application of this method to the estimation of antigen in the subnanogram range should be readily achieved by further reduction in the amount of antibody present in the system, with corresponding increase in the period of incubation and the specific activity of the tracer antigen.

The use of solid phase systems in protein chemistry has been largely confined to the field of peptide synthesis, as extensively developed by Merrifield<sup>15</sup>. The present communication reports a further application of solid phase for the micro-estimation of antigen. Its use in the technique of radioimmunoassay is a new concept<sup>16</sup> which confers several advantages over the methods which are at present in use. These advantages derive from the ability of the solid phase-antibody system to remove tracer antigen from solution at the same time as it is bound by specific antibody.

We have found that the addition of vast quantities of unlabelled human growth hormone to solid phase-antibody which has been allowed to bind labelled human growth hormone does not result in displacement of the tracer. Thus, the binding of this antigen to the solid phase-antibody appears to be an irreversible process. The consequences of this irreversible binding are of great importance, because they lead to three further effects which are of value in the procedure of radioimmunoassay. First, the speed of the antigen-antibody reaction is enhanced by the removal of the product of reaction. Second, the technique of disequilibrium is particularly applicable to this system, because the unlabelled antigen can be removed by washing the solid phase before adding the tracer. This allows a considerable improvement in sensitivity by avoiding dilution of the added tracer by the unbound antigen which would otherwise remain in the system.

Finally, the ability to wash the solid phase-antibody after reaction with an unknown antigen in serum allows



removal of serum proteins before the addition of tracer. This will avoid the "damage" which constantly occurs when tracer quantities of labelled protein hormones are incubated in the presence of serum.

We suggest that the combination of these several advantages of the solid phase-antibody system should make it a powerful tool for measuring minute quantities of antigen by a radioimmunoassay procedure.

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## MICROBIOLOGY

### Changes in Transforming DNA in *Pneumococcus*

DURING the course of an investigation involving the transforming ability of pneumococcal DNA, an interesting correlation was found between the transforming activity of lysates prepared at varying times from the same culture and the number of viable organisms in the culture. *Streptococcus pneumoniae*, strain Aml, an aminopterin resistant strain derived from strain R36A of Avery *et al.*<sup>1</sup>, was grown first in P medium and then in NS medium in the manner designed to promote competence<sup>2</sup>. At appropriate times, 1.0 ml. aliquots of the culture in NS medium were lysed in the presence of sodium citrate by the addition of sodium deoxycholate, and the DNA was precipitated with ethyl alcohol using sodium hyaluronate as coprecipitant<sup>3</sup>. In one of the experiments to be discussed another aliquot was taken at the same time as that for the lysate, and, after suitable dilution, was plated out in triplicate for a viable count. The lysates were assayed for their transforming activity with respect to resistance to aminopterin by a conventional method<sup>2</sup>, using *Str. pneumoniae* strain Cl. 3 as the receptor organism.

The results obtained are summarized in Table 1. The viable counts have been expressed as multiples of the count obtained at the time of the first sample. In the first experiment the culture did not undergo a complete division during the period of observation. The viable counts were not corrected for the average chain length, but the chain length would not be expected to increase by more than 1 per cent in this time. In the second experiment the viable count was not carried out and the figures for the multiple of the viable count quoted in Table 1 are the average from three growth curves determined in comparable conditions in the course of other experiments.

Table 1. TRANSFORMING ACTIVITY OF LYSATES PREPARED AT DIFFERENT TIMES FROM THE SAME CULTURE

Time at which lysate was prepared (min)	No. of transformants (T) ( $\times 10^3$ )	Viable count ( $\times 10^6$ )	Multiple of viable count (M)	Estimated multiple of viable count (see text)	T/M $\times 10^3$
Experiment 1					
100	3.87	8.63	1	—	3.87
105	8.38	9.70	1.13	—	7.34
110	10.2	11.2	1.3	—	7.8
115	9.30	9.77	1.13	—	8.2
Experiment 2					
80	0.9	—	—	1	0.9
100	4.3	—	—	1.51	2.85
120	42.4	—	—	1.87	22.67

The numbers of samples are small for an extensive statistical analysis, but it will suffice to comment that the results of experiment 2 show no overlap, while in experiment 1 the transforming activity at 110 min compared with that at 100 min was significant at  $P < 0.05$  in the Student *t* test.

The ratio of the number of transformants to the multiple of the viable count shows a marked increase with the increase in age of the culture from which the transforming lysate is prepared. If it is assumed that the relative number of organisms present at each time can be taken as a rough measure of the relative amount of the *AmiA-r1* gene contained in each lysate, it would appear that the increase in the transforming activity of the lysates cannot be explained solely by an increase in the amount of the gene in each aliquot. This would suggest that qualitative changes occur in the DNA during its replication which manifest themselves as changes in its transforming ability. The lysate would also contain DNA contributed from any autolysed cells, but, at the very most, this would represent only a doubling, and the DNA itself would have been exposed to DNase simultaneously released.

An alternative explanation would relate the increased transforming activity to an increase in the amount of the gene in each cell as a result of DNA synthesis disproportionate to the extent of cell division. This would imply, to account for the highest ratio observed, that gene replication of seven-fold or more occurred before cell division, and this explanation seems less likely. Further work is in progress to try to elucidate the phenomenon.

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### Controlled Investigations in Baboons (*Papio cynocephalus*) on Transmission of SV-5 Virus by Contact

THE intranasal infection of vervet monkeys (*Cercopithecus aethiops*) with SV-5 virus, which belongs to the para-influenza 2 group<sup>1</sup>, has been reported to result in a disease pattern usually observed in naturally occurring parainfluenza virus infections of man<sup>2,3</sup>. It was suggested, therefore, that this experimental model was potentially useful for the evaluation of appropriate antiviral drugs and also for investigation of the pathology and pathogenesis of acute respiratory viral disease.

Observations made in this laboratory suggest that baboons (*P. cynocephalus*) can also be intranasally infected with SV-5 virus, and, furthermore, that this virus infection can be transmitted to uninfected monkeys by contact. The contact infection was thought to be of interest because

it could be useful as a prophylactic antiviral test system. This communication summarizes the results of controlled experiments which have been conducted to compare the pattern of SV-5 infection in baboons inoculated intranasally and in their cage-mates.

Altogether, fifty-one young baboons, ranging in weight from 3 kg to 4 kg and free from signs of acute respiratory disease, were used in this work. The animals were accommodated in suitable cages, the majority in pairs and some three to a cage, in an air-conditioned animal isolation building. After preinoculation blood samples and combined nasopharyngeal swabs had been taken from each baboon, one animal in each cage was inoculated intranasally with 2 ml. of tissue culture fluid containing  $10^{6.7}$  TCID<sub>50</sub>/ml. of SV-5 virus. The baboons thus inoculated were then referred to as "inoculated" and their cage-mates as "contact". Combined nasopharyngeal swabs were then taken from all baboons at varying times, between 1 and 30 days after infection, and also on day 40. Serum specimens were taken on days 20 and 40. Each swab was tested for the presence of virus and each serum specimen for HI (haemagglutination inhibiting) antibody as previously described<sup>3</sup>. The varied amounts of fluid absorbed by each nasopharyngeal swab made it impossible to titrate virus, and therefore the virus isolation results were recorded as positive or negative. The results of virus isolations are summarized in Table 1 and Fig. 1, and the antibody response is shown in Fig. 2.

Table 1

Experimental group	Beginning of virus excretion (range in days)	Duration of virus excretion, Virus positive/group total during indicated periods (days)				Total virus positive/group total
		8-14	15-21	40	Group mean	
Inoculated	1	16/25	8/25	1/25	14.6	25/25
Contact	5-21	23/26	2/26	0/26	11.4	25/26

Table 1 shows that although the inoculated baboons were all virus positive 24 h after inoculation, the contact baboons began to excrete virus considerably later, that is between day 5 and day 21. There was also some difference between these two groups in the duration of virus excretion. While the great majority of contact baboons (twenty-three out of twenty-six) excreted virus for 8-14 days, the inoculated baboons excreted virus for longer periods. Table 1 also shows that one contact baboon remained virus negative throughout the whole observation period. This animal had a low titre (1.7) of antibody activity in its serum before exposure to virus, and there was a four-fold rise to a titre of 1:28 on day 40.

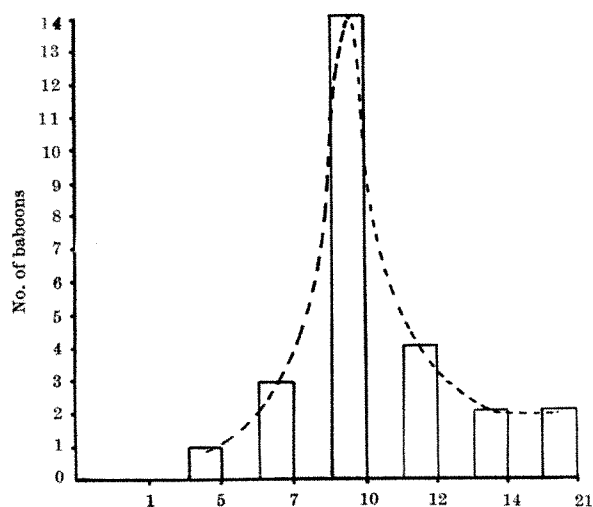


Fig. 1. Frequency distribution of time periods between exposure of inoculated baboons to virus and its appearance in the nasopharynx of contact baboons.

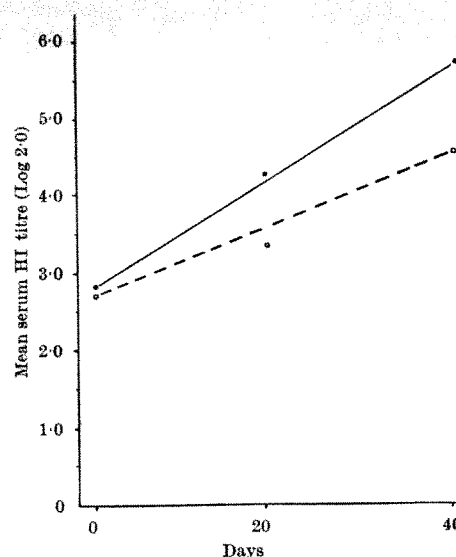


Fig. 2. Mean values of the HI antibody response of baboons to infection with SV-5 virus. —, Inoculated; ---, contact.

Fig. 1 shows frequency distribution of time periods between exposure to virus and its appearance in the nasopharynx of contact baboons. The frequency distribution resembles the normal curve with a slight skewness rising more abruptly on the short side and tailing off on the long side. The range of these periods is an important epidemiological characteristic because it determines the time interval necessary for virus transmission from SV-5 infected monkeys to their cage-mates.

As in our previous experiments<sup>3</sup>, mild clinical signs of infection were present in both groups of animals, but they were difficult to measure accurately. There were neither complications nor sequelae. The HI antibody response in both groups of experimental baboons is summarized in Fig. 2, from which it is clear that the animals developed an antibody response. In view of the prolonged virus excretion in the nasopharynx and the apparent lack of a viraemic state<sup>3</sup>, it was not surprising that the antibody response was weak.

To conclude, the contact infection of baboons with SV-5 virus seems to be a practicable model system, potentially useful for pre-clinical evaluation of antiviral drugs against viral infections of the upper respiratory tract and also for investigation of important epidemiological characteristics of these infections.

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### *Mycoplasma hominis* in Ayre's Smears

ORGANISMS of the genus *Mycoplasma* are frequent inhabitants of the female genital tract. A common species is *Mycoplasma hominis* type I, and this organism has been isolated from about 20 per cent of normal women<sup>1</sup>.

Unlike bacteria, *Mycoplasma* have no rigid cell wall and are difficult to detect in heat fixed smears. The morphology can be preserved when less traumatic methods of fixation are used, and it occurred to us that it might be possible, if enough of the organisms were present, to see mycoplasmas in cytological smears fixed in alcohol. Preliminary studies showed that when a heavy growth of mycoplasmas had been obtained from a vaginal swab, the Ayre's smear frequently had a "dirty" appearance. Ayre's smears and vaginal swabs were subsequently examined from 233 ante-natal patients. The vaginal swabs were plated on to blood agar and on to pleuropneumonia-like organism (PPLO)-agar and examined after 72 h incubation. *Mycoplasma* sp. were grown from sixty-five swabs (24 per cent); the growth was graded according to the number of colonies established and all strains grown were identified as *Mycoplasma hominis* type I by the growth inhibition test<sup>2</sup>. The Ayre's smears were stained by Papanicolaou's method. The cultural results were not known to the microscopist at the time when the Ayre's smears were examined. The combined results are shown in Table 1.

Growth of <i>M. hominis</i>		Morphological presence in Ayre's smear
Heavy	25	22 (88 per cent)
Moderate	11	6 (54 per cent)
Scanty	19	3 (16 per cent)
Negative	178	5 (3 per cent)

Where there was cultural evidence of a heavy infection of the cervix with mycoplasma, the sample obtained by the Ayre's smear technique showed, under low power, a characteristic "dirty" appearance (Fig. 1). There were innumerable moderately haematoxyphil minute micro-organisms festooning the squames and often forming bluish chains between the cells. Under the 4 mm and 2 mm objectives the organisms were mainly coccoid, the cell outline being less distinct than with bacteria. Where the organisms lay thickly they stained a purplish blue; they did not spread evenly over the glass slide as bacteria often do. Table 1 shows that there was a close correlation between the proportion of morphologically positive Ayre's smears and the heaviness of growth; where the growth was scanty, recognition of the organisms in the film was less reliable. This is regarded as evidence that the micro-organisms seen were mycoplasmas.

A "dirty" appearance in Ayre's films due to exudate, debris, cytolysis and clumps of bacteria is mentioned by Koss and Durfee<sup>3</sup> as characteristic of infection of the cervix or vagina; similarly, a "dirty" appearance in Ayre's films from cases of amoebiasis of the genital tract has been described<sup>4</sup>. Although bacteria such as lactobacilli and coliforms also appear haematoxyphilic in an Ayre's smear, and when numerous may also contribute

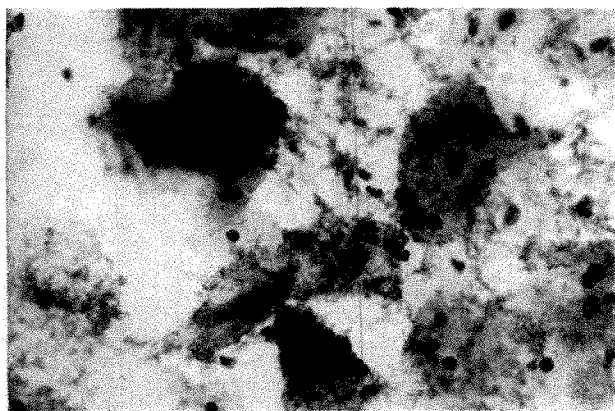


Fig. 1. Ayre's smear. Large numbers of the typically coccoid organisms seen in between and overlying the vaginal squames. Papanicolaou stain ( $\times 320$ ).

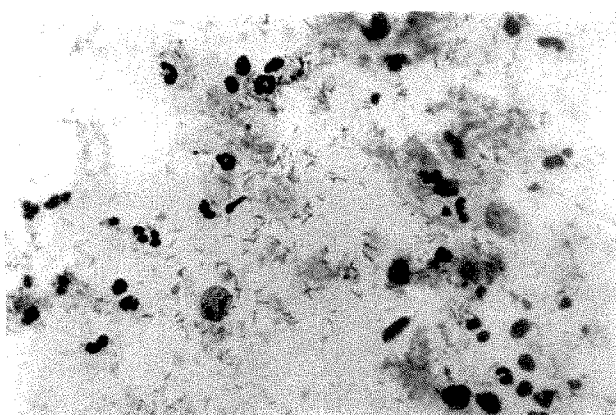


Fig. 2. Ayre's smear. Mingled with the vaginal squames and polymorphs are numerous lactobacilli. Papanicolaou stain ( $\times 400$ ).

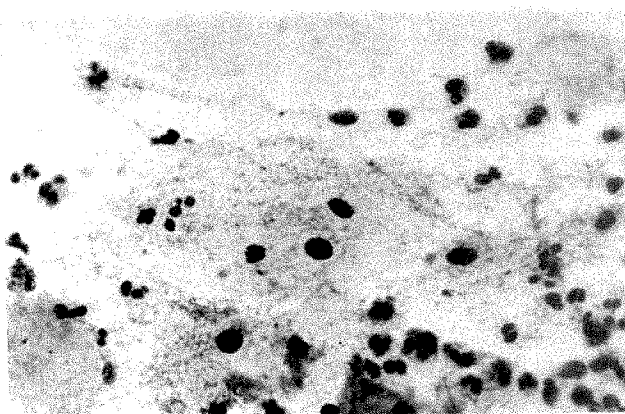


Fig. 3. Ayre's smear. Numerous small cocco-bacilli. Papanicolaou stain ( $\times 400$ ).

a "dirty" appearance, these can readily be distinguished (Fig. 2). Smaller organisms such as *Haemophilus vaginalis* were distinguished from the essentially coccoid *Mycoplasma* by their rod-like shape when examined under the 2 mm objective (Fig. 3).

The culture media used were unsuitable for isolating the *T*-strains of mycoplasma, which are also common inhabitants of the genital tract<sup>5</sup>. This may account for five slides which were classified as morphologically positive when there was no growth of mycoplasma.

Infection of tissue cultures by *Mycoplasma* affects the rate of cell growth and produces chromosome changes<sup>6</sup>. Chromosome abnormalities can be demonstrated in cells taken from dysplastic or early pre-invasive cervical epithelium<sup>7</sup>. Our results show that a heavy *Mycoplasma* infection of the genital tract can be diagnosed with fair accuracy from an Ayre's smear, and it would be possible to use this method in a retrospective study to ascertain whether the morphological presence of *Mycoplasma* in the films can be correlated with the nuclear changes associated with cervical dysplasia or *in situ* carcinoma.

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### Synergistic Fungistatic Effect of Tetrazolium-Saliva Mixtures

THE presence of an antifungal factor has been reported in normal human serum and in the conjunctival mucosa<sup>1-3</sup>. This work was initiated to determine whether saliva possesses an identical or similar substance. Stimulated whole saliva, parotid and sub-maxillary secretions were collected from ten apparently normal individuals and tested by placing aliquots in cylinders on Pagano-Levin agar, lawn seeded with *Candida albicans*, *Candida tropicalis* and *Saccharomyces cerevisiae*. No zones of inhibition were observed. Serum constituents (globulins) excreted in saliva are approximately one tenth of their concentration in serum and so it was decided to concentrate the salivary specimens to dryness by lyophilization. The solid constituents were then resuspended in sterile distilled water to give final concentrations 2.5-20 times greater than in saliva. Such concentrations manifested antifungal properties when tested as previously described. No inhibitory activity was discernible when Sabouraud's glucose agar, blood brain heart infusion agar or brain heart infusion agar were used, and so it seemed that the constituent in the Pagano-Levin medium which might be responsible for this activity was triphenyltetrazolium chloride, a redox indicator. The compound is reduced by *Candida tropicalis* to a red pigmented crystalline formazan whereas strains of *Candida albicans* usually fail to do so. At least five times the concentration of tetrazolium salt in Pagano-Levin medium is necessary for inhibition.

Lyophilized whole saliva or parotid or sub-maxillary secretions, concentrated twenty times, gave zones 25-30 mm in diameter, whereas specimens concentrated two and a half times produced zones of 12 mm diameter. Saliva from some individuals did not possess the antifungal factor and stringy mucinous salivas showed less activity than did non-viscid salivas. Individuals ill with upper respiratory infections showed little or no activity during the period of the acute symptoms of the disease.

The factor has been found to be filterable through glass fibre paper, 'Berkefeld', 'Selas' and 'Millipore' filters. Centrifuged specimens had fungistatic activity in the supernatant but not in the sediment. The factor is dialysable and can be concentrated by evaporation at 100° C. According to Louria and Brayton<sup>4</sup> sera of normal individuals contain a factor inhibitory to *Candida albicans* and *Candida stellatoidea* but not towards other *Candida* species, *Cryptococcus neoformans* or *Saccharomyces cerevisiae*. The factor these investigators have described was active after subjection to a temperature of 70° C for 1 h, dialysable, and had a molecular weight of approximately 15,000. Igel and Bolande<sup>5</sup> reported a serum factor which was non-dialysable and was inactivated at 70° C, but which was inhibitory towards the *Cryptococcus neoformans*. They also found a factor in saliva which inhibited *Cryptococcus neoformans*, but was distinct from the serum factor in that it was dialysable and the diffusate remained active after being subjected to 70° C for 2 h.

Our findings disagree with those of Louria and Brayton in that serum samples did not show inhibitory activity on our test medium, whereas saliva samples did. As suggested by the findings of Igel and Bolande, the factor in saliva is apparently distinct from that present in serum.

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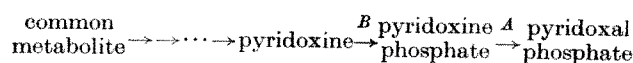
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### Phosphorylation of Pyridoxine by *Escherichia coli* B

THE recent demonstration of a pyridoxine phosphate oxidase in extracts of *Escherichia coli*<sup>1,2</sup> and the accumulation of pyridoxine phosphate by a mutant of this organism blocked in this step<sup>3</sup> suggests that this oxidation is the ultimate step (A) in the biosynthesis of pyridoxal phosphate. If this is true, the kinase step (B) may be expected to have greater specificity for pyridoxine than for pyridoxal.



This communication reports the testing of this hypothesis.

Cell-free extracts of *E. coli* were prepared by ultrasonic disruption of 1 g of lyophilized cells in 25 ml. of 0.15 molar potassium phosphate at pH 7.0. The broken cells were centrifuged for 30 min at 2° C at 40,000g and the supernatant fluid was dialysed overnight against 4 l. of distilled water at 5° C. Pyridoxal phosphate was assayed by the apotryptophanase method<sup>4</sup>. Pyridoxine phosphate was first converted to pyridoxal phosphate by the rabbit liver oxidase method of Wada and Snell<sup>5</sup> and then assayed. Pyridoxine phosphate was used to standardize this reaction. The optimum concentration of magnesium sulphate for the kinase with either substrate was 10<sup>-4</sup> molar. Protein was measured by the method of Lowry *et al.*<sup>6</sup>. Both substrates were chromatographically pure.

Table 1 shows that *E. coli* extracts can phosphorylate pyridoxine at much lower concentrations than they can phosphorylate pyridoxal. This finding and the oxidase findings are evidence in support of the foregoing biosynthetic sequence for pyridoxal phosphate.

Table 1. ACTIVITIES OF PYRIDOXINE AND PYRIDOXAL AS SUBSTRATES FOR PHOSPHORYLATION\*

mμmoles of substrate	Duration of kinase reaction (min)	Duration of oxidase reaction (min)	mμmoles product formed
(1) None added	60	30	None detected
5, pyridoxine	60	30	1.5
15, pyridoxine	60	30	5.5
25, pyridoxine	60	30	6.0
40, pyridoxine	60	30	6.5
50, pyridoxine	60	30	9.8
(2) None added	5	30	0.9
250, pyridoxine	5	30	9.0
250, pyridoxal	5	30	1.1
(3) None added	60	0	1.2
250, pyridoxal	60	0	1.2
2,500, pyridoxal	60	0	1.7

\* Kinase reaction mixture contained substrate, 250 mμmoles magnesium sulphate, 1,200 mμmoles of ATP, 2 mg of protein from cell-free *E. coli* preparation in 2.5 ml. of 0.15 molar potassium phosphate, pH 7.0. Reaction at 37° C and stopped by subjection to 100° C for 3 min.

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### Influence of Challenge Strain on Potency of Pertussis Vaccines in Mice

Eldering, Holwerda and Baker<sup>1</sup> have recently shown that, in mouse-protection tests with pertussis vaccines, the species-specific agglutinin (factor 1) is more important than the type-specific agglutinogens (factors 2 and 3). This confirms my own findings<sup>2</sup> and those of Andersen

Table 1. PASSIVE PROTECTION OF MICE, WITH FACTOR-1 ANTISERUM, AGAINST INTRACEREBRAL CHALLENGE WITH *Bordetella pertussis*\*

Challenge strain	Serum	No. of deaths/No. challenged				Total
		2	3	4	5	
353/Z	Normal	12/15	13/15	10/14	8/14	43/58
	Factor-1	1/15	1/14	4/15	2/14	8/58
W.18-323	Normal	12/15	12/15	15/15	10/14	49/59
	Factor-1	7/15	6/15	9/14	5/14	27/58

\* Data abstracted from previous publications<sup>4,5</sup>.

and Bentzon<sup>3</sup> with actively immunized mice, and emphasizes the limitation of the standard mouse-protection test in assessing the prophylactic potency of pertussis vaccines<sup>4</sup>. It is not, however, incompatible with the demonstration of type-specific immunity in mice by passive protection with mono-specific sera and subsequent challenge with selected strains of various different serological types<sup>4,5</sup>. It does not, moreover, disprove the importance of factors 2 and 3 in human prophylaxis against strains of *Bordetella pertussis* which occur naturally<sup>6-10</sup>.

Eldering *et al.*<sup>1</sup> were unable to explain one of their further observations—that vaccines, regardless of serotype, protected mice more readily against challenge with strain 353/Z (which possesses only factor 1) than against strain W.18-323 (which possesses factors 1, 2 and 3), although the two strains are of approximately equal mouse-virulence. They suggested that the matter might be explored further by passive protection experiments. Such experiments have already been performed in our laboratories<sup>4,5</sup>: antisera to factors 2 and 3 did not protect mice against either of these challenge-strains; antiserum to factor 1 protected against both strains, but it protected against 353/Z significantly better than against W.18-323. The relevant data, abstracted from experiments 2, 3, 4 and 5 in the two quoted sources<sup>4,5</sup>, are given in Table 1.

It seems unlikely that these findings are dependent on serological differences between the two challenge strains. Rather they may be explained by differences in growth rate of the two strains: on artificial culture media, 353/Z grows more slowly than W.18-323, and this may apply also to growth in mouse brain. Unpublished observations of mine support this hypothesis: out of 204 normal mice which received an intracerebral injection of 353/Z, in a dose sufficient to kill about 70 per cent of the mice within three weeks, the average time of death was 11.3 days after injection, but with W.18-323 it was only 9.6 days. Moreover, in experiments to determine the virulence of a single challenge strain, the average time of death was progressively reduced as the dose of injected organisms increased (Table 2). Presumably, reduction in the size of the inoculum injected *in vivo* has the effect of delaying growth of the bacteria just as it does in artificial culture *in vitro*.

Table 2. INFLUENCE OF DOSE OF INJECTED BACTERIA ON TIME OF DEATH OF NORMAL MICE\*

Dose of bacteria (No. of organisms of strain W. 18-323)	80	400	2,000	10,000
Average time of death† (days after injection)	11.0	10.4	9.8	8.9

\* Total of thirty-four mice injected with each dose of bacterial suspension.

† Excluding mice which survived for 21 days.

It was noteworthy, however, that even with a slow-growing strain or with a small inoculum very few mice died after the fourteenth day—suggesting that by then they had developed their own active immunity which enabled them to eliminate any surviving bacteria. The mortality in mice which received eighty organisms of strain W.18-323 (Table 2) was only 10 per cent, and a further reduction in the size of the inoculum would result in complete survival rather than increasing the average time of death (for those which died) beyond about 11 days. This is consistent with the following results obtained in mice which were protected with factor-1 serum before intracerebral challenge, if one assumes that the antiserum would be acting virtually by reducing the size of the inoculum. In the same series of experiments as those mentioned in the previous paragraph, a total of 204 mice

were injected intraperitoneally with factor-1 serum and subsequently challenged intracerebrally with the same dose of strain 353/Z as that given to normal mice; the average time of death remained at about 11 days (actually 10.8 days), as it was with normal mice, but the mortality was reduced by about 80 per cent. For a similar challenge of immunized mice with strain W.18-323, the average time of death increased from 9.6 days (for normal mice) to 10.9 days for these immunized mice, but the mortality was reduced by only about 50 per cent. With the slow-growing strain (353/Z) death takes place in unprotected mice as late as it is possible for it to occur; if the size of the challenge dose is reduced by previous immunization then the mice will tend to survive; immunization against this challenge strain will be very effective. With the fast growing strain (W.18-323), however, immunization may merely delay death, and a smaller proportion of mice will be completely protected—immunization against this challenge strain will be much less effective in terms of eventual survival.

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### Experimental Bone Tumours caused by Common Viruses

ATYPICAL osseous and articular lesions have been observed in children in the course of measles and viral neuroinfections<sup>1,2</sup>. Although no viruses were isolated from the bones of the patients, the observations prompted the following investigation of the influence of viruses on bone tissue. In this communication we present the results of experiments with the ornithosis virus O.A.P. strain Y.S. 224, Coxsackie A<sub>1</sub>, Coxsackie B<sub>1</sub>, B<sub>2</sub> and B<sub>4</sub> viruses. The method used has been described before<sup>3</sup>. The experimental suspensions were free from bacterial contamination, as checked by inoculating various culture media. White mice of the 'Porton' race weighing 15 g each were used in the experiments. The suspensions were injected into bone marrow or joints. The Coxsackie A and B viruses were used in 25 per cent carcass suspensions, and the ornithosis virus in 15 per cent brain suspensions. The numbers of animals inoculated, the dosage of the suspensions and the frequency of lesions in the bones are shown in Table 1. Mice were anaesthetized in groups at intervals of 5 days, and inoculated parts were removed for histological examination. (The material was decalcified with 7 per cent nitric acid, fixed in paraffin, and stained with haematoxylin and eosin). In two mice, 25 and 35 days after inoculation, rapidly growing tumours were observed on the temporo-frontal aspect of the head in one case, and on the front limb in the other. The longest period of observation was 50 days.

The histological changes in the bones of the mice which had been inoculated with viruses were situated mainly in the metaphyses (Coxsackie B<sub>1</sub>, B<sub>2</sub>, B<sub>4</sub> and ornithosis),

Table 1				
Strain	No. of mice inoculated	Doses of suspension (ml.)	Virus titre	Frequency of osseous lesions
Ornithosis	50	0.03	<i>L.D.</i> <sub>50</sub> 10 <sup>-2</sup>	33 (66%)
Coxsackie A <sub>1</sub>	50	0.03	10 <sup>7.2</sup> in 1 ml.	34 (68%)
Coxsackie B <sub>1</sub>	30	0.03	10 <sup>6.2</sup> in 1 ml.	15 (50%)
Coxsackie B <sub>2</sub>	30	0.03	10 <sup>6.2</sup> in 1 ml.	17 (56.6%)
Coxsackie B <sub>4</sub>	30	0.03	10 <sup>6.15</sup> in 1 ml.	12 (40%)
Control suspension	80	0.03	—	0



epiphyses and metaphyses (Coxsackie  $A_9$ ), and rarely in the diaphyses (Coxsackie  $A_9$  and ornithosis). After infection with the Coxsackie  $A_9$  virus, the changes were multilocal in most of the mice, representing the same developmental stage.

In the initial stage of the disease, viruses produced haemorrhages and focal oedema in the bone marrow. The haemorrhagic changes were followed by focal necrosis of bone marrow cells and bone necrosis. The periosteal cells or endothelial cells lining the Haversian canals showed signs of proliferation and produced large numbers of undifferentiated mesenchymal cells with hyperchromatic nuclei. These cells were able to produce osteoid, which was arranged in strands in the bone marrow cavity, at the sites of destruction of bone and elsewhere (Figs. 1-2). The osteoidal tissue calcified, producing chaotically arranged osseous trabeculae, which sometimes resembled wickerwork. These pathological bone trabeculae sometimes invaded the muscles surrounding bone (Fig. 3). In these bone tumours large groups of red blood cells were observed, often not surrounded by vascular endothelium; sometimes newly produced small vessels were present. These capillaries were situated at right angles to the corticalis. The changes were accompanied by thickening of the external periosteum, not infrequently at some distance from the tumour. The muscles near the tumour

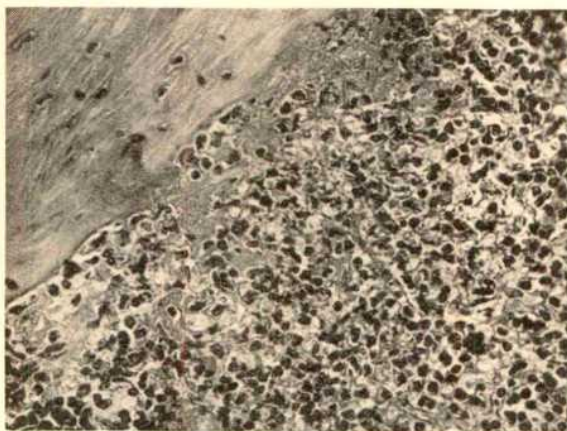


Fig. 1. Coxsackie  $A_9$ . Section from the diaphysis of the femoral bone and bone marrow cavity. Mesenchymal cells arising from the internal periosteum, producing osteoid, which calcifies and assumes the structure of chaotically arranged bony trabeculae in the marrow cavity. (Haematoxylin and eosin,  $\times 210$ .)

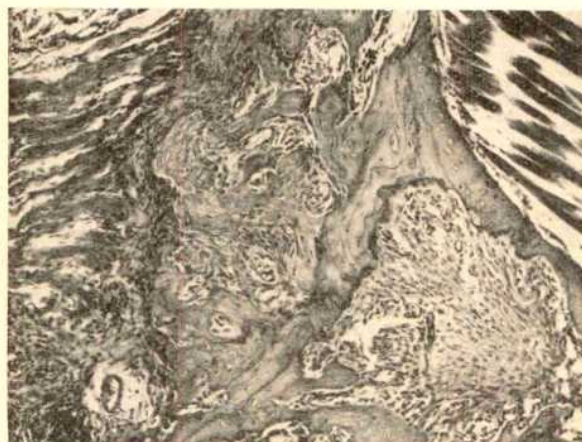


Fig. 2. Coxsackie  $A_9$ . Section from the temporal bone showing bone tumour invading the temporal muscle. The diploe of the primitive bone and intertrabecular spaces is filled with connective tissue and fusiform cells. Small blood vessels can be seen. (Haematoxylin and eosin,  $\times 90$ .)



Fig. 3. Ornithosis. Section from the diaphysis of the tibia. In the region of the destroyed compact bone of the diaphysis, chaotically arranged and freshly formed bony trabeculae invade the muscle. Primitive round osteoblasts are scattered among the trabeculae. (Haematoxylin and eosin,  $\times 90$ .)



Fig. 4. Ornithosis. Section of muscle neighbouring with the bone tumour, showing lumpy degeneration of the muscle, and proliferation of connective tissue cells with hyperchromatic nuclei. (Haematoxylin and eosin,  $\times 300$ .)

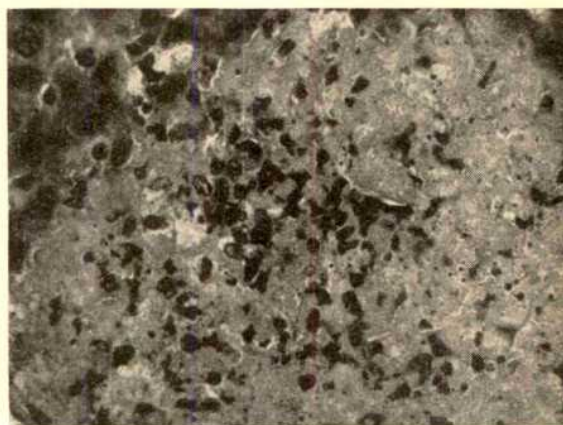


Fig. 5. Coxsackie  $A_9$ . Section of the liver. Cells surrounding and penetrating into the focus of hepatic necrosis have bizarre, hyperchromatic nuclei. (Haematoxylin and eosin,  $\times 300$ .)

showed a lumpy breakdown of muscle fibres and proliferation of the cells of the connective tissue (Fig. 4). Similar cells were observed in necrotic areas in the livers of mice inoculated with Coxsackie  $A_9$  viruses. The nuclei of these cells had bizarre shapes (Fig. 5).



The cavities of the knee joints in animals with lesions of epiphyses showed necrosis of epiphyseal cartilage, oedema of the ligaments, thickening of the synovial membrane, and sometimes filling of the articular cavity with loose, richly vascularized connective tissue. The lymph nodes of the extremities showed hyperplasia, cellular dissociation and focal necrosis, giving rise to the appearance of plaques in tissue cultures. No differences were found between the lesions in mice inoculated into the bone marrow and into the joints.

On the basis of the results obtained in these conditions, it can be concluded that the Coxsackie B<sub>1</sub>, B<sub>3</sub>, B<sub>4</sub> and A<sub>1</sub> and ornithosis O.A.P. strain Y.S.224 viruses injected into the bone marrow or joints in mice induce osseous tumours which originate in the connective tissue. We anticipate that further experiments will allow their classification and valuation of their malignity.

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## Use of Kieselguhr to increase Cell Production for Animal Virus Investigations

ONE of the difficulties involved in elucidating the structure of an animal virus is to produce sufficient virus for investigation. Wheatley<sup>1</sup> has shown that the yield of cells given by mice inoculated with Ehrlich ascites tumour could be increased by previous injection of a suspension of kieselguhr. Krebs ascites tumour cells, propagated in mice, can later be used for growing virus *in vitro*<sup>2</sup>. We show that kieselguhr can also be used to increase the yield of these cells, and furthermore, the latter resemble those produced in the conventional way in their response to virus infection. A simple method is thus available to provide larger quantities of cells for later virus investigations.

Krebs II ascites tumour cells, obtained originally from Dr. D. C. Roberts, were maintained by weekly intraperitoneal injection of  $1 \times 10^7$  cells in 0.1 ml. of phosphate buffered saline (PBS) into genetically heterogeneous white mice. Either 0.2 per cent suspension of kieselguhr in PBS, or of PBS alone (0.1 ml.), was injected at the same time as the ascites cells. After 7 days the cells were withdrawn from both kieselguhr treated and control mice, washed to remove red blood cells by centrifugation and the cells counted; the percentage of cells permeable to stain was estimated with 0.2 per cent nigrosin in PBS.

Kieselguhr treated mice produced about 1.5 times as many cells as controls (Table 1). This figure, which supports the work of Wheatley<sup>1</sup>, would be slightly higher if uncounted cells only were considered. Furthermore, ascitic fluids from kieselguhr treated mice contained fewer red blood cells and the ascites cells thus required fewer washes to prepare for use.

Table 1. EFFECT OF KIESELGUHR ON PRODUCTION OF KREBS ASCITES TUMOUR CELLS

Treatment	Cells/mouse $\times 10^{-3}$	Percentage stained	Ratio of kieselguhr to control cells	No. of mice
Kieselguhr	6.1	9.9	1.6	7
Control	3.8	16.0		7
Kieselguhr	4.9	8.9	1.3	27
Control	3.8	6.4		27
Kieselguhr	7.3	9.6	1.4	37
Control	5.1	13.8		37
Kieselguhr	7.2	10.6	1.7	37
Control	4.3	10.9		37

Both kinds of cells were tested for their ability to support virus multiplication by infecting them with encephalomyocarditis (EMC) virus<sup>2</sup>. Virus yields, estimated both by haemagglutination and by plaque assay using Krebs ascites cells (from mice not given kieselguhr) in agar suspension<sup>2</sup>, were the same, within the limits of experimental error, whether the ascites cells came from kieselguhr or from untreated mice (Table 2). Mice injected with kieselguhr gave on average 1.5 times as many cells (Table 1) and so there would be a 1.5-fold increase in virus output from the same number of mice.

Table 2. COMPARISON OF VIRUS YIELDS IN CELLS FROM KIESELGUHR TREATED AND CONTROL MICE

Treatment	Plaque forming units/ ml. $\times 10^{-4}$	Haemagglutination units/ml. $\times 10^{-4}$
Kieselguhr	1.1	8
Control	0.9	8
Kieselguhr	2.3	16
Control	1.6	16
Kieselguhr	2.8	32
Control	3.4	32

The sensitivity of ascites cells from kieselguhr treated mice when used for plaque assay of EMC virus was compared with control mice using two virus preparations. The number of plaque forming units detectable was the same, within the limits of experimental error, when either type of cell was used (Table 3).

Table 3. COMPARISON OF ASCITES CELLS FROM KIESELGUHR TREATED MICE AND CONTROLS FOR PLAQUE TITRATION

Treatment	Plaque forming units/ ml. $\times 10^{-4}$	Large plaque Small plaque
Kieselguhr	4.6	1.00
Control	4.1	1.04
Kieselguhr	5.7	1.15
Control	6.0	1.10

The virus preparations used contained mutants differing in plaque size. The sensitivity of the two types of cells was the same for both large and small plaques (Table 3).

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## PHYSIOLOGY

### Ultrastructure of the Membrane of Synaptic Vesicles

INVESTIGATIONS by Whittaker and Sheridan<sup>1</sup> on permanganate fixed preparations of isolated synaptic vesicles have recently confirmed that the membrane of such organelles has the structure of a "unit membrane"<sup>2</sup>, but have also indicated that in this case the classical model should be corrected in order to take into account the results obtained on negative stained specimens which suggest a membrane structure consisting of two 40 Å layers with an intermittent hydrophilic space between them.

In the course of experiments initiated with the aim of investigating possible morphological differences between synaptic vesicles and the axonic vesicles which are found in non-synaptic regions of peripheral nerves, we observed that in tissue specimens fixed in glutaraldehyde followed

by osmic acid the membrane of both kinds of organelle displayed a globular structure, which appeared to be more complex than either that of the unit membrane or that proposed by Whittaker. The results of observations on synaptic vesicles of frog cerebral cortex are briefly reported in this communication.

The frogs were pithed at room temperature (20°–21° C), and as soon as the brain was uncovered chilled phosphate buffered 4 per cent glutaraldehyde was poured over its surface. Small pieces of cerebral cortex were quickly removed, fixed in more of the same fixative, and treated with osmic acid after 2–16 h<sup>3</sup>. 'Epon' was used for embedding<sup>4</sup>. The sections were stained with lead monoxide<sup>5</sup>.

In cross-sections (Fig. 1) the membrane of synaptic vesicles appears to consist fundamentally of a single layer of globules with a thin electron dense contour and a light core. The size and the shape of such globules appear to vary from small spheres 40–45 Å in diameter, which are the most frequent, to larger ovoids measuring up to about 60 Å × 90 Å. The globules are connected to each other directly or through an osmiophilic granule; occasionally smaller globules form thin (about 20 Å) electron lucid bridges between globule and globule, giving them the aspect of a string of beads.

In face-on view (Fig. 1) the membrane of synaptic vesicles shows rather regular geometric designs amidst regularly and irregularly arranged globular structures. They appear to be polygonal (probably hexagonal) or round areas of unstained material limited by thin os-

miophilic lines and containing in their centres an osmiophilic granule. The diameter of such structures is about 90–110 Å, the central granule measuring about 20–30 Å. In cases in which the section is favourably oriented, the whole surface of synaptic vesicles appears to consist of an orderly mesh of such geometric designs, similar to mosaic floor. Frequently, however, thin osmiophilic septa can be resolved in the electron lucid area of such polygons, which therefore seem actually to be constituted by a rosette of non-stainable globules surrounding the central osmiophilic granule. The number of such globules is usually six in each rosette and their diameters range from 35 to 40 Å. On the basis of the observations in face-on views of the vesicles, we suspect that the large globules observed in cross-sections of the membrane are actually be polygonal units seen in oblique projections.

In conclusion, the reported observations seem to suggest that the membrane of synaptic vesicles is mainly formed by polyhedral units (probably hexagonal prisms), each consisting of a central osmiophilic granule surrounded by six osmiophobic globules, the base of the prisms being at tangent to the surface of the vesicle.

The relation of this electron microscope appearance of the membrane to the real structure cannot, of course, be resolved merely on the basis of these observations. Apart from the problem of the effects of fixation and dehydration on the tissue, there is the important problem of the size of the contribution of the embedding medium to the images obtained from sectioned material at high magnifications. In any case, the possibility that the described structure of the membrane is not the result of an artefact is supported by recent reports which propose that some biological membranes have a globular structure, by other reports which describe a hexagonal pattern in some other membranes, and by published pictures which were interpreted as showing a unit membrane structure, but a compatible with our proposed interpretation of membrane structure. Thus, Sjöstrand described a globular structure in mitochondrial membranes and in some cytoplasmic membranes of tissues fixed in potassium permanganate<sup>6</sup> and subsequently confirmed this finding in frozen dried tissues<sup>7</sup>. Nilsson described a similar globular structure in cross-sections of the outer segment disk membranes of tadpole retina fixed in osmium tetroxide<sup>8</sup>. On the other hand, hexagonal structures with a central dense spot were observed by Robertson<sup>10</sup> in face-on views of positively stained synaptic disks of Mauthner cells of the goldfish (Robertson also reported seeing a "suggestion" of a similar pattern in synaptic vesicles of the same cells). Moreover, hexagonal structures were recently described by Benedetti and Emmelot<sup>11</sup> in negatively stained isolated plasma membranes of rat liver seen in face-on views. The diameter of such polygonal structures was 95 Å in the case of Robertson's material, and 90 Å in the case of the material of Benedetti and Emmelot. Also a picture of Murray<sup>12</sup> of a face-on view of a negatively stained preparation of isolated cell membrane of *Micrococcus sarcin* shows a similar hexagonal pattern. Furthermore, we think that the cross-sections of the membrane of positively stained synaptic vesicles in the pictures given by Robertson<sup>10</sup> and by Whittaker and Sheridan<sup>1</sup> may be interpreted as showing a polyhedral-globular structure rather than a unit membrane structure. Moreover, the polyhedral-globular model may provide an explanation for the apparent differences in membrane thickness among vesicles that can be observed in the pictures of negatively stained isolated vesicles published by Whittaker and Sheridan, although no explanation for this appearance was suggested by these authors: the membrane shows a diameter of 45–50 Å when actually seen in profile, while showing a diameter of about 90 Å in face-on view. In addition, the apparent intermittent splitting of the membrane by the phosphotungstate stain, which has been observed in such pictures where the membrane shows a diameter of about 90 Å or more, could possibly be explained

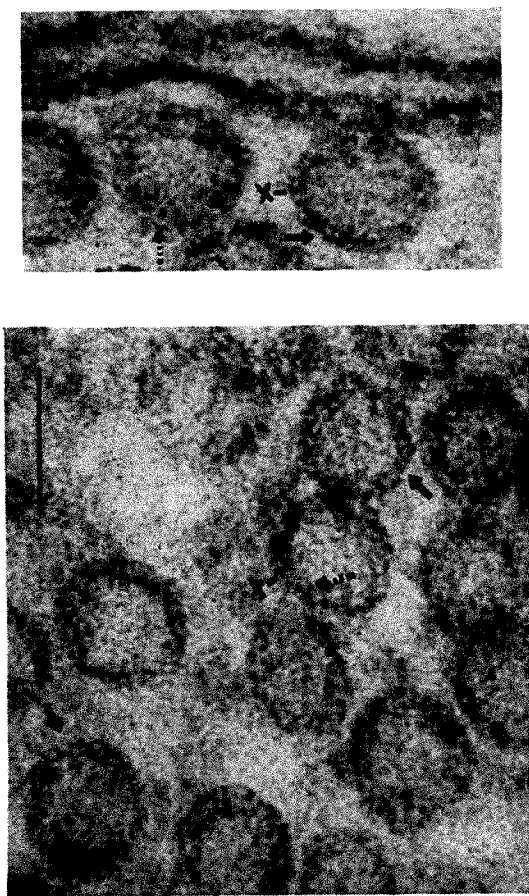


Fig. 1. Electron micrograph of frog brain cortex. The synaptic vesicles are bound by a membrane which in cross-section appears to consist of a chain of electron lucid globules (arrows). Sometimes an osmiophilic granule is interposed between two adjacent globules ("X" marks). Often, osmiophilic granules constitute the centre of clear areas limited by an osmiophilic contour (interrupted arrows). Such designs are interpreted as features of face-on views of the membrane. A similar structure is also visible in the axolemma (in the square); in this case thin septa can be seen reaching from the central granule to the osmiophilic contour and dividing the clear area into five or more globules. (Scale line = 500 Å.)

plained on the basis of an accumulation of stain in the central osmiophilic area of the hexagonal prisms seen in face-on view (rather than as due to penetration of stain into the middle layer of a unit membrane seen in cross-section, as suggested by Whittaker and Sheridan).

Osmiophobic globules in the described polyhedral-globular structure probably require a micellar arrangement of the membrane lipid. It is noteworthy that such an arrangement, as well as the formation of hexagonal patterns, has been shown to occur in reconstituted phospholipid-water systems by X-ray diffraction and electron microscope techniques<sup>13-15</sup>.

Polyhedral-globular units can also be observed in the membranes of mitochondria, as well as in other membranes, including the axolemma (Fig. 1), of our preparations of frog brain.

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## Colour Vision in the Virginia Opossum

COLOUR vision in mammals has long been considered to be restricted to primates, possibly as a result of independent evolution of the ability after it was lost during a nocturnal stage of mammalian evolution<sup>1</sup>. A variety of investigations, however, have indicated some ability to discriminate on the basis of colour in the European red squirrel<sup>2</sup>, the pigmy goat, red deer, and Nilgai antelope<sup>3</sup>, the Masai giraffe<sup>4</sup>, the common cat<sup>5</sup>, and the antelope ground squirrel<sup>6</sup>. Because the Virginia opossum (*Didelphis virginiana*) is very similar to the earliest known mammals<sup>7</sup>, the presence or absence of colour vision in this species would be of particular relevance to theories of the phylogeny of colour vision in both marsupial and placental mammals. The marsupial eye is essentially like that of a placental mammal in structure, while the retina is basically a reptilian one containing single and double cones bearing oil-droplets and a large number of filament rods. In particular, the opossum has a clearly nocturnal eye and some of the single cones lack oil-droplets and are similar to the cones of placental mammals<sup>8</sup>. A further consideration is that the marsupials are the only class of vertebrates having double cones in the retina (fish, amphibians, reptiles, and birds being the others) which have not yet been shown to have colour vision<sup>9</sup>.

The subjects were two adult female littermates from a locally caught mother, raised in the laboratory and quite tame. The taming procedure has been described elsewhere<sup>10</sup>. The apparatus is essentially the same as used previously to test position reversal learning in the opossum<sup>11</sup>. The start box opened into a choice chamber 60 cm long at the end of which were the two stimulus

panels. Each panel contained a circle of translucent plastic (3.8 cm in diameter) which was illuminated from the reverse side. The room was in darkness during the trials. The required response was to touch the nose to the stimulus; if the correct stimulus was selected, the panel was released manually and pivoted upward making available a pellet of 'Hunt Club' dog food in a cup in the floor (food was present behind each stimulus on all trials). After the pellet was taken, the panel was returned and both stimuli left in position as the subject finished eating. A heavy masking panel was then lowered, covering both stimuli. After an incorrect choice, the masking panel was lowered immediately. Lowering this panel also turned off the stimulus lights and turned on dim box and room lights. After the first few days, the subjects spontaneously returned to the start box on most trials.

Table 1. ORDER OF PRESENTATION (O.P.) AND DAYS TO CRITERION (DAYS) FOR EACH PROBLEM FOR EACH SUBJECT

Problem	Subjects		Subjects	
	First opossum O.P.	Days	Second opossum O.P.	Days
R-B	1	9	1	5
R-G	2	5	4	2
R-Y	3	6	6	2
Y-B	4	3	2	7
Y-G	5	15	5	5
G-B	6	10	3	65†
Grey*	7	35	7	18

\* Grey was paired with each of the four colours on each day.

† The subject was sick and eating erratically during most of this period.

R, Red; B, blue; G, green; Y, yellow.

The stimuli were produced by Kodak 'Wratten' filters (red No. 25, yellow No. 15, green No. 58, and blue No. 47), which are relatively broad and roughly divide the visual spectrum into four segments. They were equated for brightness for the human eye by adding neutral density filters to adjust the red, yellow, and green filters to the equivalent brightness of the blue. Neutral filters (1.0, 0.7, 0.3, and 0.0 log units in density) were paired with the colour filters on all trials in a variable fashion in all combinations such that the stimuli on a given trial could be equally bright or dim or differ in intensity by as much as 10:1. The side of the correct stimulus was determined by Gellerman series<sup>12</sup> which were changed at every session. The lighting system for each side consisted of two 'G.E.' No. 656 bulbs of 6 W and side by side 5 cm from the stimulus panel. The filters were placed in front of the light with the colour filter closest to the panel and no lens or reflecting system was used. The resulting stimulus patch was homogeneous and approximately 5.5 foot lamberts in brightness (based on 'Macbeth Illuminometer' measurements). The translucent plastic stimulus circles also prevented the subjects from being able to smell the colour filters directly. Each daily session consisted of twenty trials using a modified correction procedure: after a correct response, the colour and neutral density filters were placed according to the day's schedule; but after an error, the correction trial consisted of the same filter arrangement and this was repeated until a correct response was given. Thus eventually a correct response was given to each stimulus pair during a daily session and twenty reinforcements were thereby obtained. Only the first response to a stimulus pair was counted in the scoring since, after an error, the subject needs only to change sides to be correct. Each daily session therefore consisted of twenty scheduled trials and a variable number of corrections. Thus responding to an inappropriate cue (for example, position or brightness) could lead to a perseveration of errors and selective extinction of that response.

Both opossums learned to discriminate each colour from each of the other colours to a criterion of thirty-five out of forty correct in two consecutive sessions of twenty trials each ( $P < 0.001$ ). Table 1 gives the order of presentation of each problem and the number of training days to criterion. The first discrimination trained was blue versus red and learning for both subjects was rapid, requiring 180 for the first and 100 scheduled trials for the second opossum.

Even with the 10:1 variation in brightness provided by the neutral density filters, the possibility exists that the opossum is markedly deficient in sensitivity to the longer wavelengths. It is therefore particularly interesting that, after this training, transfer was readily shown by the second opossum when the problem was changed from blue versus red\* to blue versus yellow (eighteen out of twenty correct on the first day).

In this task, the relative wavelength of the positive and negative stimuli was kept constant in all problems for a given subject. For the first opossum the longer wavelength stimulus was always positive and vice versa for the second. With regard to specific problems, this meant that after red and yellow, the next problem scheduled was yellow and blue, and transfer was good with sixteen out of twenty correct choices on the first day. In previous testing with other subjects, an attempt was made not to change the value of a given stimulus in successive problems such that, after red and yellow, the next problem would have been blue and yellow, and this design led to markedly retarded learning. These findings suggest that the opossum discriminated between colours in a relative rather than an absolute fashion.

The final test given both subjects was to learn to discriminate between all colours and a grey stimulus. The grey stimulus, equated in brightness to the colour filters, was used with the neutral density filters in the usual fashion. Each daily session consisted of four blocks of five trials each with each block using a different colour. The order of colours used varied from day to day, and the coloured stimulus was always positive. The criterion here was sixty-eight out of eighty trials ( $P < 0.001$ ) involving twenty of each colour, with the additional restriction that no more than three errors are given to a particular colour. The initial performance of both subjects on this task was interesting: they consistently approached the stimulus that had been always positive in the preceding series (for example, red for the first opossum) and avoided the colour that had been negative. The yellow and green were responded to in an intermediate manner so that the grey stimulus seemingly was in the middle of the group of colours. Both subjects reached criterion on this problem, but it required considerable training (35 days for the first opossum, 18 days for the second). Apparently, learning of the preceding relative discrimination between colours did not aid the acquisition of the absolute discrimination of grey versus any colour. After this criterion was met the subjects were tested for an additional day using the four colours in random order against grey, and they made seventeen and eighteen correct choices respectively. Successful performance on this last task indicates that the ability of the opossum to discriminate colours cannot be explained by differential brightness of the various colours or by the stereo-colour mechanism suggested by Kohler<sup>13</sup> by which aberrations in the lens of the two eyes may result in different binocular depth cues for different colours.

A learning criterion of twenty-nine out of forty would be statistically significant at the 0.01 level, but if the opossum can gain some selective advantage from perceiving colours (which might account for the retention of this ability in a primarily nocturnal animal), then somewhat better performance should be obtainable. Errorless performance was not expected in view of the opossum's limited learning ability<sup>14</sup>, and the possibility that the least bright stimuli, particularly the red, may be nearly imperceptible to the opossum.

During the preliminary work in this series, a cat was also run as described above except that the reinforcement was ground horse meat. The animal was adapted to the laboratory and had been previously used successfully in an auditory discrimination experiment. During 10 days with twenty scheduled trials a day on a yellow-blue discrimina-

tion, the number of correct choices ranged from eight to twelve with no sign of any learning. The opossums, in contrast, usually made at least thirteen correct responses and only once had as few as eleven correct. This indicates that no striking artefacts were present and that the colour vision of the opossum is more readily employed than that of the cat.

The finding of colour vision in the opossum has some interesting implications: (a) the earliest placental mammals, which very closely resembled the opossum and were probably also nocturnal<sup>15</sup>, could similarly have retained colour vision derived from their reptilian ancestors; (b) the colour vision found in a wide range of placental mammals indicates a widespread capacity (most highly developed in the primate line); this supports the view that the colour vision of primates is a primitive mammalian characteristic; (c) because the opossum is closely related to the ancestor of the Australian marsupials<sup>16</sup>, colour vision may well be found in these animals also.

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## Parolfactory Vesicles as Photoreceptors in a Deep-sea Squid

THE recent finding<sup>1</sup> that the parolfactory vesicles of *Loligo* have the structural and biochemical properties of photoreceptors prompts this preliminary communication on the organization of these organs in a cranchid squid, *Liocranchia* sp. (see ref. 2).

*Liocranchia* is a small oceanic form (about 80 mm dorsal mantle length) which occurs at depths as great as 2,500 m. It has typical cranchid features as well as thirteen photophores round each eye. These were first described by Chun<sup>2</sup> and were also observed here: it is emphasized that their structure, which is typical of a light-producing organ, is distinct from that of the organs now described for the first time. This description is based on material collected by the *Discovery* expedition and kindly supplied by Dr. M. Clarke. Unfortunately, no material suitable for electron microscopy is available. The material was fixed with formol, stained by a Cajal silver method<sup>3</sup> and serially sectioned at 15 $\mu$ .

In the transverse plane the brain is seen to be organized in a manner typical of decapod molluscs (Fig. 1): the large, paired optic lobes lie close to the central brain, with which they are connected by the optic tracts. Associated with these tracts are several nervous and non-nervous structures<sup>4,5</sup>, the peduncle lobe, the olfactory lobe, the optic gland, the subpedunculate tissue and the parolfactory vesicles. The first four structures are similar

\* The first colour mentioned is always the one which the subject is trained to choose, that is, it represents the correct choice.



in their gross morphology to their counterparts in *Loligo*<sup>6</sup>, but the last are different, both in their organization and in their more regular arrangement.

The vesicles form a row on each side of the central brain and between it and the optic lobe. Each row lies posterior to the optic tract and extends in the dorso-ventral direction from the dorsal and lateral margin of the olfactory lobe down past the suboesophageal lobe to join, in the ventral midline, with the contralateral row. There are twenty-four vesicles in each row. If the oesophagus is taken as the centre of the brain it will be seen that those in the centre of the row are elongated along a radial axis (Fig. 1), and are about 120 $\mu$  long, while those at the dorsal and ventral extremes tend to a more circular cross-section or are elongate along a tangential axis, and may be as much as 160 $\mu$  broad. A group of vesicles from the central region is shown in Fig. 2: in each, three regions can be distinguished: A, the pigmented cup; B, the processes; and C, the cell area.

The cup comprises a thin layer (2-3 cells thick) of cells full of black granules after staining with silver. Pigment is present only in the radial and medial walls, so that a pigmented cup is formed, the occluded opening of which is directed peripherally. Laterally, the vesicles are covered by the thin membrane that encapsulates the brain in cephalopods<sup>7</sup>. Medially, the pigment cup is distinct from the cell layer of the adjacent brain. No nerve fibres have been seen associated with the vesicles.

The processes form a predominantly parallel array, the long axis of which is parallel to the long axis of the cup (Fig. 2), which itself lies on a radial axis of the brain

(Fig. 1). In the central region of the chain the cluster of processes in each vesicle is about 70 $\mu$  long and about 40 $\mu$  wide. Under the light microscope at the highest power the processes appear tubular and can be seen to bear cross-striations (Fig. 3) at right angles to the long axis. This configuration invites comparison with the parolfactory vesicle in *Loligo*<sup>1</sup> or the epistellar body in *Eledone*<sup>8</sup>, both of which show processes with cross-striations in the light microscope, and both of which reveal a rhabdomic organization in the electron microscope<sup>9</sup>.

The cell area (Fig. 2), which occupies the peripheral end of the cup, contains a compact mass of cells, the round nuclei of which are about 5 $\mu$  in diameter. Nuclei are absent in the region adjacent to the processes (Fig. 3) and it is inferred that the basal regions of the processes lie here. With the present method it was not possible to distinguish different cell types within the cell area, but the cells are so numerous compared with the processes that some of them are presumably "accessory".

Within each vesicle the long axes of the processes are parallel to the radial walls of the pigment cup. The vesicles are so oriented that their axes diverge peripherally, and each chain of vesicles is so disposed that light could be collected over an arc of almost 180° (Fig. 1). If the processes do represent "rhabdomes", then they must be activated by light that has already passed through the peripheral cell area.

The position of these organs and their structure as seen in the light-microscope leaves little doubt that they are highly developed parolfactory vesicles. It seems likely that they are photoreceptors, although further evidence is

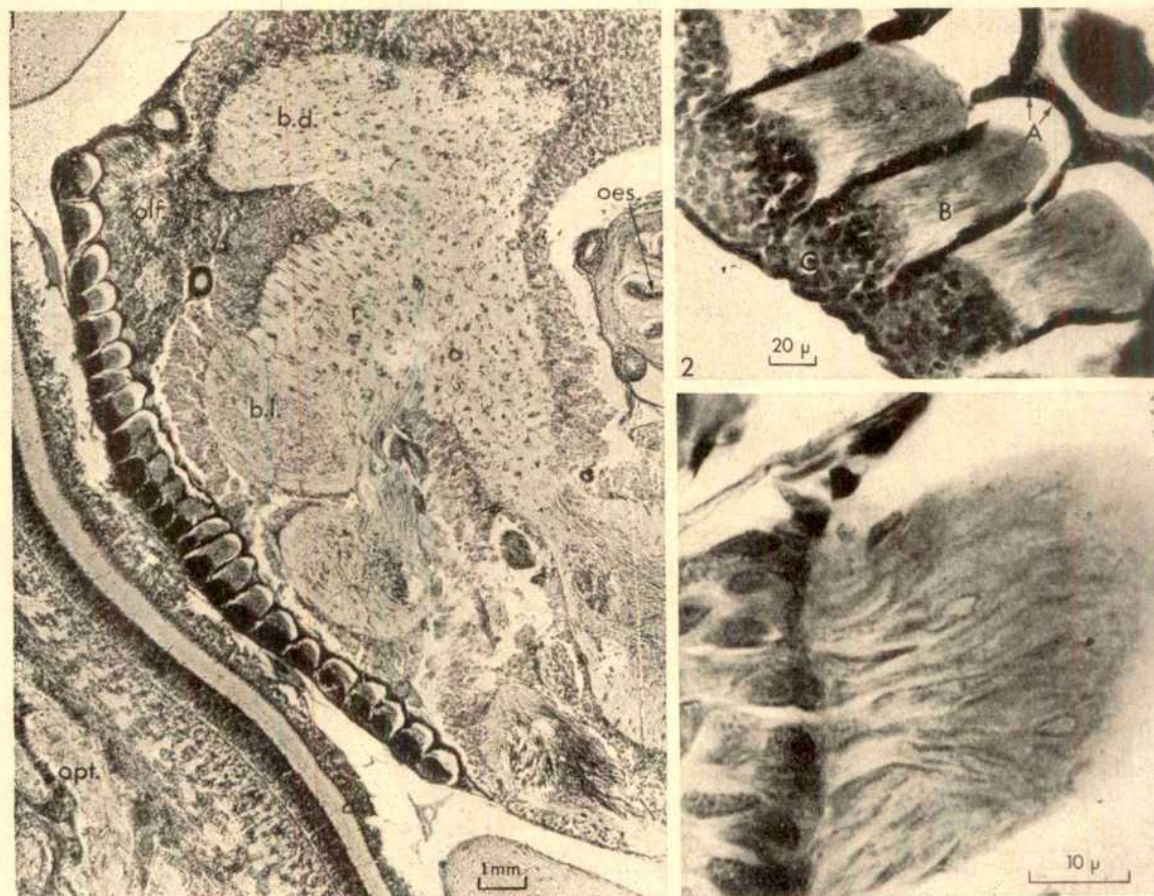


Fig. 1. The central brain and optic lobe of *Liocranchia*. Transverse section after Cajal silver stain, dorsal uppermost. b.d., dorsal basal lobe; b.l., lateral basal lobe; oes., oesophagus; olf., olfactory lobe; opt., optic lobe.

Fig. 2. Three vesicles. Cut and stained as for Fig. 1.

Fig. 3. The processes in one vesicle highly magnified. Note the cross-striations. Cut and stained as for Fig. 1.



required to be certain of this. Attention is drawn, however, to the elaborate nature of the vesicles in this genus when compared with *Sepia* or *Loligo*. First, they are more numerous and relatively larger than in *Loligo*. They are linked into a continuous chain, perhaps to maximize the collection of photons. Second, the processes are packed in an ordered array, instead of lying randomly as in *Loligo*<sup>9</sup>. Does such a parallel array serve to maximize the collection of light, when little light energy is available? Third, the vesicle here has a marked polarity. Fourth, pigment granules occur in the medial part of the vesicle wall.

Several questions present themselves. Are the parolfactory vesicles developed here in association with the low intensity of light in the habitat? Are they associated with the presence of photophores? Are they unique to the Cranchiidae? With fragmentary evidence it is possible only to speculate, but several other deep-sea and photogenic forms have been examined and only in one of them were the parolfactory vesicles especially well developed. This was *Bathotaurina* sp., also a cranchid and also photogenic. It is interesting that the parolfactory vesicles here are intermediate in form, in that they are larger than in *Loligo* and more asymmetrical, but they do not, like *Liocranchia*, possess a parallel array of processes. If well developed parolfactory vesicles are a feature of the Cranchiidae we have still to discover the peculiar feature of the organization of this group that demands this development.

The function of accessory photoreceptors situated deep within the body of an animal with well developed eyes has been considered by Nishioka *et al.*<sup>1</sup>. It is now clear that the parolfactory vesicles in cephalopods are not vestigial photoreceptors; nor can there be doubt that in this class of molluscs extra-ocular photoreception is widespread.

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### Release of Catecholamines in the Guinea-pig by Substances Involved in Anaphylaxis

MANY substances are released in anaphylaxis, including histamine<sup>1</sup>, bradykinin<sup>2,3</sup> and slow-reacting substance<sup>3-5</sup>. In some species, histamine<sup>6</sup>, bradykinin<sup>7-10</sup> and possibly slow-reacting substance<sup>11</sup> liberate catecholamines into the circulation, and so the anaphylactic reaction may also be expected to lead to catecholamine release, and some evidence suggests that it does<sup>5</sup>. This communication demonstrates directly that, in the guinea-pig, histamine, bradykinin, slow-reacting substance and anaphylaxis all liberate catecholamines into the circulation; their relative effects are estimated and their roles discussed.

We used a modification of the blood-bathed organ technique for detecting active substances in circulating blood<sup>12</sup>. Guinea-pigs of either sex weighing 500–1,050 g were anaesthetized with pentobarbitone sodium (60 mg/kg intraperitoneally). Cannulae were inserted into the trachea, into both jugular veins and into a carotid artery,

and heparin (3,500 U) was injected intravenously. The animal was then ventilated with air or oxygen by a miniature Starling respiration pump. Blood from the carotid artery was superfused at a constant rate between 2.1 and 2.5 ml/min over the assay tissues by a roller pump. The blood was returned to the animal through both jugular veins—to one through a second channel in the roller pump and to the other under gravity. The extracorporeal circuit contained 3–5 ml. of blood. To detect catecholamines, 2–5 cm lengths of rat stomach strip<sup>13</sup> and of chick rectum<sup>14</sup> were used. This combination of tissues is highly specific for catecholamines<sup>15</sup> and readily distinguishes between adrenaline and noradrenaline<sup>16</sup>. Isometric contractions of the muscles were transduced with Ether strain gauges, adjusted so that the tissues had an initial tension of approximately 3 g. Arterial blood pressure and intratracheal pressure were transduced with Statham gauges connected to side tubes on the carotid and tracheal cannulae; rectal temperature was transduced with a thermistor. All records were displayed on an eight channel Beckman Offner dynograph. Drugs were injected either intravenously into a jugular vein or through a fine nylon catheter passed down the left carotid artery to the arch of the aorta. The position of the catheter was checked *post mortem*.

To prepare the slow-reacting substance in anaphylaxis, the lungs were excised from guinea-pigs previously sensitized to ovalbumen, and perfused through the pulmonary artery for 60–75 min at 1.25–1.75 ml/min with antigen (4 mg/ml. of ovalbumen) in Tyrode containing cysteine (1.2 g/l.) and succinic acid (120 mg/l.). Freeze-dried slow-reacting substance was prepared from the perfusate, after removal of histamine and some other impurities. Slow-reacting substance prepared in this way had previously been shown to be substantially free from acetylcholine, angiotensin, bradykinin, histamine, 5-hydroxytryptamine, prostaglandin F<sub>2α</sub> and substance P, and to differ from the "slow-reacting substance in anaphylaxis" of Brocklehurst<sup>4</sup> in that they have a bronchoconstrictor effect in the guinea-pig<sup>17</sup>. Two control samples for the experiments with this slow-reacting substance were prepared in exactly the same way as the substance except that, in one, the lungs were taken from unsensitized guinea-pigs and, in the other, the perfusion fluid did not contain antigen.

Intravenous injection of histamine, bradykinin or slow-reacting substance liberated a burst of catecholamine into the circulation, as shown by relaxation of the blood-bathed organs. The catecholamine was mainly, if not all, adrenaline, because the extent of the relaxation of the rat stomach strip, relative to that of the chick rectum, could be exactly matched by intravenous injection or infusion of adrenaline. The amount of adrenaline liberated increased with increasing dose of histamine, bradykinin or slow-reacting substance. The following estimates were obtained of the dose of each agent required to give a relaxation of the assay tissues to the same degree as that produced by 0.1 µg of adrenaline: histamine, 4.4 µg (range 2–5 µg in fourteen experiments); bradykinin, 0.75 µg (range 0.1–2 µg in twelve experiments); slow-reacting substance, 1.5 mg (range 0.5–6 mg in fifteen experiments). After a single intravenous injection of bradykinin, histamine or slow-reacting substance, release of adrenaline sometimes lasted for 10–15 min. The two control samples were inactive at two to three times the dose at which slow-reacting substance liberated adrenaline (Fig. 1). After exclusion of the adrenal glands from the circulation, doses of histamine, bradykinin or slow-reacting substance that had previously been effective in the release of adrenaline were ineffective, indicating that the adrenal glands were the source of the catecholamines detected in the circulation.

Ovalbumen (0.05–0.5 mg given intravenously) liberated adrenaline in sensitized guinea-pigs (Fig. 2), whereas it was ineffective in animals that had not been sensitized to it. This release lasted for 15 min to > 60 min and cor-

responded in intensity to an intravenous infusion of  $0.02-1 \mu\text{g}/\text{min}$  of adrenaline. When the adrenal glands were excluded from the circulation, antigen no longer released adrenaline into the blood stream. To test whether desensitization, rather than exclusion of the adrenals, explained the failure of the second dose of antigen to liberate adrenaline, three sensitized guinea-pigs were prepared, but the adrenals were removed from the circulation before giving antigen. Although these animals were very susceptible to ovalbumen ( $0.02-0.2 \text{ mg}$  given intravenously), as shown by intense bronchoconstriction followed by death, no catecholamine release was detected. The adrenals were therefore also the source of the circulating adrenaline released by antigen.

When histamine, bradykinin, slow-reacting substance or antigen was given intravenously, a rise in intratracheal pressure (taken to indicate bronchoconstriction) preceded the relaxation of the blood-bathed organs and a return towards normal pressure accompanied the appearance of catecholamines in the extracorporeal circulation. When bradykinin or histamine was injected into the aorta, the

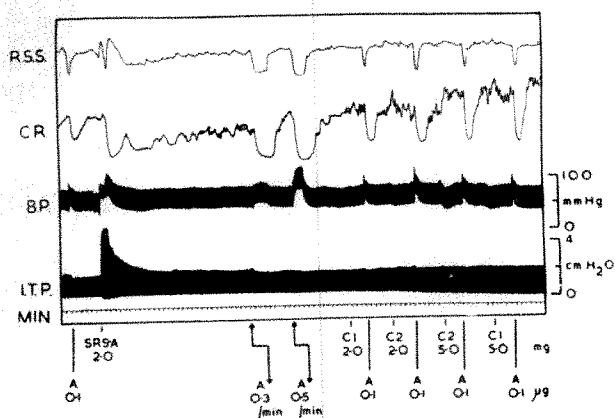


Fig. 1. Guinea-pig (1,030 g) anaesthetized with pentobarbitone (60 mg/kg intraperitoneally). The tracing shows the movements of a rat stomach strip (R.S.S.) and chick rectum (C.R.) superfused with carotid arterial blood from the guinea-pig. Carotid blood pressure (B.P.) in mm of mercury, intratracheal pressure (I.T.P.) in cm of water and time in min. All doses are intravenous; the system is calibrated with single doses or infusions of adrenaline (A). Slow-reacting substance (SRS-A) raised intratracheal pressure and liberated adrenaline, but the control samples for this substance (C1 and C2) did not, even when the dose was increased to 5 mg. Administered directly to the blood-bathed assay tissues as a control, slow-reacting substance had little effect on chick rectum, but caused rat stomach to contract.

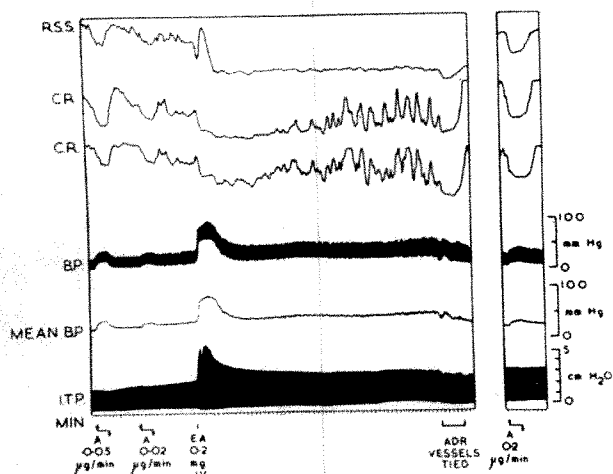


Fig. 2. Guinea-pig (540 g), previously sensitized to ovalbumen, anaesthetized with pentobarbitone (60 mg/kg intraperitoneally). Records are arranged and labelled as in Fig. 1. An intravenous dose of ovalbumen (EA, 0.2 mg intravenous) raised the intratracheal pressure and the blood pressure. There was also a prolonged release of adrenaline, equivalent to more than an intravenous infusion of  $0.2 \mu\text{g}/\text{min}$ . The release stopped when the adrenal glands were excluded from the circulation. Administered directly to the blood-bathed assay tissues as a control, ovalbumen had no effect.

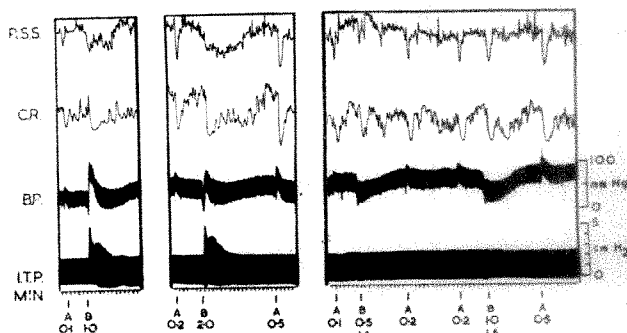


Fig. 3. Guinea-pig (825 g) prepared and records arranged and labelled as in Fig. 1, except that some doses are intra-arterial and all are in  $\mu\text{g}$ . Intravenous doses of bradykinin (B) raise intratracheal pressure and liberate adrenaline; intra-arterial doses of bradykinin liberate adrenaline without raising intratracheal pressure. Administered direct to the blood-bathed assay tissues as a control, bradykinin had no effect on chick rectum, but caused rat stomach to contract.

release of adrenaline could be observed without accompanying bronchoconstriction; but, with slow-reacting substance or antigen, release of adrenaline was always accompanied by bronchoconstriction. Fig. 3 illustrates these actions of bradykinin by both routes of injection.

Figs. 1, 2 and 3 show that liberation of adrenaline is often accompanied by a rise in blood pressure. In several experiments, the rise in blood pressure produced by histamine, bradykinin, slow-reacting substance or antigen was greater than would have been expected from the amount of adrenaline liberated, suggesting that there may also have been a sympathetic nerve stimulation. Indeed, both histamine<sup>18</sup> and bradykinin<sup>19</sup> have been shown to excite ganglia. If anaphylaxis also leads to sympathetic stimulation, the bronchoconstriction would thereby be reduced; some evidence for this is provided by the fact that propranolol is more effective than adrenalectomy in potentiating anaphylactic bronchoconstriction in the guinea-pig<sup>20</sup>.

The liberation of adrenaline by bradykinin, histamine or slow-reacting substance could arise from a direct or an indirect stimulation of the adrenal medulla. That hexamethonium (2-7 mg/kg given intravenously in two experiments) or pentolinium (5 mg/kg in three experiments) failed to block the release of adrenaline by bradykinin shows that, at least with bradykinin, the stimulation was not transmitted through a ganglionic pathway blocked by these drugs. Histamine, bradykinin and slow-reacting substance release adrenaline into the circulation of the guinea-pig, and these substances are liberated during anaphylaxis<sup>1-5</sup>, and so they could partly or wholly account for the release of catecholamine by antigen. Whatever the mechanism, there is no doubt that this release, coupled with any sympathetic stimulation that may also occur, will lessen the intensity of the bronchoconstriction. If histamine, bradykinin and (or) slow-reacting substance mediate the bronchoconstriction of human bronchial asthma, a partly compensating release of catecholamines may also be expected to occur. This expectation is strengthened by the finding that  $\beta$ -adren-ergic blocking agents intensify asthma<sup>21</sup>. Drugs given to mitigate asthma by antagonizing the bronchoconstrictor effect of an endogenous substance should therefore not, at the same time, block any liberation of catecholamine from the adrenal glands by that substance. The fact that antihistamine drugs block release of catecholamines by histamine<sup>6</sup> may help to explain their relative inefficacy in asthma.

Some or all of the endogenous substances shown here to release catecholamines into the circulation are believed to take part in local reactions to injury. The liberation of catecholamines by these substances may perhaps be regarded as a protective mechanism operating when the amount of mediator is excessive.

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### Depressor Effects with Sympathomimetic Amines after Blockade of Cardiovascular $\alpha$ -Receptors

THE reversal of the pressor response to adrenaline after ergotamine was first observed by Dale<sup>1</sup> in cats. Reversal of the pressor effects of noradrenaline has also been observed after ergotamine or 'Dibenamine'<sup>2</sup>. Nickerson and Nomaguchi<sup>3</sup> demonstrated that directly acting catecholamines and indirectly acting sympathomimetic amines such as hydroxyamphetamine or tyramine lowered the blood pressure of cats after blockade of  $\alpha$ -receptors.

The depressor effect of noradrenaline in cats after blockade of  $\alpha$ -receptors has been attributed to an action on cardiovascular  $\beta$ -receptors<sup>4</sup>. The present experiments have been made in pithed rats and chloralosed cats using a number of directly and indirectly acting sympathomimetic amines to study further the site, mode of action and chemical structure of the molecules necessary to produce lowering of the blood pressure after blockade of  $\alpha$ -receptors.

After treatment with phenoxybenzamine or 'Hydergine', depressor effects were obtained in rats with the catecholamines (–)-noradrenaline, (±)- $\alpha$ -methyl noradrenaline, (–)-adrenaline, dopamine and epinine but not with (–)-phenylephrine which lacks the ring hydroxyl in the 4 position. The depressor effect only appeared if the mean carotid arterial blood pressure was greater than 65 mm of mercury, and the necessary sustained increase in vascular tone was achieved by giving ergotamine before phenoxybenzamine. In agreement with Nickerson and Nomaguchi<sup>3</sup>, molecules with *N*-alkyl or  $\alpha$ -carbon substituents (adrenaline,  $\alpha$ -methyl noradrenaline) had greater depressor potency than those lacking them (noradrenaline, dopamine, epinine). L-Dopa with an acidic and basic terminal also had depressor activity, but catechol which lacks the aliphatic side-chain did not have depressor

potency. Presumably, attachment to receptors through the cationic head was necessary for depressor as well as pressor activity. The depressor effects of the compounds were abolished by an antagonist at  $\beta$ -receptors, propranolol, and pressor effects then reappeared.

After ergotamine and phenoxybenzamine, the indirectly acting amines  $\beta$ -phenylethylamine or tyramine also elicited depressor responses and these were abolished by propranolol. The depressor effects of these indirectly acting amines were prevented by pretreating the rats with reserpine or cocaine, which deplete<sup>5</sup> or render inaccessible<sup>6</sup> the noradrenaline stores in sympathetic post-ganglionic nerves in circumstances which enhance the depressor effects of the directly acting noradrenaline.

Karim<sup>4</sup> suggested that the depressor response to noradrenaline which developed in cats following blockade of  $\alpha$ -receptors was caused by activation of  $\beta$ -receptors in the splanchnic blood vessels. In six cats, however, the depressor responses to noradrenaline and dopamine persisted unchanged either after evisceration which removed the splanchnic blood vessels, or after evisceration and ligation of the abdominal aorta immediately below the diaphragm which excluded the systemic circulation to the lower limbs and the lower half of the trunk. In a further four eviscerated cats, the circulation was confined to the heart and lungs by ligation of the carotids, the subelavians, the internal mammary arteries and the root of the aorta between the left first intercostal and subclavian arteries. Depressor responses were still elicited by dopamine and by noradrenaline after phenoxybenzamine, and pressor effects restored by propranolol.

These investigations showed for the first time that the depressor responses to directly and indirectly acting sympathomimetic amines which appeared after blockade of  $\alpha$ -receptors were caused by an action on cardiovascular  $\beta$ -receptors. The responses were influenced by the chemical structure of the amines and by the degree of vascular tone. The depressor responses to indirectly acting sympathomimetic amines  $\beta$ -phenylethylamine and tyramine were apparently mediated *via* noradrenaline release in a similar way to their normal pressor effects. The depressor responses to the sympathomimetic amines were a result of action on either the heart or the pulmonary vessels, the former being more likely.

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### Suppression of the Early Inflammatory Response in the Sheep by Strophanthin G

It is well established in rats that the increased vascular permeability which follows the intrapleural injection of turpentine is due to the local release of histamine and is continued by the activation of other mechanisms<sup>1,2</sup>. Our experiments on sheep have shown a similar pattern of immediate histamine release and later continuation of the increased vascular permeability. Following the report by Judah, Ahmed and McLean<sup>3</sup> of the similarity of action between the antihistamine promethazine and the cardiac glycoside strophanthin G, strophanthin G was used as a possible suppressor of the early inflammatory changes in the sheep.

Romney Cheviot X lambs weighing about 20 kg were used: they were "blued" by intravenous injection of pontamine blue (25 mg/kg body weight of a 5 per cent

solution in isotonic saline). Turpentine was used as the irritant to produce inflammation: the intradermal injection of 0.1 ml. of turpentine into the clipped back of "blued" sheep was followed by marked leakage of dye at the injection site. The size of the blue area varied from 3.5–5 cm in diameter at the end of 1 h. In "blued" sheep pretreated with the antihistamine mepyramine maleate (25 mg/kg body weight in 5 per cent solution, injected intramuscularly), the leakage of dye did not develop until 1 h after the intradermal injection of turpentine. Pretreatment of sheep with mepyramine maleate also suppressed exudate formation after the intrapleural injection of 0.5 ml. of turpentine into the right pleural space. The antihistamine treatment, however, failed to give complete suppression of exudate formation for longer than 2 h.

Histamine depletion using compound 48/80 gave similar results. Sheep were depleted of their stores of histamine by repeated injections of compound 48/80 using the method of Spector and Willoughby<sup>1</sup>; a 0.1 per cent w/v solution of compound 48/80 in saline was given intraperitoneally, morning and evening, in ten doses beginning with an evening dose. The dose used was 0.6 mg/kg body weight for the first eight injections and 1.2 mg/kg for the two final injections. Skin tests were carried out 2 h after the last injection. In sheep depleted of histamine both the early inflammatory changes in the skin and exudate formation in pleural cavity were suppressed. Compound 48/80, however, like mepyramine maleate, failed to suppress the inflammatory changes for longer than 1 h in the skin and 2 h in the pleural cavity. Pretreatment of sheep with strophanthin G (100 µg/kg body weight intravenously) suppressed the leakage of dye for 1 h after the intradermal injection of 0.1 ml. of turpentine, after which marked blueing took place. Pretreatment with strophanthin G also suppressed the formation of pleural exudate for 2 h after the intrapleural injection of turpentine. These results are similar to those observed with mepyramine maleate in the sheep.

These experiments show that a biphasic response of increased vascular permeability develops in the sheep after injury with turpentine, the first stage being inhibited by antihistamines. The antihistamine compound mepyramine maleate is a specific antagonist for histamine, and has not been shown to antagonize any other compound which increases capillary permeability<sup>1,4-7</sup>. In the sheep, the suppression of the early inflammatory changes by pretreatment with mepyramine maleate indicates that it is the local release of histamine which initiates the increased vascular permeability. The experiments with compound 48/80 lend further support to this view. Suppression of the early inflammatory changes in the skin and pleura by strophanthin G suggests that strophanthin G may be blocking the effect of local histamine release. It is thought that anti-histamines act by blocking the effects of histamine at tissue receptor sites, thereby preventing the normal physiological reactions to this substance<sup>8,9</sup>. The protective effects of antihistamines in preventing liver cell necrosis have been reported<sup>10,12</sup> to be due, in part, to their interaction with the system in the cell membrane which regulates active transport of sodium and potassium<sup>11</sup>. Both the antihistamine promethazine and strophanthin G have been reported to protect the rat liver against thioacetamide induced necrosis<sup>3,10</sup>. More recently, it has been reported that promethazine and strophanthin G show approximately equal activity in inhibiting potassium transport<sup>3</sup>. In the sheep the suppression by strophanthin G of the early inflammatory changes induced by histamine could be due to inhibition of the sodium/potassium ion transport mechanism of the capillary wall and this could, in return, suggest that part of the action of the antihistamine mepyramine maleate against histamine may be due to its action on the ion transport mechanism of the cell membrane.

Our results support the view put forward by Judah, Ahmed and McLean<sup>11</sup> that the antihistamines owe their

effects to their interaction with that system in the cell membrane which regulates the active transport of sodium and potassium ions. The results suggest that strophanthin G may prove useful in the investigation of the inflammatory reaction, particularly histamine liberation and the mechanism of exudate formation, in the sheep.

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### Characteristic Pulse Wave caused by Organic Nitrates

NITROGLYCOL poisoning at dynamite factories has been a cause of concern for some time, but no specific and reliable diagnostic method for it has yet been established.

This communication reports a specific measurement of both nitroglycol and nitroglycerine poisoning. In a series of experiments, organic nitrates were given to human subjects and their effects on the pulse wave were examined by plethysmography. This method is suitable not only for detecting the degree of nitroglycol and nitroglycerine exposure before symptoms appear but also for measuring the degree of poisoning.

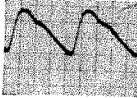

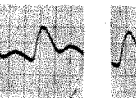
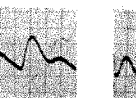
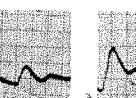
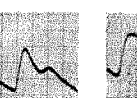

A variety of organic nitrates were administered sublingually as tablets, and nitroglycol was given as a 0.5 per cent aqueous solution, to healthy males aged 30–40 years. A reflexion photoelectric plethysmograph was used to record the condition of the blood stream in a finger. The recordings were made with the subjects lying on a bed. The most important index of toxicity is the contour of the pulse wave. This was characterized by a sharpening of the systolic curve of the pulse wave and by a depression of the diastolic notch, forming a distinct dent there. In addition to the pulse wave, the blood pressure and the electrocardiogram were measured at the same time.

The dose of the compounds administered varied from experiment to experiment, ranging from 0.5 to 8 mg for nitroglycol, 0.3 to 1.8 mg nitroglycerine, 10 to 20 mg for pentaerythritol tetranitrate, 4 to 6 mg for triethanolamine trinitrate diphosphate, 5 to 15 mg for isosorbide dinitrate, 10 to 20 mg for 2,2-dihydroxy-methyl-butanoltrinitrate, and 0.2 to 0.6 ml. for amylnitrite.

Before the experiments, all the subjects showed a similar pulse wave contour, designated as "normal" in Fig. 1. As the result of the administration of organic nitrates, the characteristic and similar altered pulse waves shown in the wave *BCD* of Fig. 1 were recorded irrespective of the variety of organic nitrate used. Fig. 1 shows these alterations of the pulse wave in a subject given 0, 0.9, 1.8 or 4.2 mg of nitroglycol sublingually; these contours of curve were classified as normal, slight, middle,



Table 1. PULSE WAVES, BLOOD PRESSURES, AND SUBJECTIVE SYMPTOMS AFTER NITROGLYCEROL ADMINISTRATION AND EFFECTS OF OXYGEN INHALATION

							
Time after initial administration of nitroglycerol (min)	0	2	5	7	11	16	30
Blood pressure (mm of mercury) (systolic/diastolic)	114/76		106/90	94/84	96/96	108/84	116/74
Symptoms	None	None	Slight headache	Slight headache	Chill, eye-lid spasm, dyspnoea	None	None

Administration of nitroglycerol: the initial dose was 0.5 mg, the second, third, and fourth doses were 0.5, 0.5, and 3.5 mg which were given 2, 3, and 7 min after the initial administration, respectively.

Oxygen was given 11 min after the initial administration of nitroglycerol.

and severe with reference to the pulse wave, pulse pressure and the symptoms shown.

The onset, duration and intensity can be assessed using this method by checking the change of pulse wave after administering organic nitrates. As shown in Fig. 1, wave B, a distinct change in the pulse wave was seen only 1 min after the sublingual administration of 0.9 mg nitroglycerol, while no subjective symptoms appeared. Table 1 shows the result of a typical experiment, in which a similar change occurred 2 min after administration of

0.5 mg nitroglycerol. In this experiment, three additional doses were given 2, 3 and 7 min after the initial dose. In addition to the typical changes seen in the pulse wave, a marked depression of systolic blood pressure occurred together with a moderate elevation of diastolic pressure. The fall in systolic blood pressure was so drastic that the pulse pressure dropped to zero 11 min after the first administration of the compound. Table 1 also shows the beneficial effect of inhaling oxygen: this gave a rapid restoration of blood pressure and eliminated the toxic symptoms.

To find whether organic nitrates can be absorbed through the skin, 1 g of dynamite was applied to the palm of one hand and changes in the pulse wave were checked on a finger tip of the other hand. There was no significant change in the pulse wave even after 30 min, but when the dynamite was applied and then covered by a surgical rubber glove the pulse wave began to show a characteristic change after about 5 min. The observation may indicate a possible absorption of vaporized nitroglycerol through the skin, although further experiments are needed if any unequivocal conclusion is to be reached.

The fact that the pulse wave is sensitive to organic nitrates was also confirmed during the examination of workers engaged in producing dynamite.

Various vasodilative and hypotensive agents such as 'Persantin', 'Segontin', 'Dyphyllin', benzylimidazoline, 'Hydergine', guanethidine sulphate, and hexamethonium chloride were examined in a similar way for comparison. These agents caused no changes in the pulse wave, but 50 ml. of whisky containing 40 per cent ethanol occasionally brought about a change which somewhat resembled that due to the organic nitrates.

In an additional experiment, the effect of asphyxia on the pulse wave was examined. As shown in Fig. 1, cessation of breathing for 1.5 min resulted in flattened pulse waves which were entirely different from those found with the organic nitrates.

This method might be considered too sensitive, but the contour of the pulse waves and the pulse pressure lead to a correct diagnosis of the condition of persons exposed to the toxic vapour which may be absorbed both by inhalation and through the skin. On the other hand, electrocardiograms seem to be of little use in this respect because they are not altered by administration of nitroglycerol and other organic nitrates.

Detailed reports on a field investigation at an explosive factory with reference to the use of protective gloves and the anti-anginal effect of organic nitrates will be published elsewhere.

I thank Mr. K. Muraki, Dr. Y. Ikoma, Mr. T. Tanaka and Professor M. Kuratsune for their help in this investigation.

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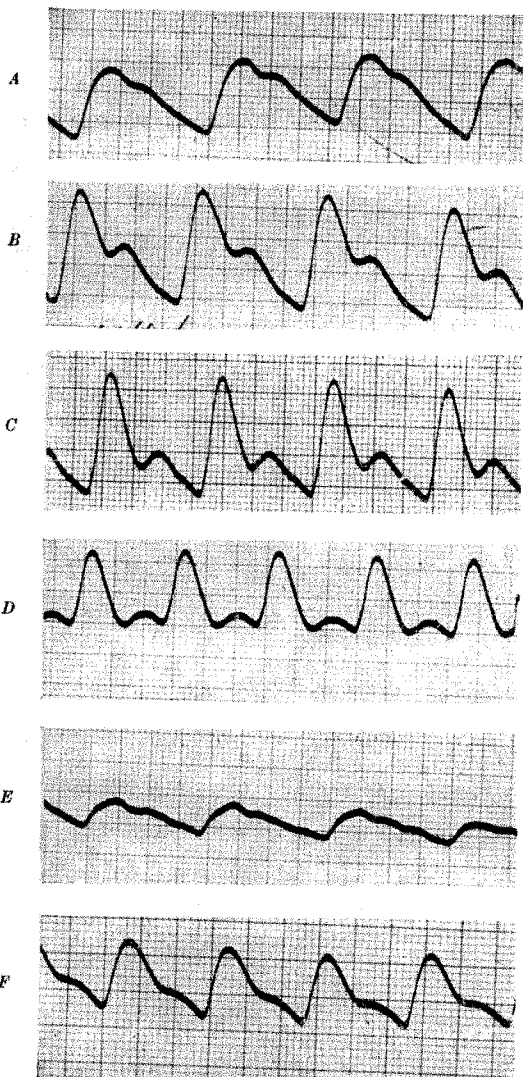


Fig. 1. Change of pulse wave by administration of nitroglycerol and other substances. A, Normal, before administration of nitroglycerol; B, slight poisoning, 1 min after administration of 0.9 mg of nitroglycerol; C, middle poisoning, 3 min after administration of 1.8 mg of nitroglycerol; D, severe poisoning, 3 min after administration of 4.2 mg of nitroglycerol; E, after 1.5 min of breath stopping; F, 13 min after administration of 50 ml. of whisky.

## RADIOBIOLOGY

## "Division Probability" and Radiation-induced Cell Death

THE "non-surviving" members of an irradiated sample of cells frequently vary considerably in their mitotic behaviour—some fail to divide, others divide once, and abortive clones of various sizes are not infrequently produced. This heterogeneous response has usually been considered evidence that lethal damage induced by radiation is of several types. Whitmore and Till<sup>1</sup>, however, have suggested that "these different responses may be manifestations of the same type, and indeed even the same degree of damage". They have proposed a unifying concept of cell death termed division probability, which, if correct, would drastically alter the current interpretations of dose-survival curves.

Briefly, Whitmore and Till postulate that, *a*, radiation has the effect of reducing the probability that a cell will give rise to two viable offspring, and that, *b*, this reduced probability of successful division,  $p_2$ , is approximately the same for all cells of an irradiated sample and is maintained relatively unchanged through successive divisions after irradiation. In consequence, they would regard it as a matter of chance whether growth is terminated or proceeds indefinitely. Furthermore, if their theory is correct, the same type of damage would lead to termination of growth both before any cell division had occurred and after a varying number of progeny had been produced. This concept also provides a plausible explanation of the fact that colonies produced by "surviving" cells vary in size and rate of growth—the fraction of cells actually undergoing division at any one time would vary from colony to colony due to the operation of chance in preceding generations. Whitmore and Till have considered some of the implications of the presumed fixed division probability and have demonstrated its application by calculating values of  $p_2$  from experimental data.

It would be unfortunate if such calculated values were brought into general use before it had been shown that the mitotic behaviour of irradiated cells actually conforms to that expected from the concept. The necessary test is best carried out through straightforward observation of repeated cell divisions in an organism that is amenable to cell-pedigree analysis. One such organism is yeast.

Superficially, the response of yeast cells to irradiation agrees with that expected from the concept of a constant division probability in at least three respects. *a*, "Non-surviving" cells produce an array of abortive clones which contain from one to several hundred cells. *b*, Surviving cells produce colonies which are extremely variable in size and rate of growth. *c*, Clones produced by surviving cells frequently contain a mixture of dividing and non-dividing cells. Detailed studies of these three characteristics of radiation damage have been made by various investigators, and the degree to which they support or reject the concept is considered later.

(*a*) The frequency distribution of abortive clones does not always conform with that expected of the concept. An observed distribution of abortive clones ranging in size from one to thirty-two may be compared in Fig. 1 with the distributions expected of various  $p_2$  values.

Experimental results were obtained in the following manner: diploid cells of *Saccharomyces cerevisiae* were exposed to 40,000 r, an exposure which yields about 50 per cent survival in terms of counts of visible colonies on agar plates. Individual interdivisional cells were then isolated by micro-manipulator to the surface of solid medium. The relative frequency of abortive clones and the number of cells which each contained were determined by periodic examination throughout several days. For accurate counts, it was sometimes necessary to separate the cells of a clone. Expected values were calculated by computer, making use of the fact that after  $D$  generations

the relative frequencies of abortive clones containing exactly  $D$  non-dividing cells is fixed, and is

$$L(D)p_2^{D-1}(1-p_2)^D$$

where

$$L(0)=0, L(1)=1, L(2)=1, L(3)=2$$

and

$$L(D)=2L(D-1)+\sum_{j=2}^{D-2} L(j)L(D-j) \text{ for } D \geq 3$$

(T. D. Newton, personal communication.)

The greatest conflict between observation and expectation lies in the fact that the expected modal number of cells at termination of cell division is 1 for any value of  $p_2$ , whereas the actual modal number is 2. The fit would be improved by assuming that the unit of death is 2, that is, that death usually occurs, not before completion of division, but in both products of a division. This assumption is, however, unsatisfactory because it has been shown that the modal number does shift to 1 at higher doses<sup>2,3</sup>.

(*b*) Comprehensive studies of the temporary and of the hereditary consequences of irradiation indicate that the sizes and the rates of growth of yeast clones produced from irradiated cells are influenced by at least three factors regardless of the possible existence of a certain probability of non-division. (i) The size which a clone attains in a specified time is strongly affected by the temporary mitotic delay imposed by radiations in early divisions, and the extent of this delay is known to vary widely within samples of uniformly irradiated cells<sup>4</sup>. For the irradiated samples mentioned in this communication, the interval between exposure and the second division was found to vary between 2 and 24 h in instances where no other damage was apparent. The normal generation time was 2.25 h. (ii) Mitotic stimulation induced by irradiation also occurs in many cells after the second cell division<sup>5</sup>. This further increases colony variability because the extent of the stimulation is variable within a sample of cells and is only imperfectly correlated with the length of inhibition beforehand. (iii) Many surviving cells contain mutations which affect the rate of cell division<sup>6,7</sup>. Recessive lethal or near-lethal mutations, which depress growth rate on the average by 8 per cent,

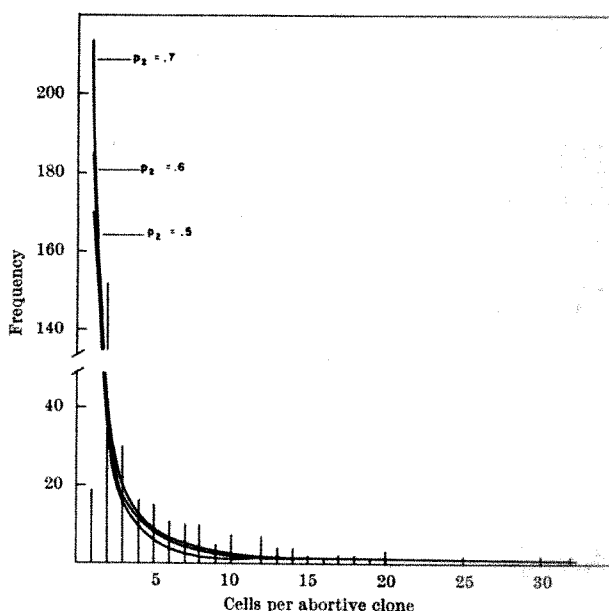


Fig. 1. The frequency distribution of 306 abortive clones of *S. cerevisiae* which contained fewer than thirty-three cells, and the distributions expected from the operation of division probability with various values of  $p_2$ .

are induced with a frequency of about 20 per cent by exposures which give 46 per cent survival. Other mutations which reduce growth rate in the heterozygote occur with a frequency which is at least three times greater.

The effects of delayed death within clones produced by surviving cells are no doubt superimposed on the influences already mentioned. But it is clear that variation in clone size or growth rate cannot be used either as evidence of the existence of division probability or to calculate its parameters.

(c) Better evidence of the operation of division probability in yeast comes from pedigree analyses of surviving cells. The fact that irradiated cells produce clones which contain both dividing and non-dividing cells has been recognized for many years<sup>8-10</sup>. More recently, the phenomenon has been studied in detail<sup>11,12</sup>. In such studies, pedigrees are obtained by systematically separating the progeny of individual irradiated cells by means of a micromanipulator. This procedure reveals both the source and the pattern of production of non-dividing cells in mixed clones. Two pedigrees, which might be regarded as typical, are shown in Fig. 2. Such pedigrees are characteristic of more than half the survivors after exposures which give 50 per cent survival. Non-dividing cells appear throughout several generations as would be predicted by the theory, and the data emphasize a possible part played by chance in determining whether or not radiation damage is phenotypically expressed. Further, there is no doubt that radiation can produce a type of lethal damage in yeast which is not revealed by conventional plating procedures.

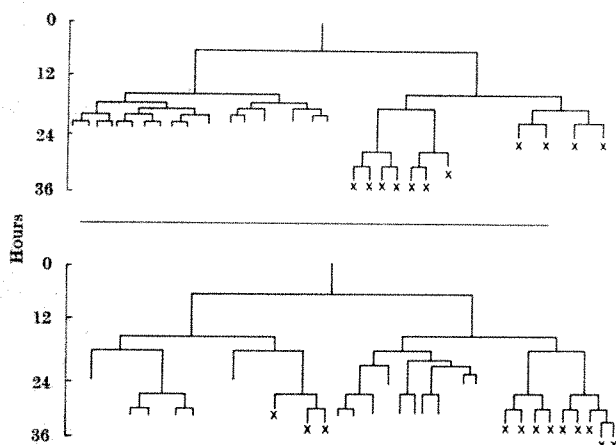


Fig. 2. Pedigrees of two X-irradiated cells of *Saccharomyces cerevisiae*. Each vertical line represents a separated cell, that on the left being the mother cell in any division. An 'X' denotes that no further division occurred. Otherwise, reproduction continued with eventual production of a visible colony.

Nevertheless, the pattern that has emerged with accumulating data is not that expected of the concept. *a*, Non-dividing cells usually appear in clusters rather than as isolated cells. *b*, The size of a cluster varies from one to more than 100 cells. *c*, These clusters appear as sectors and seem to be a consequence of residual growth after the initiation of some lethal event in an earlier generation. *d*, The risk that a cell will be the progenitor of a lethal sector decreases in successive generations. Nevertheless, instability has been noted after as many as eight generations. *e*, The degree of instability is not uniform for all cells of an irradiated sample.

These characteristics do not exclude the existence of a rapidly shifting probability of non-division, but they rule out a constancy in the value of  $p_2$  and in the number of generations between the occurrence of a lethal event and the termination of cell division. To be acceptable for yeast, the concept of division probability would have

to be altered so drastically as to lose the practical usefulness claimed for it.

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### "Congenital Anomalies" induced by X-Ray

It was recently reported in your pages that prenatal X-irradiation produces a higher incidence of congenital anomalies in the offspring of primiparous mice than in those of multiparous mice, and that although the difference is not great, it is "indeed significant". In addition to gross external malformations, the classification "congenital anomaly" was also assigned by the writers to resorbed conceptuses, dead fetuses, and stunted survivors, although there is no justification for this practice, because these disparate phenomena may result from unlike effects of irradiation. Thus, collecting them together under one heading probably obscures rather than clarifies the nature of the damage done by prenatal irradiation.

I should like to go further and examine the authors' data<sup>1</sup>. When resorbed, dead and stunted offspring are omitted, it turns out that primiparous mice had 440 surviving young, of which 180 (40.9 per cent) were malformed; while the multiparae had 165/454 (36.3 per cent) malformed ones. This difference is indeed not great, but neither is it significant ( $\chi^2 = 1.78$ ,  $P \geq 0.2$ ).

It thus appears that the teratogenic susceptibility of the two groups of embryos was similar and that the previous reproductive history of the females was of no consequence.

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## AGRICULTURE

### Effect of Environment on Wheat Gliadin

CHEMICAL and physical investigations have shown that gluten composition differs from one wheat variety to another<sup>1-5</sup>. The total protein as a percentage of the weight of grain of a particular variety is affected by environmental factors and may be twice as great in some samples as in others grown in different conditions. Variations of this kind within a variety can result in an alteration in the proportions of the broad protein classes<sup>6,7</sup> and could alter the distribution of components within classes. The work reported here was designed to test as rigorously as possible whether the second kind of change occurs within the gliadin class of proteins. It is desirable to know whether or not this type of change can occur if information on the composition of the gliadin is to be readily applicable to genetic investigations of inheritance of wheat quality.

The varieties of wheat used in this work and their ranges of protein contents were as follows: 'Gabo' (8.1 per cent to 17.9 per cent), 'Spica' (9.6 per cent to 16.0 per cent), 'Javelin' (8.9 per cent to 12.7 per cent), 'Quadrat' (8.3 per cent to 12.0 per cent). Thirty-three samples were obtained from six widely separated locations

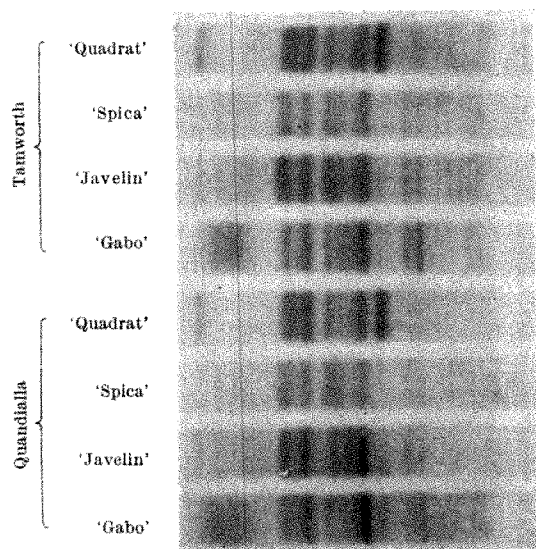


Fig. 1. Starch-gel patterns of the acetic acid extracts of flour from four wheat varieties grown at two locations. Gels contained 14 per cent of hydrolysed starch and were prepared in 0.017 molar aluminium lactate buffer, pH 3.1, containing 2 molar urea. Period of running 5 h at 15 V/cm.

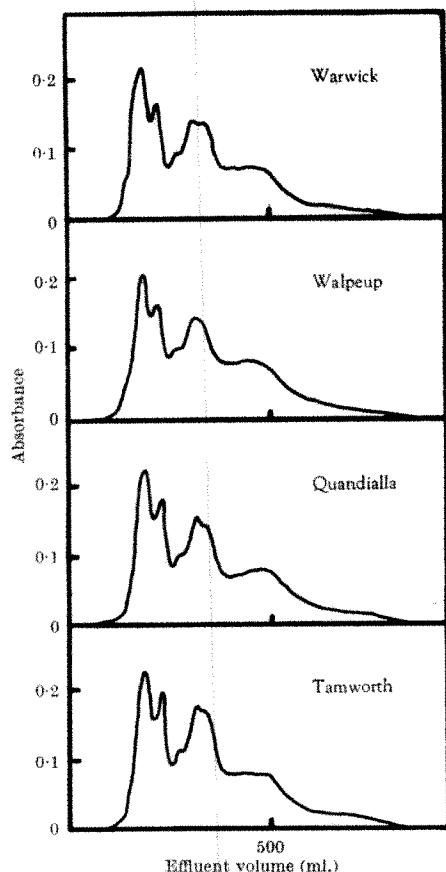


Fig. 2. Elution profiles of the proteins in soluble acetic acid of flours milled from 'Gabo' wheat grown in four different localities. Extracts containing 10 mg of nitrogen were applied to carboxymethyl cellulose columns (1.5 cm  $\times$  25 cm), equilibrated with 0.01 molar lactate buffer, pH 4.1, and maintained at 40° C. Protein was eluted by a 1,000 ml. linear salt gradient (0.02 molar sodium chloride) in the above buffer. Protein was estimated by the Lowry procedure.

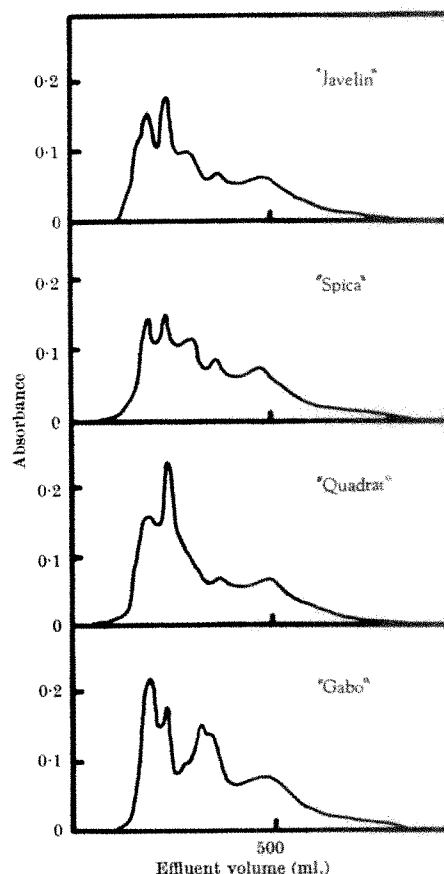


Fig. 3. Elution profiles of extracts from four different wheat varieties grown at a single location, Narrabri. Experimental details as for Fig. 2.

(Merredin, Narrabri, Quandialla, Tamworth, Walpeup and Warwick) with a wide range of environmental conditions in four Australian states. The variety 'Gabo' was grown at all sites in two successive growing seasons, while at one (Merredin) the varieties were grown on two distinctly different soil types. Proteins soluble in acetic acid extracted<sup>8</sup> from flours milled from each of the samples were subjected to chromatography on carboxymethyl cellulose<sup>9</sup> and starch-gel electrophoresis<sup>10</sup>.

The results shown in Figs. 1-3 are typical of those obtained for the thirty-three samples examined. Starch-gel patterns for the four varieties grown in two of the locations are presented in Fig. 1. Fig. 2 compares the chromatographic profiles of the variety 'Gabo' grown in four different locations, and Fig. 3 illustrates chromatographic differences between varieties grown at a single location. It is clear that varietal differences are much more important than environmental factors in their effects on the starch-gel and chromatographic patterns (Figs. 1-3). The similarity between the electrophoretic and chromatographic patterns of the varieties 'Javelin' and 'Spica' was unexpected as they are not closely related genetically.

Variation between samples of the same variety (Figs. 1 and 2) may be a result of environmental factors, but it should be emphasized that these differences were small compared with the varietal differences and may be the result of experimental factors which are difficult to control. For example, apart from the inherent difficulties in reproducing exactly the chromatographic and electrophoretic techniques, the presence of variable amounts of other proteins, lipids and carbohydrates during extraction can result in the selective extraction of some gliadin components in preference to others.

The present investigation has not completely excluded the possibility of environmental effects on the gliadin composition of a variety, but it has demonstrated that the

combined effects of differences in locality, soil type, and season resulting in up to a two-fold variation in total protein content have little effect on the distribution of gliadin components. This suggests that the composition of gliadin could be a useful marker in work on wheat genetics.

We thank Dr. P. C. Williams of the Wagga Agricultural Research Institute for the wheat samples used in this investigation.

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### Larval Phoresis of Chironomidae on Perlidae

A SPECIES of Chironomidae (Diptera) the larvae of which were living on the wing pads and legs of the immature stages of *Acronuria abnormis* (Newman, 1838) (Plecoptera, Perlidae) in streams around Ithaca, New York, has been reported<sup>1,2</sup>. Nothing has been added to our knowledge of this peculiar association since then. From May 1963 to January 1964 I observed what seems to be the same species in a stream near Wolf Lake, Pontiac County, Quebec. From larvae collected on their hosts, the other stages of metamorphosis have been reared and described as representative of a new species and a new genus of Orthoclaadini [sub-familia Orthoclaadiniinae], *Plecopteracoluthus downesi*<sup>3</sup>. The larvae fed on small particles, not on the Plecoptera themselves; thus the association is not parasitism, as had been suggested earlier, but rather it is phoresis.

The stream in which these larvae were found flows through two lakes which influence its temperature scale. During the summer months it has a moderate daily range, in spite of very different air temperatures by day and by night. The temperature of the stream, always measured in the early afternoon, was 12° C in May, 28.5° C in July, 8° C in October, and -0.5° C in late January (spot readings). In the faster regions the stream bed consists of rocks and big stones which are rather unstable, and there is almost no submerged vegetation. According to the classification of running water systems by Illies<sup>4</sup>, the region would be called the *hyporhithron*. The Plecoptera usually lived underneath the biggest stones, which were exposed to the strongest current. Of those that were carrying Chironomidae, 83 per cent were *Acronuria abnormis* (Newman, 1838), 6 per cent *Paragnetina media* (Walker, 1852), 5 per cent *Paragnetina immarginata* (Say, 1823), 3 per cent *Acronuria lycorias* (Newman, 1839), and 2 per cent *Togoperla media* (Walker, 1852), all belonging to the familia Perlidae. In early May 20 per cent to 40 per cent of the Perlidae were inhabited by *Plecopteracoluthus downesi*, and during the summer the percentage increased until in late autumn 82 per cent of all specimens of Perlidae collected were inhabited. From a sampling made on January 29, 1964, about 73 per cent of larvae of Plecoptera were found inhabited, and another 10 per cent were bearing cases but had lost the larvae themselves. Examining the host species separately it was found, in a sample of October 1963, that 96 per cent of all specimens of *Acronuria abnormis*, 45 per cent of *Paragnetina immarginata* and 37 per cent of *Paragnetina media* were inhabited, thus showing that *Acronuria abnormis* is the preferred host.

Of all the inhabited Perlidae that were examined, 50 per cent were bearing one older larvae and 10 per cent one younger larvae, while 40 per cent carried from two to four larvae each in various assortments of old and young. There are probably four larval stages, because four size classes could be identified in each sample that was taken, except in May when only the older larvae were noticed. The "younger larvae" probably represent the first and second, the "older larvae" the third and fourth, larval stages.

All larval stages live in gelatinous cases attached tightly to the body of larvae of Perlidae. The cases are often sparsely dotted with small dirt particles. They have a front and a rear opening, through either of which the larvae may emerge for about half their length when feeding. Most commonly (90 per cent), however, the larva lies head forward. The cases of the younger larvae are attached, almost always, between the hairs and bristles of the cerci or the metatibiae, more infrequently on the pro- or mesotibiae or at the surface of a wing sheath. The older larvae live almost exclusively on the surface of one of the wing sheaths, preferably that of the mesothorax. There was, however, no clear relation between the age of the larvae of Chironomidae and the age of host, except that most of the older larvae were on older hosts, probably because of their more convenient size. There seems to be no strict conformity in the life cycles of the members of this association. When rearing these insects in the laboratory, it has been observed that the larva will leave its case when the host larva is moulting and will move on to the newly emerging instar just as the old skin is being sloughed off. Thus, it can be assumed that the larva does not complete its development in the time needed for one instar of its host but can deal with the difficulties that may arise at the moults. On the other hand, the larval period probably has to be completed within the span of the aquatic (immature) stages of its host. On migrating to the new instar, the larva builds another case; and within such a case, eventually, pupation takes place. The pupa has not been found on free-living specimens and seems to last a very short time only, perhaps about 48 h. Before the imago emerges, the pupa leaves the case and swims to the water surface.

From the facts observed, it is clear that this association is not accidental but has ethological and ecological significance. There is little reason to call the association a symbiosis, for the host does not seem to derive any special benefit, other than the cleaning of its hairs, referred to below. The fact that the host species are carnivorous would be against the association and could indicate a danger when attachment is taking place. For the larvae of *Plecopteracoluthus downesi*, however, the association may have two advantages. The first one is in the obtaining of food. The contents of their guts showed that the larvae are detritus feeders but they are not adapted by structure or habit to filter such food from the water current, nor does their fast moving water habitat provide places where such fragments could accumulate. The larvae of Plecoptera, and above all *Acronuria abnormis*, possess dense and long fringes on the legs, cerci and some other parts, in which silt and organic particles accumulate. When keeping specimens of *P. downesi* on *Acronuria abnormis* in the laboratory, I could see the larvae stretching out from their cases, bending the front part of the body as though seeking for food, and picking up small pieces by the movement of their pseudopodes anteriores and mouthparts. This behaviour would be even more effective in the younger larvae, which live in the hair-fringes of the cerci or tibiae.

The second advantage may be that the small larvae of *P. downesi* are not able to cover great distances, as are their hosts. Thus, if a stone is displaced by the current, the hosts will more easily be able to avoid damage and to find a proper living site again than their inhabiting Chironomidae would be. This suggestion agrees with the hypothesis of Corbet<sup>5,6</sup> for the associations of *Simulium*.



with Odonata and Ephemeroptera; he considers that "the determining factor in the evolution of this association has been the need for *Simulium* in fast-flowing, unstable watercourses to secure a pupation site which gives protection against disorientation and damage". In the case treated here, however, it is not only the pupa that seems to benefit from the security guaranteed by the phoretic association but also, and perhaps even more, the larval stages; the larva, inside its case, is not only almost equally unable to move but much longer lived. There is also another factor which seems to be of strong selective value for the evolution of this association of Chironomidae and Perlidae. During the winter the larvae of *Plecoptera coluthus downesi* are almost immobile, while their hosts are still moving around. On January 29, 1964, the water temperature of the biotope was  $-0.5^{\circ}\text{C}$  and the host larvae were still active. The larvae of the Chironomidae inhabiting them did not move at all, however, and were apparently in a state of hibernation. In the laboratory, at a temperature still near freezing point, they did not react with the usual quick movement when touched with a needle. Even in water of about  $5^{\circ}\text{C}$ , it took about a quarter of an hour before they began to react. Thus, if larvae were dislodged during the winter they would not be able by themselves to avoid being damaged or swept away and would not be able to seek another shelter. All these problems are solved, however, when living in phoretic association with Perlidae. Thus, I consider that the one main factor in the evolution of the association of Chironomidae and Perlidae is the provision of accommodation for the larvae, which would otherwise be unable to endure the winter in perilous currents.

This work was carried out during my tenure of a post-doctoral fellowship from the National Research Council of Canada. I thank Mr. J. A. Downes and Dr. P. S. Corbet, of this Institute, for valuable suggestions and for reading the manuscript.

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## PSYCHOLOGY

### Intracranial Self-stimulation in the Chick

In 1954 Olds and Milner<sup>1</sup> showed that rats would work to administer trains of electrical pulses through implanted electrodes to sites (for example, medial forebrain bundle, hypothalamus<sup>2</sup>) in their brains (self-stimulation). Self-stimulation has since been reported in the domestic cat<sup>3</sup>, rhesus monkey<sup>4</sup>, bottlenose dolphin<sup>5</sup> and domestic dog<sup>6</sup>. Euphoria has followed stimulation in the hypothalamus and tegmentum in man<sup>7</sup>. In vertebrates other than mammals, the only demonstration of self-stimulation appears to have been in goldfish (*Carassius auratus*)<sup>8</sup>.

In the present study very young domestic chicks were chosen for use because of recent demonstrations<sup>9</sup> of the relative permanence in the fowl of the effects of early learning on the choice of social or sexual partner ("imprinting"), or of food, which suggested that the neurophysiological basis of positive reinforcement would be of special interest in this species at this age. A second reason for the choice was the fact that positive reinforcers or conditioned stimuli for such reinforcement evoke vocalization<sup>9</sup>, thus providing a means of investigating the central effects of intracranial stimulation, independent of changes in the rate of the operant response which provides the stimulation.

The site chosen lay in the medial forebrain bundle, very slightly dorsal to the level of the anterior commissure. An insulated stainless steel electrode, exposed at its tip over the width of its cross-section (0.01 in.), was permanently implanted on the fourth day of life. Stimulation was applied between this and an electrode exposed for 3 mm of its length, which ran superficially in the forebrain, just under the skull. (Stimulation was applied between the tips of a bipolar electrode in *E1*.) Flexible leads, which were suspended from an overhead counterbalanced arm, and connected to contacts, which could rotate about a central bearing shaft, in circular mercury filled tracks, allowed free locomotion, including maintained circus movements. Pairs of square pulses of opposite polarity, 1 msec duration, and 0.2 m.amp peak to peak amplitude, were used in trains lasting 0.5 sec, at a rate of 100 pairs of pulses/sec, except where otherwise indicated. The current delivered to the brain was monitored on a 'Tektronix 5024' oscilloscope.

Male chicks (Warren sex-link) 18 h old were separated into isolation cages (8 in.  $\times$  8 in.  $\times$  14 in.) where they lived thereafter. When testing was about to begin (day 5, 6 or 8: Fig. 1), two identical keys were exposed in the wall of the cage, each consisting of a red sphere, 5 mm in diameter. (Two keys were presented to allow later experiments in which chicks chose between currents of differing duration or intensity.) If either key were pecked or pulled, it set in action a Grason-Stadler electronic clock, which timed the duration of stimulation, and a counter. A key operation produced exactly the same sequence of sounds from this equipment for a control (*C*) as for an experimental bird (*E*). All six experimental birds were run with a paired control from the same hatching, implanted at the same time, and with an exactly similar history. All controls were connected to overhead leads in the same way as the subjects during testing. *E4* on the first day and *C2* on the second day of testing served as yoked controls. Other controls received no stimulation.

All six experimental birds showed a marked increase of key operation, to an amount well above both their own previous rate of operation and that of their control, in the first 10 min after stimulation was made available (Fig. 1). (The duration of testing was not standard, because a session was not terminated until adequate recordings of vocalization during self-stimulation and protocols of associated behaviour had been obtained.) *E4* showed a slight but definite increase as a yoked control. The rise in rate of operation by *E4* on the second test day, however, when stimulation was made contingent on key

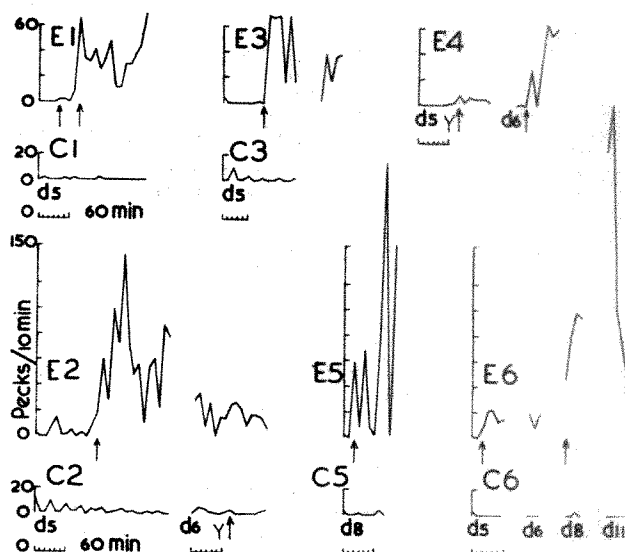


Fig. 1.

operation, was very much greater, and reached a level much above that reached by any control. This, and the fact that the other yoked control showed no increase at all after the onset of stimulation, indicates that the main effect of stimulation on the rate of operation in the subject is not caused by a general increase in activity.

The birds were killed on the fourteenth day of life, and their brains sectioned. All the subjects had correctly placed electrodes, but it was clear, as the tip of each track showed incipient healing over 0.5–1 mm, that the electrode tips had lifted during the last few days of life over this distance (within the medial forebrain bundle) from the original placement because of skull growth. This may explain the sudden and marked increase in rate on day 8 in the case of *E6*, rather than the lengthening of the train of pulses to 0.75 sec on that day. A seventh experimental bird, which never began to self-stimulate, was also the only *E* in which the electrode lay outside (and anterior to) the medial forebrain bundle.

The rates of stimulation, at their highest (260 pecks/10 min), are comparable with some reported maximal rates for equivalent areas in the rat (400 operations/10 min for the septal area<sup>9</sup>; 150/10 min for the medial forebrain bundle<sup>10</sup>). Moreover, the figures for the chick are not for highly trained animals, and are lowered by being averaged over 10 min because even the longest bouts of operation were not longer than 6–7 min and most were considerably shorter. Between bouts chicks slept and fed.

The fact that each peck resulted in a train of pulses, and that pecks were often given in rapid repetition, resulting in almost continuous stimulation while the pecks continued, makes it clear that all experimental birds would have worked for, and perhaps preferred, trains of considerably greater length (2–3 sec) than 0.5 sec, as is often true of the rat<sup>11</sup>. (This has been confirmed in later choice experiments, in which continuous stimulation was impossible. Trains of 0.1 sec would not maintain self-stimulation, and were consistently rejected in favour of 0.5 or 1.0 sec. Some chicks preferred 2.0 sec to 0.5 or 1.0 sec.)

The chicks gave, in association with self-stimulation, calls of a type also given to a variety of sources of stimulation which chicks tend to approach (for example, the imprinting object, food or signs announcing food<sup>8</sup>).

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### Effect of Self-instruction on Perceptual Judgment

A MENTAL set, or "expectancy", may exercise a selective influence on the perception of ambiguous stimuli<sup>1</sup>. Such a set can be induced by a bodily need such as hunger, or by verbal instructions. The present study investigates whether an instruction which an individual gives to himself has more effect on subsequent perception than an instruction which he receives from an external source. There were three independent groups of subjects.

(1) In the self-instruction or reasoning group (RG), the subjects were presented with this conditional statement: "If the line on the left is longer than the line on the right, then the line on the right is red". A red line on their right

Table 1. FREQUENCY OF JUDGMENTS

	RG	IG	CG	
Longer	12	4	1	17
Equal	3	11	14	28
	15	15	15	45

was then exposed. The subjects were then asked what they could say about a concealed line on their left, and they all replied that it must be longer. They were then shown a red line of the same length on their left, and asked to judge its length relative to that of the previously exposed line. If they said it was longer, they were asked whether it actually looked longer.

(2) In the external instruction or information group (IG), the procedure was similar but instead of being invited to make an overt inference from a conditional statement, the subjects were told that the line on the left would be longer than the line on the right.

(3) In the control group (CG) the subjects were merely asked to judge the relative length of the two lines.

The two lines, strips of red 'Sellotape' fixed on white cardboard, were both 15 cm long and 0.5 cm wide. They were displayed on end in the same horizontal plane separated by 20 cm on a desk in front of the subject.

It was predicted that the frequency of perceptual judgments that the left line was longer would vary as follows: RG > IG > CG. A sample of forty-five subjects was selected at random from the student population of the University of London and assigned in rotation to the groups ( $n=15$  in each group). The subjects were tested individually and there was no time limit on any phase in the task.

Table 1 shows the number of subjects in each group who judged the left line as either longer than or equal to the right line. Three subjects, one in each group, judged it as shorter and were included in the equal category.

The prediction, RG > IG > CG, was confirmed with a high degree of significance (Kendall's  $S=330$ ,  $P=0.00005$ , one-tailed), but it will be noted that the difference between RG and IG is mainly responsible ( $P=0.005$ , one-tailed, Fisher's exact test), the difference between IG and CG being negligible. Eleven out of twelve subjects in RG, and three out of four in IG, who judged the left line longer, said it actually looked longer.

The results are consistent with Bruner's "hypothesis theory" of perception<sup>1</sup>, but they suggest that self-instruction is much more potent than external instruction. They are also consistent with the theory of "cognitive dissonance"<sup>2,3</sup>. The subjects in RG had committed themselves to an overt inference and might have been reluctant to contradict themselves in their perceptual judgments. The subjects in IG had not committed themselves—they were passive recipients of information. Their perceptual judgments would not have entailed self-contradiction; they would only have entailed contradicting the experimenter. Whether the results of this exploratory study were caused by perceptual or cognitive factors, they corroborate the notion that a belief, in which there is personal involvement, may distort our knowledge of reality.

It should finally be noted that all the subjects in RG were self-instructed from having made a logically invalid inference. Previous research<sup>4,5</sup> has shown that intelligent adults can be made peculiarly susceptible to this particular fallacy in a test situation.

I thank Miss Adele Kosviner, who conducted the experiment.

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<sup>1</sup> Bruner, J. S., *Psychol. Rev.*, **64**, 123 (1957).

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 27

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Prof. G. A. Barnard: "The Bayesian Controversy in Statistical Inference".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. B. M. Weedy and Mr. J. P. Perkins: "Steady-State Thermal Analysis of a 400 kV-Cable Through Joint"; Dr. B. M. Weedy: "Thermal Aspects of Changes in the Environment of Underground Cables".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. R. Pollard: "Storage Systems for Telephone Switching".

UNIVERSITY OF LONDON (in the Engineering Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. G. Kendall, F.R.S.: "The Arithmetic of the Kingman Semi-Group".\*

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Charlotte Auerbach, F.R.S.: "Mutagenesis Research in Edinburgh. II, Mutagen Specificity".\*

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 6.15 p.m.—Dr. R. Gold and Mr. M. Paneth: "Management of Pulmonary Embolism".\*

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Meeting on "The Automotive Industry—Are Reinforced Plastics Making Progress?"

## Tuesday, February 28

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 4 p.m.—Dr. J. B. Rose: "Synthesis of Polyarylene Sulphones and Ketones".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. H. W. Kropholler, Dr. D. J. Spikins and Mr. F. Whalley: "Control of Distillation Columns".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. C. W. Kilmister: "What is Applied Mathematics and What Should It Be?" (Inaugural Lecture).\*

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Prof. J. R. Vane: "The Release and Assay of Hormones in the Circulation". (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

SOCIETY FOR ANALYTICAL CHEMISTRY, THIN-LAYER CHROMATOGRAPHY GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6 p.m.—Discussion on "The Use of Thin-Layer Chromatography for Identification Purposes" opened by Dr. A. S. Curry.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Eccleston Hotel, Victoria, London, S.W.1), at 7 p.m.—Commercial Meeting.

## Wednesday, March 1

INSTITUTION OF ELECTRICAL ENGINEERS (joint colloquium with the I.E.E.E. and the Royal Television Society, at Savoy Place, London, W.C.2), at 9.30 a.m.—"Colour Cameras".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. R. A. French: "The Migration of Butterflies and Moths in Relation to Weather and Season" (Margary Lecture).

UNIVERSITY OF LONDON (at the Institute for Diseases of the Chest, Brompton Hospital, London, S.W.3), at 5 p.m.—Dr. M. Turner-Warwick: "Rheumatoid Factors in Lung Disease".\*

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Sir Benjamin Rycroft: "The Evolution of the Corneal Graft".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. H. V. Dunnington: "Stratigraphical Distribution of Oil Pools in the Iran-Iraq-Arabia Basin".

UNIVERSITY OF LONDON (at Senate House, London, W.C.1), at 5.30 p.m.—Sir Rudolph Peters: "Fluoride and Its Hazards in Nature".\*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. H. R. Oakley: "The Disposal of Communal Waste".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Waltham Forest Technical College, Forest Road, London, E.17), at 6.30 p.m.—Mr. H. E. Barnett: "Measurements and the British Calibration Service".

## Thursday, March 2

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. C. M. Yonge, F.R.S.: "Living Corals".

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. S. Rapoport (Berlin): "The Biochemistry of the Red Blood Cell". (Further lecture on March 6).\*

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "The Influence of Transport Facilities on the Growth of S.E. England". Introduced by Major A. C. Dalgleish.

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Institution of Mechanical Engineers, at Savoy Place, London, W.C.2), at 5.30 p.m.—Meeting on "Control—a Unifying Force in Engineering Education".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dr. G. L. Asherson: "Autoimmunity". (Last of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilation, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. B. Lundvik (Stockholm): "Versatile Applications of the Screw Compressor".

## Friday, March 3

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 10.55 a.m.—Symposium on "The Experimental Taxonomy of Flowering Plants".

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Dr. B. Stevens: "Photoaddition of Molecular Oxygen to Unsaturated Compounds".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 2.15 p.m.—Annual General Meeting followed by Dr. A. A. Smales: "Our Society, Our Science—an Analysis" (retiring President's Address).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. William Sargent: "The Mechanism of Brainwashing and Conversion".

## Monday, March 6

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. M. K. McPhun: "U.H.F. Tunnel-Diode Amplifier"; Dr. D. L. Hedderly, Mr. J. Hooper and Mr. M. K. McPhun: "Short-Hop Radio-Relay Systems Using Tunnel-Diode Repeaters".

UNIVERSITY COLLEGE LONDON (in the Chemistry Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. S. Bosanquet: "The Sum of an Unconvergent Series". (Inaugural Lecture).\*

UNIVERSITY OF LONDON (in the Main Lecture Theatre, Physics Building, Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. Sir Vincent Wigglesworth, F.R.S.: "Insect Hormones" (further lectures on March 7 and 8).\*

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. D. A. Dowden: "The Coherence of Pure and Applied Heterogeneous Catalysis".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. R. A. Hibbert: "Mongolia Today".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT, SENIOR RESEARCH ASSISTANT or SENIOR DEMONSTRATOR (preferably with experience in electronics) in the DEPARTMENT OF APPLIED PHYSICS—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 4).

ASSISTANT LECTURER (with interests in mineralogy and petrology) in GEOLOGY—The Registrar, The University, Hull (March 10).

SENIOR ASSISTANT LIBRARIAN (with a degree and experience of dealing with scientific material, and preferably a professional qualification)—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey (March 10).

ASSISTANT LECTURER or LECTURER (with interests in any branch of pure mathematics) in the DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (March 11).

RESEARCH FELLOW (graduate in mathematics, physics or electrical engineering) in the DEPARTMENT OF ELECTRICAL ENGINEERING, for studies in the synthesis of lumped linear electrical networks—The Registrar, The University, Leicester (March 11).

SCIENTIFIC OFFICER (with a good honours degree in zoology or applied entomology, preferably with postgraduate experience) to carry out laboratory and field studies on the behaviour and performance of insecticides in soil to improve their efficiency—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (March 11).

READER IN CLINICAL PHARMACOLOGY at St. Bartholomew's Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 13).

ASSISTANT LECTURER or LECTURER (preferably with interests and experience in any field of experimental psychology) in PSYCHOLOGY—The Secretary, University of Stirling, Stirling, Scotland (March 15).

LECTURER or ASSISTANT LECTURER (with a strong mathematical background and able to initiate a research group in mathematical logic, theory of probability or philosophy of mathematics) in PHILOSOPHY OF MATHEMATICS in the DEPARTMENT OF HISTORY AND PHILOSOPHY OF SCIENCE—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (March 15).

LECTURERS or ASSISTANT LECTURERS in the DEPARTMENT OF PHILOSOPHY, University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 15).

SENIOR LECTURER in the DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leicester (March 15).

SENIOR LECTURER in the DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leicester (March 15).

READER IN PHILOSOPHY at Birkbeck College—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 16).

RAMSAY CHAIR OF CHEMISTRY (in the field of inorganic chemistry)—The Secretary of the University Court, The University, Glasgow (March 18).

ASSISTANT LECTURER (preferably with interests either in invertebrate physiology or in zooplankton) in MARINE BIOLOGY in the Marine Science Laboratories—The Registrar, University College of North Wales, Bangor, North Wales (March 20).

LECTURER IN STATISTICS in the DEPARTMENT OF APPLIED STATISTICS—The Registrar (Room 39, O.R.B.), The University, Reading (March 25).

LECTURER or ASSISTANT LECTURER (with research and teaching experience in chemical microbiology) in the DEPARTMENT OF BIOCHEMISTRY—The Assistant Registrar (S), P.O. Box 363, The University of Birmingham, Birmingham, 15 (March 31).

LECTURER or ASSISTANT LECTURER (with interests in the biochemistry of micro-organisms) in MICROBIAL BIOCHEMISTRY—The Registrar, University College of North Wales, Bangor, North Wales (April 10).

LECTURER or ASSISTANT LECTURER (with interests in the biophysical and biochemical aspects of soil-plant inter-relationships) in SOIL-PLANT NUTRITION—The Registrar, University College of North Wales, Bangor, North Wales (April 10).

LECTURERS or ASSISTANT LECTURERS (2) in the DEPARTMENT OF CHEMICAL ENGINEERING—The Assistant Registrar (Sci./Eng.), University of Birmingham, Birmingham 15 (April 14).

BIOCHEMIST (with an Hons. B.Sc. in biochemistry or chemistry) to join a team working on iron metabolism in liver disease—Dr. Roger Williams.

Clinical Research Wing, King's College Hospital, Denmark Hill, London, S.E.5.

**LECTURER** (with good qualifications in teaching and/or research and either interests and experience in some aspects of intermediary metabolism or in the applications of analytical techniques (e.g., gas chromatography) to biochemical problems) in **BIOCHEMISTRY** in the **BIOCHEMISTRY SECTION** of the **DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY**—The Registrar, University of Strathclyde, George Street, Glasgow, C.1.

**MEDICAL DIRECTOR** (preferably registered practitioner with experience in drug evaluation and assessment) of the **National Drugs Advisory Board**—The Secretary, National Drugs Advisory Board, 6 Fitzwilliam Place, Dublin 2, Republic of Ireland.

**SENIOR LECTURER** (preferably with experience or interest in biochemistry or microbiology) in **BOTANY**—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

**SENIOR PHYSICIST** (with good academic qualifications and considerable experience together with an interest in modern methods of teaching)—The Headmaster, Clifton College, Bristol, 8.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

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## Mr. MACNAMARA and Mr. CROSLAND

It is ironical as well as sad that two of the most able men in the British and American governments should find themselves in a trans-Atlantic slanging match (see page 853) about technology and the brain drain. Mr. Macnamara, of course, has so far done no more than make a public speech which erred most seriously in being frank about issues which are usually ignored, but which was also well larded with the unavoidable complacency of those who can clearly see the solutions of problems which do not concern them directly. Mr. Crosland, rattled perhaps by three weeks of nagging about university fees for students from overseas (see page 853), and possibly wishing that there were more of the good red meat of politics in the administration of British education, was responding with the impatience of those who have learned by painful experience that it is easier to specify solutions to British problems than to attain them. And nobody, of course, will be surprised that both Mr. Macnamara and Mr. Crosland have right on their side.

It is, for example, splendid that Mr. Macnamara should have argued that the phenomenon which has become known as the technology gap is more a matter of management than of technology. If only enough people of his stature would say this more often, there would be less danger that technology will become a kind of magic wand for pretending that real problems do not exist. The most common form of this delusion is that which reads the link between technology and prosperity the wrong way round. Simply because the United States abounds with ambitious—and expensive—technological projects, and because the United States is prosperous, there is a perennial temptation for Europeans to believe that a few technological spectacles would make them prosperous too. Europe is littered with the relics of projects originally undertaken because of this magical belief. Mr. Macnamara has in particular done well to attack the notion that expenditure on defence technology is a sure way of creating prosperity. The frail doctrine of spin-off, or of technological fall-out as it is sometimes called, has nothing but optimism to support it—even in the United States. It is intellectually offensive because it implies that thoughtless effort is as productive as that which is consciously and conscientiously undertaken. If Mr. Macnamara can help to stamp it out from Europe, and keep it at bay in the United States, he will be a public hero.

Mr. Macnamara also dealt with a less obvious misconception of the value of technology—the tendency to regard it as advantageous in its own right, detached from its objectives. It is in this spirit that Signor Fanfani, the Prime Minister of Italy, started the

present excitement about the technology gap by raising the possibility of a kind of exchange of technology within the North Atlantic community as if technology were a kind of foodstuff to be shared out by some modern equivalent of the Marshall Plan. Mr. Harold Wilson, the British Prime Minister, has given support to this concept of technology as a kind of international commodity by commending British membership of the European Economic Community with the argument that Britain has a great technological dowry to bring to any marriage. But as Mr. Macnamara says, what counts is not skill but the capacity to use it. Modern techniques of management have their roots in technology, but they are also an attitude of mind. They are necessary, if not sufficient, conditions for closing whatever gap exists. Luckily, in Britain, the Ministry of Technology seems to have learnt this lesson, and may yet be able to educate the Prime Minister.

So why, then, was Mr. Crosland angry? The chances are that he would cheerfully accept Mr. Macnamara's homilies on the importance and the limitations of technology. Why then does he protest at the comparison on education? The trouble is that Mr. Macnamara's comment was too facile. It was not sensible of him to imply that if the nations of Europe wish to become more prosperous, they should make it their first responsibility to provide more education for more people for longer. In its way, this is as much of a fallacy as the belief that spectacular technology is the way to riches. Good systems engineer that he is, Mr. Macnamara should know that the problem is to maximize the economic benefit from the educational system, not the amount of education as such. In Britain, the problem happens to be exceedingly difficult because the traditions of selective and specialized education mean that the system can be at once inflexible, inequitable and sometimes superb. Mr. Crosland's problem is that the pace of change is limited not merely by the structure of the system but by the amount of effort and money that can be invested in it. The Swann report last year showed, for example, how the expansion of British universities in the past three years has used up a substantial number of people who would otherwise have found their way into industry—or possibly to the United States. The often intolerable frustration of the national disparities that Mr. Macnamara accurately identified is the constant awareness that desirable social amenities, such as a universal system of higher education, which are themselves sources of industrial strength, must be forgone for the sake of more compelling but less pleasing forms of investment. This is what Mr. Macnamara over-



looked. But he has done well to provide such an eloquent reminder that if the British feel badly about the comparison with the United States, less prosperous nations are likely to feel even more unhappy.

## RIBONUCLEASE

THE article by Dr. David Harker and his colleagues which appears on page 862 carries further the discussion of the tertiary molecular structure of ribonuclease. The fact that the team at Buffalo has been working on this problem has, of course, been common knowledge for some time. In spite of all the successes of the past few years, however, deriving the shape of a large protein molecule by X-ray analysis is still an exacting and a lengthy task. The difficulty is that an unambiguous identification of the positions of the atoms does require a sufficiently detailed analysis for individual atoms to show up on an electron density map. Sometimes, of course, a close similarity between two protein molecules may mean that information about one helps with understanding the other, but that does not often happen. This is why it was good to read in several newspapers in January that Dr. Harker's group had acquired a good head of confidence in their description of the ribonuclease molecule. At the same time, an article by Dr. C. H. Carlisle and his collaborators from Birkbeck College, London, was on the way into print in *Nature*. Now that both models have been published, it is plain that they differ from each other in several important ways. The fact that Dr. Harker's analysis implies a resolution of 2 Å, while Dr. Carlisle's is equivalent to 5.5 Å, will naturally make the American model seem the more convincing. One possibility, for example, is that Dr. Carlisle's model will come to seem more like Dr. Harker's as the analysis continues. Alternatively, further detailed study may suggest more radical changes to be necessary. These are not matters which can be decided lightly, but only by well-informed appraisal of whatever evidence may be available. It may be a great help that there is a third group, at Yale, working hard and quickly on the problem.

There remains the question of what these great endeavours are intended to accomplish. The fact that ribonuclease turns out to have the same general shape as the protein molecules the tertiary structure of which has already been determined, with hydrophobic groups inside and hydrophilic groups outside, is, of course, important if no longer surprising. But each new example of tertiary protein structure is bound to be a further help in the attempt to understand how tertiary structure is determined by the overall arrangement of amino-acids. The special interest of ribonuclease is that there is only one other enzyme molecule—lysozyme—the tertiary structure of which has been determined with accuracy and confidence. Obviously there will now be hopes that a comparison of the two enzyme molecules will provide clearer pointers

to an understanding of how enzyme molecules function than would be possible if there were just one model to work with. Unfortunately it is a long time since there were expectations that the sight of an accurate tertiary structure would indicate quite quickly the seat of its biological activity.

## WHAT NOW, APOLLO?

THIS is a critical time for the future of piloted space flights in the United States. Congress has at last begun picking over the budget of the National Aeronautics and Space Administration for the coming financial year. The process is more uncertain than usual because it is not yet clear how radically the space agency will be required to change its plans for the immediate future to prevent a recurrence of the kind of trouble which killed three astronauts on January 25. The signs are not, however, encouraging for the optimists. The third and most substantial report of the Board of Inquiry into the accident at Cape Kennedy, issued last week-end, has some ominous passages about complacency and miscalculation. Mr. James Webb, the chief executive officer of NASA, has openly acknowledged that the risk of fire was underestimated. It would not be surprising if Congress now took a cooler view of the urgency of sending men to the Moon than has previously been its custom. But healthy scepticism this year is likely to be particularly influential, if only because the foundations for the continuing programme of the NASA are going to be laid this year and the next.

What should a good committee man do? First of all, he is likely to ask how much the Apollo trouble is likely to affect the programme now mapped out by the White House for what is called the Apollo Applications Programme intended to occupy the early seventies. Briefly, the intention is to capitalize on the investment there has so far been in the family of Saturn rockets. In this scenario there are to be one or two flights each year by people to the Moon, and more frequent journeys by instruments to the planets. Optical telescopes in orbit about the Earth, with real astronomers in attendance, are included in the plans. But all this may now seem dangerously ambitious. Congress may choose to counsel caution. It would certainly be wise not to commit NASA to a specified level of expenditure without more information about the viability of the system for breathing oxygen in Apollo. Whether, in all this, the Air Force Project for a "Manned Orbiting Laboratory" will seem to be tarred with the same brush as Apollo because it has a similar oxygen system, or whether it will seem an attractive alternative, is not at this stage clear. And it is always possible, of course, that the Board of Inquiry will eventually have a hopeful tale to tell. But whatever happens, this is a valuable time for Congress to strike a blow for moderation in the exploration of space by people.

## NEWS AND VIEWS

### War of Attrition

MR. ANTHONY CROSLAND, Secretary of State for Education and Science, is unrepentant about his decision to raise university fees for overseas students. In a debate in the House of Commons on February 23, he defended his position with some skill, though he may have been rash to describe some Vice-Chancellors as "near hysterical". He also seems to be clutching at straws by quoting support from local authority associations; it would have been surprising indeed if they had supported the general increase in fees which Mr. Crosland offered as an alternative to the increases for overseas students, since any general increase in fees would fall most heavily on the local authorities. And if Mr. Crosland can find time to raise the matter informally and without commitment with the local authorities, people are bound to ask, as Mr. Nicholas Scott did in the House of Commons, why it was impossible to consult the Vice-Chancellors at all. Mr. Crosland was aided by a subdued Opposition, perhaps because some Conservatives felt their alliance with inflamed student opinion downright unnatural.

For all the fine words, universities have seemed slow to take action, beyond bombarding the minister and their M.P.s with telegrams. The University of Bradford, with 194 overseas students on its books, is prepared to remit fees to overseas students who are already at the university and can show evidence of hardship. Most of the other universities are waiting for a clarification of the position before they take any action. From Mr. Crosland's point of view, all that would seem necessary to forestall action would be to keep the arguments imprecise. Whether this is what happened at the meeting between the Vice-Chancellors and Mr. Crosland on February 28 is not clear, but in the circumstances the meeting could hardly be more than a trudge around well prepared positions. Another meeting will follow on March 7; so far time seems to have been spent in deciding exactly what an overseas student is, and how Mr. Crosland's hardship fund will work.

A lesson in good timing was supplied by the Fulbright Commission, which announced on the morning of the meeting a cut in the number of Fulbright Scholarships tenable both at American and at British universities. The decision, taken as a direct result of the increase in fees, is intended to share the extra cost between British and American scholars. Instead of 211 British postgraduates going to American universities in 1967-68, there will be 194. Similar steps may also be expected from Europe, where there are more British students than there are European students in British universities. In the European Economic Community it is the policy to encourage the movement of students even during undergraduate courses. Britain, as a candidate for Common Market entry, will not seem to have behaved diplomatically.

The fury caused by Mr. Crosland, of course, does not consist wholly of moral righteousness; the Vice-Chancellors, after all, favour a general increase in

student fees. Face to face with the Department of Education and Science, the universities have found that much of their freedom is a polite fiction, preserved by the University Grants Committee. What is surprising is that this should have come as such a shock to them.

### What Macnamara said

THE view that what is called the technological gap between the United States and Europe is more a matter of management than technology was one of the themes in a speech by Mr. Robert Macnamara, United States Secretary of Defence, at Millsaps College, Jackson, Mississippi, on February 24. Newspaper reports of Mr. Macnamara's diagnosis of the reasons why scientists leave for the United States provoked an angry reply last week-end from Mr. Anthony Crosland, Secretary of State for Education and Science in the United Kingdom.

On the disparity between the United States and Europe, Mr. Macnamara said that "it is not so much a technological gap as a managerial gap . . . The brain drain occurs not merely because we have a more advanced technology here in the United States, but rather because we have more modern and effective management. God is clearly democratic. He distributes brain-power universally. But he quite justifiably expects us to do something efficient and constructive with that priceless gift. That is what management is all about—it is the gate through which social, political, economic, technological change—indeed change in every dimension—is rationally and effectively spread through society . . ."

Mr. Macnamara went on to say that Europe could not close the gap by boycotting American technology, by high tariffs, or by prohibiting investment. Restricting emigration would not help either. There was no future in the establishment of a defence industry "on the dubious economic theory that only through massive military research and development can a nation industrialize with maximum speed and benefit". In the long run, Mr. Macnamara said, only education could help. "Europe is weak educationally and that weakness is seriously crippling its growth. It is weak in its general education; it is weak in its technical education; and it is particularly weak in its managerial education".

He went on to say that in the United Kingdom, France, Germany and Italy, fewer than 20 per cent of schoolchildren remain at school after 15, but that 45 per cent of American children were still at school at 18. In the United Kingdom, he said, "there are some 336,000 students enrolled at university level. Only 10 per cent of college age people stayed on for higher education, with seven per cent in Germany, seven per cent in Italy, and fourteen per cent in France. But in the United States, said Mr. Macnamara, there were four million college students, amounting to 40 per cent of the population of college age.

What seems to have irritated Mr. Crosland most is the suggestion that the uniform distribution of brain-power is all that matters. "One thing that God does not distribute equally is wealth." He said that the reason that "brains move from every country of the world to America is that America . . . is the richest country in the world and can pay much for brains". The brain drain, he said, was now a serious matter of

conscience for the richer countries, particularly the United States.

## Aircraft for Aldabra?

THE Council of the Royal Society has taken the unusual step of making public its "considerable concern" about the future of Aldabra Island, in the Indian Ocean north of Madagascar. The immediate anxiety is that the British defence authorities are interested in the island as a possible air staging post in the Indian Ocean. In a statement published last week the council of the Royal Society says that Aldabra is the only island in the Indian Ocean which has remained "essentially unaffected by man", with the result that its flora and fauna contain many species not found elsewhere. Indeed, the island supports something like 10,000 of the land tortoise (*Testudo gigantea*) which is now found in its natural state only on Aldabra and the Galapagos Islands. Among the notable sea-birds are frigates (*Fregata minor* and *Fregata ariel iredalei*) which are likely to be a hazard to aircraft if an airstrip is ever built. The land birds include a flightless rail (*Dryolimnas cuvieri*). Although frigate birds occur elsewhere in the Indian Ocean, Aldabra is the principal breeding ground for them as for the green turtle (*Chelonia mydas*) and the hawksbill turtle (*Eretmochelys imbricata*). It is also estimated that 10 per cent of the 170 plant species on the island do not grow elsewhere. There is little doubt that Aldabra is an isolated island ecosystem of unparalleled interest.

The interest of the Ministry of Defence stems from the recognition in the early sixties that a continued British presence East of Suez would require air staging posts in the Indian Ocean. The map of the Indian Ocean suggests that Aldabra Island would be valuable if Aden were untenable by the Royal Air Force. The Ministry of Defence says that other islands in the neighbourhood have been considered as staging posts, and says that it has promised the council of the Royal Society that the "scientific case for the preservation of Aldabra will be fully considered before a decision is made". The Ministry also says that if it should decide to make a staging post at Aldabra, it will do everything it can to protect the species living there, and that even though the frigate birds would be a hazard, there is no question of exterminating them. The trouble with this kind of assurance, of course, is that the experience of the nineteenth century, when the atolls of the Indian Ocean were widely exploited for their guano, coconuts and turtles, shows how little outside interference is necessary to disturb the balance of the ecosystem.

Aldabra is by all accounts an inhospitable place, which accounts for its isolation. At present the island supports a population of 80 to 100 migrant workers from the Seychelles. One of the particularly objectionable features of the proposal to build an airstrip is that this would have to be sited on the part of the atoll ring at present separated from the human settlement and which is also frequented by the land tortoises. Although the fact that the Royal Society has chosen to make its anxiety public is a sign that it is afraid of reading in the newspapers one day that Aldabra is to be an airstrip, relations with the Ministry of Defence are friendly enough for the Royal Navy

to have agreed to ferry to the island in August this year the two scientists whose reports in 1966 drew attention to the dangers that an airfield would bring. It is now planned that a scientist should remain on the island at least until Easter 1968.

## Research into Environment

THE Centre for Environmental Studies has now been formally established as an independent charitable trust for advancing education and research in the planning and design of the physical environment. Announcing this in a written answer in the House of Commons on February 21, Minister of Housing and Local Government Mr. Anthony Greenwood, said that the Ford Foundation had given \$750,000 to the centre and, if Parliament approved, the Government intended to give a grant of one and a half times this amount for the first five years, up to a maximum of £600,000. Eight members of the ten strong governing body had already been appointed; the Chairman would be Lord Llewelyn-Davies, and the Vice-Chairman Sir William Fiske. One international governor had been appointed in consultation with the Ford Foundation—Mr. M. Meyerson—and others who had accepted appointments as governors were Mr. C. D. Foster, Lord Helford, Lord Jellicoe, Mr. W. Taylor and Professor R. M. Titmuss. Dr. A. H. Chilver had been appointed Director of the Centre from April 1, 1967.

Lord Llewelyn-Davies said this week that the centre, based initially on London, would have three functions: discovering (by discussions and conferences) the areas in which research is needed, sponsoring research in universities, and interesting itself in information retrieval and dissemination. As well as architectural planning, it would hope to support research in economics, sociology and geography. There had, he said, been pressure to recognize this area of research, and the new centre was the result.

## University of Cranfield?

THERE may be an educational precedent of some importance in the way in which the College of Aeronautics at Cranfield has applied to the Privy Council for a charter that would allow it to award its own higher degrees in engineering subjects. Discussions between the college and the Department of Education and Science have been going on ever since the Robbins Report recommended, in October 1963, that the college should be brought within the university system. At present the college is a direct pensioner of the Department of Education and Science. Inquiries at the University Grants Committee and the Department of Education and Science suggest that it is not yet clear whether the granting of a charter will mean that the college is financed like the universities, through the University Grants Committee, or like the polytechnics, through the Department of Education and Science. It does, however, seem that the college has successfully resisted suggestions that it should accommodate itself under the wing of an existing university or, alternatively, that it should make use of the degrees being awarded by the National Council on Academic Awards. The way in which the college has been pointing out that it lies a mere three miles from the site of the proposed city of Milton Keynes may indicate that

it has great ambitions for the future. Indeed, the college is hoping that its student population of 350 or so—all of them postgraduates—will increase to about 1,000 in the seventies.

## Asbestos in the Lungs

THERE are at present 250 registered cases in Britain of mesothelioma, the cancer tumour associated with asbestos dust contamination. A tumour of the lung lining (and occasionally the peritoneum), it can reach remarkable dimensions, is terminal and has not so far been known to develop without a relationship (however remote) with dust from asbestos of the crocidolite type—blue asbestos.

Four fibrous silicates with the generic name asbestos are in commercial use. These are: crocidolite (blue); amosite (brown); anthrophyllite (white); and chrysotile (also white) which is by far the commonest. Asbestos is in increasing demand for many industrial uses, especially in shipyards and for pipe-lagging. The material must be mined, milled and fabricated and at each of these stages a fine penetrating dust is produced, but this is not the full story. So far, only the dust from blue asbestos has been definitely implicated as carcinogenic. The relationship was established in the 1950s in South Africa, where the principal blue asbestos mine is located. It was found that on average the disease takes 40 years to develop, but that fatal exposure could be as short as three months in childhood. More than half the cases investigated had not worked in the asbestos industry but had been open to environmental exposure only. It is disturbing that these cases arose from exposure in the early days of the Cape industry—it started in 1890—when annual crocidolite production was only 10,000 tons. Now more than 80,000 tons a year are produced, and much of this milled for short fibres.

To define the relative hazards, a world-wide survey is being organized by the International Union against Cancer (UICC), with the active participation of the MRC Pneumoconiosis Unit at Penarth, Glamorgan-shire, where one of the South African researchers, Dr. J. C. Wagner, is now working. To help in comparable laboratory experiments, an international asbestos dust bank of standard samples of the economically important asbestos types has been established. For the epidemiological research it is necessary to go to the asbestos mining countries to look at the communities exposed to only one type of asbestos fibre. Surveys are already in hand in South Africa (crocidolite, chrysotile, amosite), Finland (anthrophyllite) and Canada (chrysotile), and it is hoped that the other chrysotile producers, the Soviet Union and Cyprus, will soon join in.

Follow-up studies of industrial populations where a single type of asbestos has been used are also valuable. In Germany crocidolite was extensively used in the construction of the First World War Grand Fleet. 4,000 people worked in a crocidolite factory in London, in the early 1900s, and Californian shipyards where amosite was employed in World War II. British follow-up studies have been greatly hampered by the eccentric mode of national record keeping. It has taken four years to trace 98 per cent of the men from the London factory, and many of the women employed have escaped the net completely because records lose track of

them at marriage. The Director of the Unit at Penarth is severe on the subject: "In this country we don't make use of modern methods in either record-keeping or retrieval" and compared the situation unfavourably with the United States and Finland—Finland in particular provides "magnificent death certificates". "At present, the Ministry of Health is not interested in occupational disease," he said.

## Pugwash in Australia

DR. PATRICIA LINDOP writes: The first South-east Asian Regional Pugwash Conference was held in Melbourne from January 23–27, when scientists from Australia and New Zealand, Japan, Indonesia, Malaysia and Ceylon, India, Pakistan and Singapore discussed the application of science, technology and industry to the development of the region. The conference was yet another experiment and step forward in the evolution of the Pugwash Movement.

During the past ten years, the chief Pugwash activity has been in international conferences discussing, particularly in an East–West context, problems arising from development of weapons of mass destruction, and ways towards reducing the likelihood of their use, by disarmament. Some attention has also been given to the other potential source of international tension—the increasing disparity between the developed and developing nations. It was felt that to tackle this problem effectively, and within the available resources of time and money, it was necessary to deal with some of the problems on a regional basis. The need to discuss regional development as well as South-east Asian security made the initiative of the Australian scientists in organizing this first meeting most timely. Unfortunately, but perhaps understandably, the Chinese scientists did not come to Australia, although it is hoped that they will be interested in future regional meetings.

The meeting itself set one again wondering what exactly is Pugwash and its effectiveness? Each individual scientist probably looks for or finds something different in Pugwash, and the only common factor is in the desire to prevent the misuse of science. But the Pugwash-type discussion is quite different from other more formal international conferences on the same or similar topics. For example, in Melbourne there was no real discussion of "aid" to neighbouring countries—only of the necessity for co-operative action and mutual education and development. There was objective and constructive criticism of the mechanisms of the existing aid schemes from both donor and recipient countries. A need in all countries was for a scientific committee to vet and co-ordinate co-operative projects in science and technology, and to ensure the better use of scientists and equipment. At present, even in a donor country such as Australia, scientific consultation is on an informal and piecemeal basis. In countries of the region where the scientific cadre is at present too small to have much influence in government planning, the support of scientists from other countries in the region in providing an objective assessment of projects was greatly welcomed. And during the few days of the meeting the atmosphere changed from one of helplessness to a realization that the problems were the same in all other countries in the region, whether economically more or less

developed; and that a co-operative approach with good international communication and consultation in the region could be most effective.

## Cow Green Lost

THE battle for Cow Green was finally lost in the House of Lords on February 23, when the Tees Valley and Cleveland Water Bill was given its third reading. This ended what Lord Grenfell described as an exhaustive and somewhat exhausting inquiry into the Bill sponsored by the Water Board and Imperial Chemical Industries, Ltd., seeking to build a reservoir in Upper Teesdale (*Nature*, 212, 442, 656, and 764; 1966). The committee set up to defend Cow Green, while regretting that it has lost the battle, believes that people are now more aware of the danger to amenity and to botany which schemes of this sort represent. The Defence Committee will now disband, but a wary eye will be kept on developments by a joint working party from the Council for Nature and the Council for the Preservation of Rural England. This group is particularly concerned with water resources, and apart from reporting back to its parent bodies it may also publish its findings from time to time.

## Navigation on Show

UNDER the unsuitably melodramatic title of "Man is not Lost" the National Maritime Museum has launched an exhibition to celebrate the bicentenary of the first issue of the Nautical Almanac and Astronomical Ephemerides for 1767. These tables enabled seamen to determine their position at sea accurately by measuring the angular distance of the Moon from certain fixed stars, and until Harrison's chronometer came into widespread use in the 1830s it was the only satisfactory method of determining longitude. Although vitally important in the history of navigation, the Nautical Almanac is not the most interesting subject on which to base an exhibition, and the organizers are to be complimented on the way they have tackled the problem. Choosing to stress the historical importance of the Almanac they have set the exhibition out under the following headings: the founding of the Royal Observatory of Greenwich in 1675; the compilation of the Nautical Almanac by Maskelyne in 1765 and its publication in 1767; the development of the method of position lines for determining latitude and longitude in the mid-nineteenth century; modern methods, which began to be used in 1914, and which included the applications to air navigation in 1937; and astronomical navigation today. The exhibits are all intelligible and well presented, if sometimes incompletely supported by information. The outstanding exhibit is a model which the visitor can operate to measure lunar distances in the same way as they were measured in the past.

## Floral Stamps

IN its choice of designs for the latest set of eight special postage stamps depicting the British flora (on sale on April 24) the Post Office has reached a compromise between the demands of minuscule design and those of botany. The Rev. W. Keble Martin, whose designs for

the new 4d. stamps were selected, is a keen amateur botanist who has been drawing flowers for sixty years. Mary Grierson, who designed the 9d. and 1s. 9d. stamps, is the official artist at the Royal Botanic Gardens, Kew.



The artists were asked to submit designs showing familiar members of the British flora. Their final choice was dictated by the need to depict flowers which would be clearly visible against a white background when reduced to the size of a postage stamp. The results are perhaps not every ecologist's dream of a representative selection of the British flora, but hedgerows, woods, fields and waste places—all common British habitats—are well represented, if rather sedately, by twelve familiar flowers. The stamps show flowers rather than whole plants, for extraneous leaves and stems have been eliminated, and this also applies to the names of the plants, omitted for reasons of design and to avoid taxonomic repercussions.

## Molecular Weights without Tears

by a Correspondent in Molecular Biology

THE development of photoelectric absorption optics for the analytical ultracentrifuge has for the fortunate possessor of such equipment remarkably simplified the problems of molecular weight determination, and makes it possible to perform accurate measurements on unprecedentedly small (microgram) quantities of proteins and other substances. In this system, the ultracentrifuge in effect incorporates a double-beam spectrophotometer, the sample and reference (solvent) being placed in the two compartments of a double-sector centrifuge cell. By scanning the contents of the cell at a wavelength at which the solute absorbs light, the complete concentration distribution can be obtained. At sedimentation equilibrium this leads at once to a molecular weight; the tedious routine of a separate concentration run (which is required by other optical systems that measure only concentration gradients) and dialysis of the solution against solvent are obviated. Moreover, unless the extinction coefficient of the solute is low, the concentration required for the measurements is so small that no extrapolation to infinite dilution is needed, and a single experiment suffices for the determination. This and other benefits accruing from the use of this technique, such as possibilities for the study of ligand binding, have been explored by Schachman and his associates.

In the latest paper in this series, Edelstein and Schachman (*J. Biol. Chem.*, 242, 306; 1967) now describe



how a single experiment with photoelectric absorption optics can yield both partial specific volume and molecular weight. The former quantity is required for all ultracentrifugal molecular weight procedures. Its independent measurement requires a substantial amount of material, and solutions of precisely known dry-weight concentration. For proteins, a moderately reliable value can be calculated if the amino-acid composition is known and if there are no prosthetic groups; in many cases, however, and especially perhaps for nucleic acids, the partial specific volume is arguably the limiting factor in molecular weight determinations.

Edelstein and Schachman measure in a single experiment the sedimentation equilibrium distribution in  $H_2O$  and  $D_2O$  solutions of identical concentration in separate cells. They show that a correction, which is effectively the same for all proteins, can be applied to account for the small change in molecular weight resulting from hydrogen-deuterium exchange. From the standard equilibrium plots ( $\ln c$  against  $r^2$ ) for the two solutions, the partial specific volume is easily derived, and an accuracy of 1 per cent is reported. The precision of the method can be further enhanced if  $D_2^{18}O$  is available as a solvent; for a typical protein the slope of the equilibrium plot is then nearly halved compared with  $H_2O$  solution. Values of the partial specific volume and molecular weight obtained by these procedures for adenosine and a range of proteins are in remarkable agreement with accepted data. Edelstein and Schachman point out that their method can be applied even in the presence of other sedimenting species if the substance under study happens to have an absorption band in a separate region.

In referring to the work of Simon and Konigsberg (*Proc. Nat. Acad. Sci., U.S.*, **56**, 749; 1966) in this column on December 10, 1966, their conclusions were incorrectly represented. Whereas the first report by these authors (*Fed. Proc.*, **25**, 648; 1966) stated that cross-linked haemoglobins had been prepared which were incapable of dissociating into sub-units, the later article instead reports intramolecular cross-linking, and no bonds between sub-units. The annihilation of the haem-haem interactions evidently results therefore from the chemical disturbance of the  $\beta$ -chains as such and not from inhibition of sub-unit exchange.

## Survival of Starved Bacteria

by a Correspondent in Microbiology

ORGANISMS might be expected to respond to adverse environments by a suspension of synthetic activities so as to become more independent of their surroundings but, in practice, bacteria rarely respond in this way. The synthesis of cell components may continue, possibly at altered rates, during prolonged starvation. Continued synthesis under starvation conditions leads to aberrant protoplasm which may ultimately prove lethal. In the current number of the *Journal of Bacteriology* are published a series of papers by Kennell, Kotoulas and Marchesi (*J. Bact.*, **93**(1), 334, 345 and 357; 1967) which discuss various aspects of nucleic acid and protein metabolism in starved *Aerobacter aerogenes* cultures. Starvation conditions were established by harvesting exponentially growing cells and

resuspending them in the growth medium lacking magnesium. Although natural environments are probably rarely or never deficient in  $Mg^{++}$ , this starvation system was studied because on present evidence magnesium is the most frequent metal co-factor for metallo-enzymes and is intimately concerned with ribosome stability.

In the  $Mg^{++}$  deficient medium, *A. aerogenes* continued synthesizing nucleic acids and proteins for more than 70 hours provided that the source of carbon was not limiting. During the initial 24 hour period, the DNA content of the culture increased ten-fold, but the viable count showed only a three-fold increment during the first few hours and thereafter remained approximately constant. The ribosome population became "frozen" when the cells were incubated in the starvation medium. This constancy was maintained by the rate of ribosome degradation (induced by  $Mg^{++}$  deficiency) being paralleled by an equivalent rate of synthesis. Ribosome degradation was sequentially ordered and particles derived from degradation did not appear to be exchangeable with those in the process of assembly. Ribosomal proteins which had been synthesized during the preceding phase of growth in the complete medium were lost steadily during starvation and accounted for the total protein loss from the starving cells. Magnesium starvation of uracil-requiring strains of *A. aerogenes* produced confirmatory results: during uracil starvation a smaller fraction of the synthesized proteins were ribosomal and the amount of protein which decayed during the subsequent  $Mg^{++}$  starvation was proportionally reduced.

Cytochemical changes accompanying  $Mg^{++}$  starvation are also described by these authors (*J. Bact.*, **93**(1), 367; 1967). A progressive decrease in cell size occurred during the initial period when an expanding viable population was paralleling DNA synthesis. However, the subsequent phase of continued DNA synthesis and constant viable population showed cells becoming elongated into filamentous morphologies and, in addition, a second population of very small cells appeared. The latter, probably the result of disparate cell division, comprised an increasing proportion of the total population but (i) were unable to grow or divide when returned to complete media, and (ii) were devoid of biosynthetic activity as assessed by uracil- $H^3$  incorporation into nucleic acids. Furthermore, these cells appeared almost completely lacking in ribosomes. After 20 hours of starvation, the very small cells accounted for more than 80 per cent of the population and were enough to explain the disparity between the total DNA content and the viable complement of the starved culture.

A continuation of normal cell division throughout starvation would lead to a diluting out of the ribosomes in the population so that the number per cell would be insufficient to maintain the metabolic potential and viability of the population. Kennell and his colleagues conclude that the very small cells arising from this apparently inevitable division activity are sacrificed in order to preserve a line of viable cells—the filamentous cells. The regulation of such a differential survival mechanism is an intriguing problem and it remains to be seen whether this type of differentiation is general in starving bacteria under different starvation conditions.

## Parliament in Britain

MR. R. MAUDLING, in opening a debate in the House of Commons on February 23 about the proposed increase in fees for overseas students, once again questioned the magnitude of the anticipated savings of £2.5 million per year. The Secretary of State for Education and Science, Mr. A. Crosland, who followed, gave figures as to the increase in the subsidy to overseas students over the last 10 years. He stated that over 95 per cent of the fees of undergraduates were paid out of local authority awards, and claimed that to increase the fees of all British university students to the point where they represented 20 per cent of recurrent costs as proposed by the Robbins Committee and the Estimates Committee would involve an addition of £8 million to the rates. There was a consensus of opinion that the number of overseas students should be maintained at least at its present level, and in winding up for the Government, Mr. G. Roberts could only add that what had been said would be carefully considered and that the total grant to universities could assume an increase of £250 per annum per overseas student.

In reply to questions in the House of Commons later in the week, Mr. A. Crosland gave an analysis of the geographical origin of the 16,256 full-time overseas students at British universities in 1965-66: 1,873 were from Western Europe, 9,081 from the Commonwealth, 1,643 from the United States and 3,659 from other countries. Of those from Commonwealth countries, 1,289 were estimated as from advanced and 7,792 from developing countries. Of the 16,256, 2,189 were supported by United Kingdom public funds; 970 from other United Kingdom funds, including University awards; 2,415 by the Governments of developing countries; 1,906 by other overseas governments and services; and 8,776 were privately financed. In all 10,890 came from developing countries and 5,416 were privately financed. No figures are available of overseas students who hold no awards but 4,855 from Commonwealth countries were privately financed.

In a written answer in the House of Commons on February 13, the Minister of State, Mr. G. Roberts, gave the expenditure of the Medical Research Council in 1963-64, excluding capital expenditure, specifically on cancer research as £958,000, of which £531,000 was on the Council's units and external staff, £373,000 on grants to university research workers and £54,000 on grants to other research workers; for 1964-65 the corresponding figures were £1,029,000, £548,000, £415,000 and £66,000; for 1965-66, £1,178,000, £643,000, £461,000 and £74,000; and for 1966-67, £1,270,000, £696,000, £484,000 and £90,000. No estimate could be given of the proportion of Government funds applied to this purpose from grants given on the advice of the University Grants Committee to universities and medical schools or of the amount spent by hospitals within the National Health Service.

BARONESS PHILLIPS, answering a question in the House of Lords, said that in 1965-66 there were 2,471 undergraduate students of agriculture and 562 post-graduates in British universities. Rationalizing the teaching of agriculture in Britain was the responsibility of the U.G.C., she added.

## University News:

### Aston in Birmingham

DR. E. BRAUN, at present head of the Department of Physics in the West Ham College of Technology, has been appointed professor of physics as from September 1.

### Birmingham

THE personal title of professor of comparative endocrinology has been conferred on Sir Francis Knowles, reader in comparative endocrinology at the university.

### Cambridge

DR. P. W. ANDERSON of the Bell Telephone Laboratories will be visiting professor in theoretical physics at the Cavendish Laboratory in the University of Cambridge from October 1967. During his stay in Cambridge he will teach only two terms out of three each year so that he can maintain his relationship with the Bell Telephone Laboratories. Dr. Anderson works principally in solid state physics.

### Edinburgh

DR. CHARLOTTE AUERBACH, at present reader in the Department of Animal Genetics, has been appointed to a personal chair in genetics, with effect from October 1.

### Manchester Institute of Science and Technology

DR. L. R. G. TRELOAR, at present reader in the Department of Polymer and Fibre Science, has been appointed to a newly established chair of polymer and fibre science.

### Nottingham

DR. J. S. L. LEACH, at present reader in physical metallurgy in the Imperial College of Science and Technology, has been appointed to the Cripps chair of metallurgy, and to the headship of the department.

## Announcements

THE Council of the Institute of Physics and the Physical Society has made the following awards for 1967: *Guthrie Medal and Prize* to Sir James Chadwick, formerly Lyon Jones professor of physics in the University of Liverpool and Master of Gonville and Caius College, Cambridge; *Glazebrook Medal and Prize* to Sir Charles Sykes of Firth Brown Limited; *Thomas Young Medal and Prize* to Professor D. Gabor of Imperial College of Science and Technology, London; *Charles Chree Medal and Prize* to Dr. J. H. Chapman of the Defence Research Telecommunications Establishment, Canada; *Duddell Medal and Prize* to Dr. K. D. Froome and Mr. R. H. Bradsell of the National Physical Laboratory; *Charles Vernon Boys Prize* to Dr. A. H. Cook of the National Physical Laboratory; *Bragg Medal and Prize* to the late Mr. D. McGill, at the time of his death organizer of the Physics Section of the Nuffield Foundation Science Teaching Project.

THE Council of the Edinburgh Geological Society has nominated Mr. W. Mykura, Institute of Geological Sciences, Edinburgh, for the Clough Award for 1966/67, in recognition of his geological work in the south of Scotland.

THE British Society of Audiology has recently been established, and the chairman of the provisional council is Dr. J. D. Hood of the Otological Research Unit, National Hospital, Queen Square, London. The aims of the society are to promote learning and advance education in the subject of audiology, to provide a common platform for discussion among the various disciplines involved in audiological work and to promote the status of audiology as a discipline. Further information concerning the society may be obtained from Dr. L. Fisch, Hearing Clinic, Vicarage Farm Road, Heston, Middlesex, or Mr. A. Boothroyd, Department of Audiology, University of Manchester, Manchester 13.

## 1967 GORDON RESEARCH CONFERENCES

	Colby Junior College, New London, New Hampshire	New Hampton School, New Hampton, New Hampshire	Kimball Union Academy, Meriden, New Hampshire	Tilton School, Tilton, New Hampshire	Proctor Academy, Andover, New Hampshire	Crystal Inn, Crystal Mountain, Washington
June 12-16	Hydrocarbon chemistry	Molecular electronic spectroscopy	Science and Technology of biomaterials	Animal cells and viruses		
June 19-23	Nuclear chemistry	Nucleic acids	Magnetic resonance	Biochemistry and agriculture	Lasers in medicine and biology	
June 26-30	Catalysis	Proteins	Cell structure and metabolism		Lipid metabolism	
July 3-7	Polymers	Coal science	Coenzymes and metabolic pathways	Chemistry of heterocyclic compounds	Lysosomes	Environmental sciences; air
July 10-14	Textiles	Statistics in chemistry and chemical engineering	Chemistry, physiology and structure of bones and teeth	Chemistry and physics of space	Biomathematics	Chemistry and physics of isotopes
July 17-21	Scientific information problems in research	Radiation chemistry	Physical metallurgy	Chemistry and physics of coatings and films	Chemistry and metallurgy of semiconductors	Molecular pathology
July 24-28	Corrosion	Organic reactions and processes	Chemistry at interfaces	Microbiological deterioration	Chemistry and physics of paper	Dynamics of quantum solids and liquids
July 31-Aug. 4	Elastomers	Steroids and other natural products	Solid state studies in ceramics	Nuclear structure physics	Chemistry and physics of liquids	Medicinal chemistry
August 7-11	Separation and purification	Inorganic chemistry	Toxicology and safety evaluations	Organic photochemistry	*	Plasma physics
August 14-18	Food and nutrition	Analytical chemistry	Chemistry and physics of solids	Photonuclear reactions	*	Laser interaction with matter
August 21-25	Ion exchange	Geochemistry	Chemistry and physics of cellular materials	Thin films	*	
Aug. 28-Sept. 1	Cancer	Science of adhesion	Chemistry of molten salts	Glass	*	

\* Week not available.

THE 1967 Gordon Research Conferences will be held in New Hampshire and Washington, between June 12 and September 1, 1967. Each conference will run from Monday to Friday each week, with meetings in the mornings and evenings. Application forms for the conferences can be obtained from W. G. Parks, Director, Gordon Research Conferences, Department of Chemistry, University of

Rhode Island, Kingston, Rhode Island 02881, and all application forms should be returned at least two months before the date of the conference. The conferences are intended to bring together scientists for discussions in the latest developments in each field, and not for the review of known information.

A NEW journal entitled "Thin Solid Films", designed to cover all aspects of the science and technology of thin solid films, is now being published by Elsevier Publishing Company.

THE thirty-second Parsons Memorial Lecture entitled "The Measurement and Control of Small Displacements", organized by the Institute of Physics and the Physical Society, will be given by Professor R. V. Jones on April 24 at the Royal Radar Establishment, Malvern. Further information can be obtained from the Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

ERRATUM: In the communication entitled "Production of Oat Callus and its Susceptibility to a Plant Parasitic Nematode" by Dr. J. M. Webster (*Nature*, 212, 1472; 1966), the third sentence in the second paragraph should read "Glucose (20 gm/l.) or the same quantity of sucrose was used . . .".

ERRATUM. In the article entitled "Metabolism of Methylcarbamate Insecticides by the NADPH<sub>2</sub>-requiring Enzyme System from Houseflies" by M. Tsukamoto and J. E. Casida (*Nature*, 213, 49; 1967), the following corrections should be made: page 49, left-hand column, line 18, mammalian liver microsome-reduced nicotinamide adenine dinucleotide phosphate (NADPH<sub>2</sub>) system; line 23, the liver microsome-NADPH<sub>2</sub> system; line 26, insect microsome-NADPH<sub>2</sub> systems; line 37, an insect enzyme-NADPH<sub>2</sub> system. Page 49, right-hand column, line 14, the abdomen-NADPH<sub>2</sub> system; line 17, twenty-four insecticides and synergists labelled with carbon-14 were investigated. Page 50, right-hand column, line 59, the fly abdomen-NADPH<sub>2</sub> system; line 67, O-O-dimethyl. Page 51, left-hand column, line 16, (+)-trans-chrysanthemumate; line 17, allethronyl; line 21, the fly abdomen-NADPH<sub>2</sub> system; line 25, (5-allyl-1-methoxy- . . .). Page 51, right-hand column, line 2, chemicals; line 13, homogenate or microsomes of the resistant fly abdomen. Reference 7, *The Physiology of Insecta*.

## CORRESPONDENCE

## Economic Geochemistry

SIR,—In the note on January 7 (*Nature*, 213, 7; 1967), the statement that geochemistry has limited application and is still struggling for recognition is entirely erroneous.

The facts of the matter are that since the Second World War geochemical techniques have developed to the point that they are standard exploration practice in most parts of the world. At a conservative estimate, geochemical samples are being collected for prospecting purposes at a rate well in excess of 3,000,000 a year in the West, and probably nearer 10,000,000 a year in the Soviet Union.

Mineral discoveries, wherein geochemical prospecting has played a vital role, include base metal deposits in Eastern Canada and important copper mineralizations in Central Africa and the South-west Pacific area, to mention but a few.

In the academic field, applied geochemistry is now included in any comprehensive course of training in mineral exploration and a number of research centres exist in both government establishments and in the universities at home and overseas.

The foregoing in no way implies any criticism of the technical publication you mention, which gives a most interesting description of a novel method of prospecting for use in a particular type of terrain. I am quite certain that the authors would in no way subscribe to the view expressed in that part of your note concerned with the current status of applied geochemistry in general.

Yours faithfully,

JOHN S. WEBB

Department of Geology,  
Imperial College of Science and Technology.

# Who is Carl Zeiss?

by our Special Correspondent

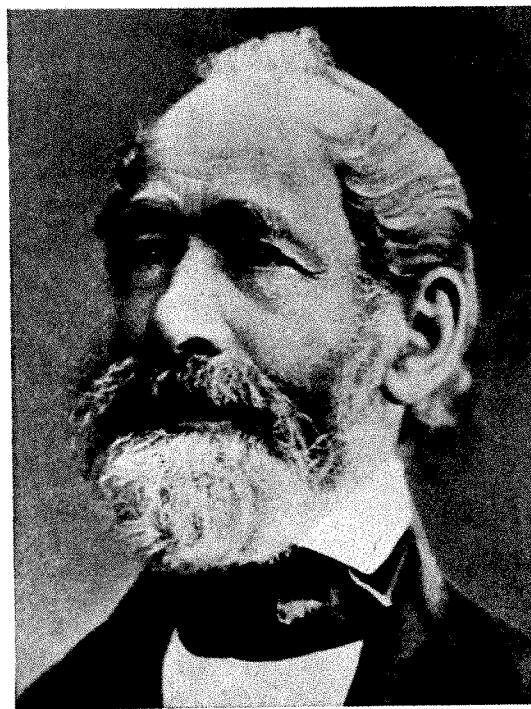
*Jena, February.*

As a source of street names in eastern Europe, Karl Marx is rarely challenged. In the East German town of Jena, however, he is outdone by two local figures, Carl Zeiss and Ernst Abbe. Together these two created the firm of Carl Zeiss of Jena, manufacturing optical and fine mechanical instruments. Under Abbe's influence the company became philanthropic, and fits comparatively easily into the socialist system, but it has a history of adapting to circumstances. Before the Second World War, it supplied equipment to the German armed forces, as the present managers gloomily admit. Since then, they insist, no military equipment has been made at Jena. The company, now nationalized, makes 4,000 items (excluding its range of 200,000 spectacle lenses) and sells them throughout the world, despite considerable harassment in capitalist countries.

The instruments made by Zeiss of Jena fall into two categories, optical and metrological. The optical glass is made at Jena, by processes jealously guarded but which seem to owe more to 120 years of empiricism than to modern control technology. Apart from its well known microscopes—the latest, the all purpose “Nu”, operates either with reflected or transmitted light (or with both at once), has a pancratic (zoom) lens and all the usual facilities, and sells for about £2,000 in the United Kingdom—the company produces surveying instruments, refractometers, interferometers spectrophotometers, and the like. Zeiss also make binoculars and cameras, and a range of measuring equipment less directly useful to scientists.

Zeiss of Jena find it more than usually hard to trade with the West. In addition to the indignities caused by cold war diplomacy, there is confusion, to say the least of it, over the use of the Carl Zeiss name. When Jena became part of the Russian sector, a number of Zeiss workers, encouraged by the departing Americans, defected to the West. Claiming the right to move the Carl Zeiss Foundation to Heidenheim, a small town near Ulm in West Germany, the emigrées started production in Ober Kochen, and were supported by a ruling from a West German court. The range of equipment produced by Zeiss of Ober Kochen is similar but, says Jena, less comprehensive. After a difficult start, the Ober Kochen concern has flourished, aided by Jena's early pre-occupation with the eastern market. In recent years Jena has been expanding its business in the West, and this has brought the conflict into the open.

Judgments in favour of the Ober Kochen concern in West Germany, Austria and the Netherlands are balanced by judgments in favour of Jena in East Germany, Czechoslovakia and Yugoslavia. Ober Kochen does not market its products in eastern Europe, but Jena continue to market in West Germany under the trade name “Aus Jena”. Advertising by either concern, apart from adding to the legal confusion, tends to rebound to the advantage of the other side. In the United Kingdom the battle is in a state of suspended animation, after a High Court decision last year establishing the right of Jena to be represented. Ober Kochen had argued that the absence of diplomatic relations between Britain and East Germany prevented legal representation of Jena in a British Court. Jena, predictably, is full of praise for the British judicial system, and may feel sufficiently encouraged to go ahead with a full scale civil action. In the



United States the Ober Kochen concern is dominant, because Jena, unable to obtain visas to send service engineers, will sell only their smaller equipment there. In Canada, the city of Montreal was recently presented with an Ober Kochen planetarium, while the Royal Ontario Museum bought one from Jena.

Carl Zeiss of Jena occupies a special position in the East German economy. Nationalized in 1949, it employs more than 20,000 people and is the largest centre of research and development in the country. Unlike most East German concerns, it operates its own overseas marketing organization, and its success—it is the largest earner of foreign currency in the country—may enable other East German companies to obtain the same privileges. In the home market it is obliged by law to make a profit on every item it sells, but abroad this control is lifted, and Jena is able to undercut competitors, sometimes by as much as 30 per cent.

Research at Jena seems concentrated in product development. Ernst Gallerach, Director General of the company since August 1966, refuses to make any distinction between pure and applied research. “Pure science does not exist; it exists only when implemented”, he says. This means that while the firm retains its traditional strength in classical optics, it is slow to adopt anything newer. Lasers and fibre optics have been taken up rather half-heartedly, but holography is dismissed as an academic toy. For market research, the company relies on careful reading of the literature, conversations with customers and information from university scientists in East Germany. It needs to be convinced that an instrument is thoroughly reliable and commercial before it will set about designing it, and hence tends to leave important basic research work to companies in the West. The company has recently been re-organized, and as part of this plan is buying a computer from International Computers and Tabulators. Western computer manufacturers need not reach for their passports, however; Mr. Gallerach says that most future purchases by East Germany will be from the Soviet Union.



# A New University in Scotland

by our Special Correspondent

THE newest of the new universities to be created from nothing in Britain will receive its first students in September this year. The university is not English but Scottish. Its plans for academic development in the next few years are important not merely because the University of Stirling is well placed in time to profit from the lessons which have been learned in setting up other new universities in Britain, but also because those in charge, and particularly the Vice-Chancellor, Dr. T. L. Cottrell, are anxious that there should be a close integration between the teaching of science and the teaching of other material. In particular there is to be a course in technological economics intended to weld together an awareness of physical science and an awareness of the processes of applying science in industry. Although other universities in Britain are trying out schemes to leaven the teaching of science with matter from other disciplines, particularly economics, the plans at the University of Stirling go further than those elsewhere.

The university itself plans to start in a comparatively small way, with fourteen professors (most of whom have now been appointed). The first cohort of students will number a mere 150, plus 30 graduate students. The university is being created physically around a lake in a heavily wooded part of the Scottish countryside two miles north of Stirling. Fig. 1 shows the site of the university before building had begun. The university will differ from others in Britain not merely in its concern to have a course integrating science and industrial affairs but also in more general ways. For example, it is planned that the pattern of teaching shall be broken up into semesters each consisting of a 15 week period, one before and one after the winter vacation. In addition students will be required to spend at least two weeks at work in the university during the winter vacation. The result will be that if a student is to qualify for a degree, it will be possible to ask that he should have completed a certain number of semester courses.

Outwardly this means that the organization of the University of Stirling will resemble in some ways the pattern of academic life in many universities in the United States. In practice, the Vice-Chancellor intends that the contact between students and academic staff, and in particular the thoroughness of supervision, will make it possible to avoid any sense that the acquisition of seminar-courses or units will be a process comparable to the acquisition of trading stamps and other such tokens.

With luck, it is hoped that each semester course will occupy roughly the same amount of the student's time. Students will be helped to choose suitable courses by a director of studies. There will be major and subsidiary courses, the first occupying three consecutive semesters and the second two semesters. There will also be minor courses occupying merely one semester. In addition it is planned that every student shall be put through a course in "Approaches and Methods" largely concerned with the use of libraries and bibliography, communication, and what are called in the prospectus "rhetoric, logic and scientific method, statistics and social arithmetic". To complete Part I of a degree, a student will have to complete eight semester courses, which may for example mean



Fig. 1.

two major subjects and a subsidiary subject or—at the other extreme of diversity—one major, one subsidiary and three minor courses.

The second part of the degree course will make the total time three years for the general degree and four for the honours. The plans for technological economics are intended to cater for the education of people who may become administrators, operational researchers, or scientists working in industry. Naturally, the university provides more orthodox courses in biology, physics, chemistry and mathematics, and it will be interesting to see how great the demand is for the integrated course. Those taking the course in technological economics may begin with a school background of science specialization or may have had no science at school but only mathematics. All students following this programme will take a major course in economics in their first three semesters (1.5 years), two semesters of mathematics and a minor course in a subject with "introduction to techniques of industrial science" in the third semester of their stay at the university. In addition, they will follow a major course in physics, chemistry or biology or—if they have no background of science at school—in the major course "Introduction to Physical Science". The design of the Part I course is intended to enable students to switch for Part II either to a science subject or to economics if they so choose. Those wishing to continue with technological economics will finish off their degree with mathematics and statistics, a further study of economics, a continuation of their Part I science course, and special studies of industrial techniques and industrial projects.

In the planning of the course the Vice-Chancellor and his Academic Planning Board have had in mind the need somehow to train people who are skilled in science and also aware of the industrial importance of their subject. These people could go into works investigation teams, or economic planning departments in science-based industry, but there may be many other openings because, in general industry, commerce and public administration are in need of people who have a foot in each camp. But this part of the plan which the University of Stirling has designed for itself will be determined as much by circumstances, the inclinations of students, and the pedagogical innovations of the staff as by the aspirations of the planners. It is, in other words, an important and potentially illuminating experiment.



# Tertiary Structure of Ribonuclease

by

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A model is proposed of the polypeptide chain in bovine pancreatic ribonuclease based on a 2 Å electron density map involving 7,294 reflexions and data from seven heavy atom derivatives. The molecule seems to be roughly kidney shaped with dimensions of about  $38 \times 28 \times 22$  Å with a deep depression in the middle of one side. The model disagrees with that based on a 5.5 Å map suggested by Dr. C. H. Carlisle *et al.* (*Nature*, **213**, 557; 1967).

We started our investigations of the tertiary structure of the enzyme ribonuclease in about 1950 at the Protein Structure Project at the Polytechnic Institute of Brooklyn and have continued them in the Biophysics Department of the Roswell Park Memorial Institute since 1959. During the past four years, they have resulted in three dimensional electron density distributions of the protein molecule in the crystalline state; these maps progressively showed more detail as more X-ray diffraction data at higher resolution were used. Recently we have calculated a map at 2 Å resolution, using the data from the free protein crystal and from seven heavy atom derivative crystals. We believe that this map clearly shows the structure of the molecule. Comparison of this map with the primary structure of the protein, as elucidated by biochemical methods during the past few years, makes it possible to locate the amino-acid residues of which the molecule is made. Many of these residues, including four cystine disulphide bridges, can be located unambiguously. In contrast to myoglobin, the absence of any appreciable amount of  $\alpha$  helix makes a complete description of this molecule difficult; it must await a more detailed map. The purpose of this article is to show the general course of the polypeptide chain in the ribonuclease molecule, and to describe some features of biochemical relevance. We have also reason to believe that we have located the active site of this enzyme molecule.

## Primary Structure

All our X-ray diffraction investigations have been carried out on bovine pancreatic ribonuclease. The primary structure of this protein has been determined<sup>1</sup>, so that we know the number, nature and sequence of the amino-acids with a high degree of certainty. This covalent structure is shown in Fig. 1 and it can be seen that the protein contains a single polypeptide chain of 124 residues in a specific sequence. This chain is internally cross-linked by four disulphide bridges. The molecule does not possess any free SH or similar groups which facilitate specific heavy atom tagging for X-ray studies. Much information<sup>2</sup> from chemical investigations suggests probable non-covalent interactions between the side groups, when the main chain is folded up into its native configuration; some of these interactions seem essential for the integrity of the active centre.

## X-ray Data

Crystals of ribonuclease used in these investigations were grown from 55 per cent 2-methyl-2,4-pentanediol (MPD) at pH 5.0 (usually in the presence of phosphate buffer) and were monoclinic in space group  $P2_1$  with lattice constants shown in Table 1. There are two ribonuclease molecules per unit cell and the unit cell contains about 40 per cent by weight of the solvent. The molecular weight based on the covalent structure is 13,683.

The diffraction data were collected using either the Eulerian cradle<sup>3</sup> on General Electric diffractometers

Table 1. MODIFICATION II SPACE GROUP  $P2_1$ ,  $Z=2$ 

		<i>a</i> (Å)	<i>b</i> (Å)	<i>c</i> (Å)	$\beta$	Intensity measure- ments completed 1/19/67 (Å)
Free protein (STD)	(3P65)	30.13	38.11	53.29	105.75	1.8
Cis-diglycine pt.	(9P12)	29.87	38.39	53.19	105.95	2.0
K <sub>2</sub> PtCl <sub>4</sub> + D-serine	(9I22)	30.08	38.28	53.23	105.66	2.0
UO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub> + L-valine	(9I24)	30.13	38.18	52.77	105.53	2.4
UO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub> + arsenazo	(9P28)	30.09	38.12	52.81	105.55	2
Sodium arsenate	(K2-P9)	30.11	38.01	52.95	105.70	2

(XRD-3, XRD-6) or, later, the G.E. goniostat. Copper  $K\alpha$  radiation was used ( $\lambda=1.5418$  Å). Earlier, the arcs were manually set from precomputed tables using the stationary crystal, stationary counter method, counting the X-ray quanta at the peak position, with intervening balanced pairs of nickel or cobalt filters (Ross filters); each counting time was 10 sec. Approximate absorption corrections were applied to the intensities. During the past year some of the high resolution data used in these investigations were measured using similar techniques but by setting the arcs by an automated XRD-5 with prepunched cards containing arc settings for reflexions sorted in an order suitable for efficient collection of data. Statistical studies of data collected from the same or similar crystals indicated an accuracy of 4–5 per cent in  $|F|$  for reflexions within the 3 Å resolution sphere. In the 3–2 Å range, however, the overall reproducibility fell to about 10 per cent, mainly as a result of the comparative weakness of many of the reflexions. A copper target was operated at 20 m.amp and 40 kVp, was used as a source of radiation. Wherever possible, measurements were extended to Bijvoet reflexion pairs to take account of anomalous dispersion.

## Structure Determination

Attempts were made to determine the structure of ribonuclease by means of the method of multiple isomorphous series<sup>4</sup>—a technique which had led to the solution of the myoglobin<sup>5</sup> and lysozyme<sup>6</sup> structures. A search for suitable heavy atom derivatives, conducted over the past few years, revealed a few likely ones, some of which, though satisfactory for low resolution work, were either not easily reproduced or not suitable at 2 Å resolution. Thus not all the derivatives used in computing the 4 Å map except the *cis*-diglycine platinum (CDG) were used in the later investigations. This (CDG) derivative contained two main heavy atom sites per molecule; anomalous scattering measurements were made in this case. The 2 Å map also included isomorphous derivative data from five other crystals and limited data to 3 Å from *tris*-(ethylenediamine) platinum (IV) chloride (TEP) for which the anomalous scattering data had been measured earlier.

During the course of the past 3 years, electron density maps were prepared at four resolutions and a summary of these maps is given in Table 2. It is emphasized that some of these derivatives had heavy atoms in similar positions, and the similarity of the derivatives in some

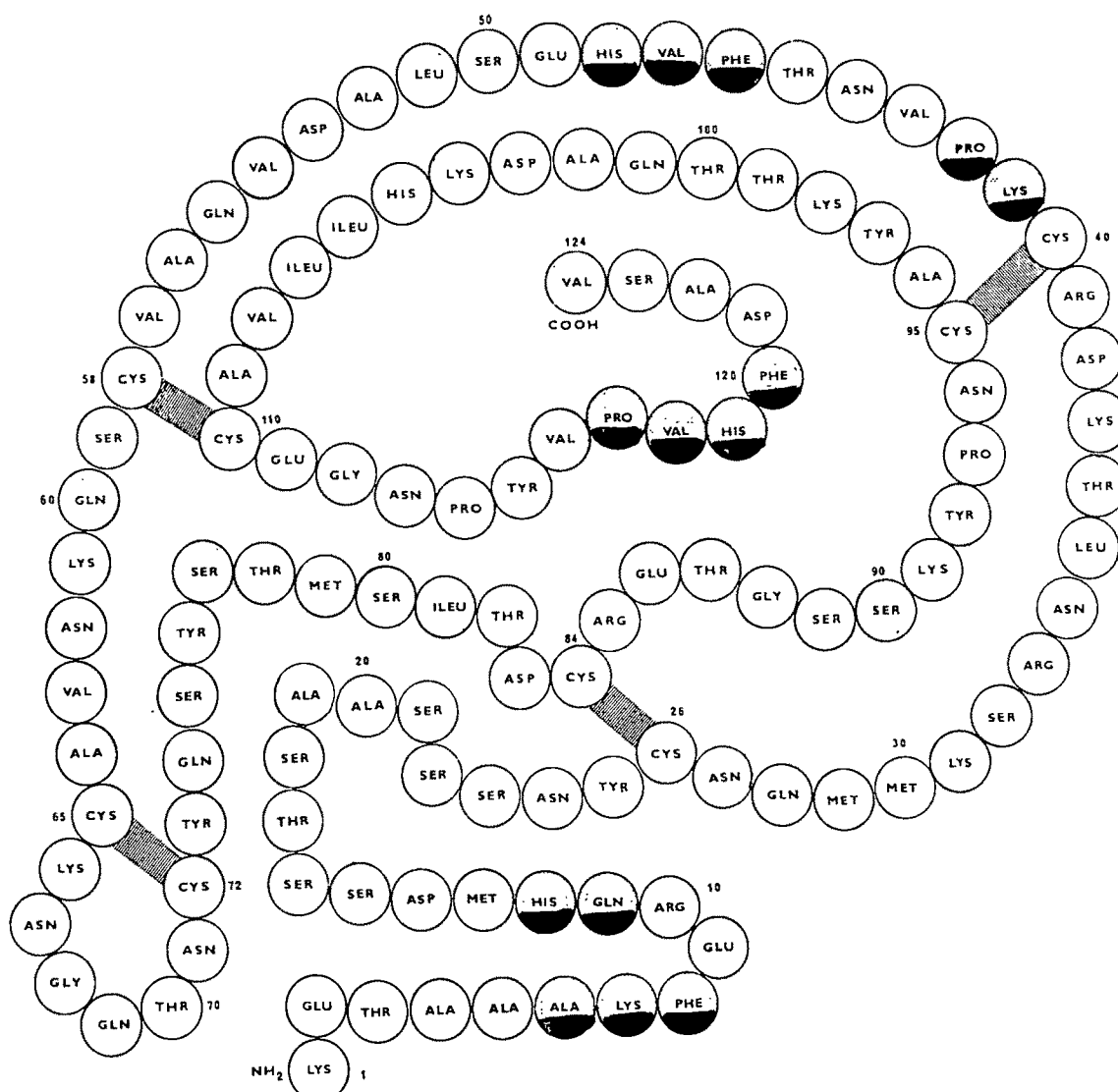


Fig. 1. Covalent structure of bovine pancreatic ribonuclease. (After ref. 1.)

pairs is so great that one could even have replaced the two independent sets of results by a single one. In fact this was done for the two sets of data for CDG derivatives (soaked in different concentration) in the 2 Å map, even though they were treated as independent derivatives in the 2.4 Å map. Table 3 gives a list of the derivatives, the heavy atom sites and occupancies used in the phase evaluation for the 2 Å map. These heavy atom positions were determined and refined by Patterson, difference Fourier and least squares methods<sup>8-10</sup>. The protein phase angles were evaluated by a combination of isomorphous series and anomalous dispersion data<sup>11,12</sup> from the different derivatives, taking care to give proper weights to the

phases obtained using any given derivative, and to the reliability of the data from that derivative. The Fourier maps were computed in *xy* or *yz* sections and the results printed out in coded form suitable for direct contouring on the output sheets from the IBM 7044 computer.

Many features of the molecule are visible in the 3 Å model computed with 2,340 protein reflexions: (a) the position of the amino end which sticks out quite independently of the rest of the molecule; (b) the depression on the surface of the molecule where the phosphate ion is located (as will be shown later); (c) the position of three S-S bridges of high density near which the main chain density shows the topology expected in these

Table 2. PROGRESS IN FOURIER RESOLUTION OF ELECTRON DENSITY MAPS ACHIEVED IN THIS INVESTIGATION

Date	Resolution	No. of "reflexions" used in map	No. of derivative crystals from which data were collected and used in phase evaluation. At higher resolution only some had their anomalous scattering effects measured	No. for which anomalous scattering was also used	Comments
July 1963	4 Å	1,020	7	7	Shown general shape of molecule and also two regions of high density which looked like S-S bridges
June 1964	3 Å	2,340	In addition to above data from seven derivatives, isomorphous series and anomalous scattering data from CDG and TEP	2	Shown molecular boundary, three S-S bridges, amino end of chain and, later, we could also infer the position of the active site from arsenated RNase
Aug. 1966	2.4 Å	4,895	Data from eight derivative crystals including two separate sets of measurements for CDG	1	All S-S bridges now unambiguous. Whole of main chain could be traced with reasonable certainty, except for a few regions of ambiguity
Dec. 1966	2 Å	7,294	Data for seven crystals; the two separate sets of CDG data were combined into one	1	Ambiguities in tracing the main chain are removed and we can locate all residues with a reasonable degree of certainty

\* Each "reflexion" combines information from several derivatives.

Table 3. HEAVY ATOM POSITIONS AND OCCUPANCIES USED IN EVALUATING THE 2 Å MAP

	<i>x</i>	<i>y</i>	<i>z</i>	Occupancy electrons
(1) <i>cis</i> -Diglycine platinum (II) (CDG)				
1	0.207	0.028	0.938	53.7
2	0.410	0.500	0.425	54.9
3	0.285	0.905	0.871	17.9
4	0.252	0.711	0.008	11.2
5	0.483	0.676	0.495	7.3
(2) <i>tris</i> -(Ethylenediamine) Pt (IV) (TEP) chloride				
1	0.126	0.654	0.975	27.4
2	0.216	0.850	0.990	19.0
(3) D-Serine-Pt (II) complex and Pt(en) <sub>2</sub> Cl <sub>2</sub> (PTD)				
Two main sites same as	0.193	0.036	0.940	39.8
two main sites of CDG	0.416	0.500	0.429	38.1
3	0.286	0.905	0.875	39.7
4	0.250	0.715	0.008	11.2
5	0.487	0.500	0.353	9.0
6	0.490	0.681	0.495	6.2
(4) Complex of L-valine with uranyl ion				
1	0.255	0.710	0.008	28.0
2	0.486	0.682	0.506	21.3
3	0.253	0.818	0.495	9.5
4	0.802	0.523	0.061	6.2
5	0.418	0.495	0.430	9.5
(5) Uranyl complex of 1,8-dihydroxy-2,7-bis ( <i>o</i> -arsonaphenylazo)-naphthalene-3,6-disulphonic acid UAZ				
1	0.264	0.710	0.009	46.5
2	0.484	0.678	0.504	39.8
3	0.250	0.808	0.495	19.0
4	0.797	0.534	0.082	7.3
5	0.418	0.500	0.430	15.7
6	0.418	0.558	0.590	11.2
(6) Arsenated RNase NA1				
1	0.443	0.461	0.388	23.5
(7) Arsenated RNase NA2. Basically same as NA1				
1	0.451	0.462	0.386	24.6

regions. It was also seen that the region near the amino end is partly helical and that the molecules are well separated by regions of very low density.

The 2 Å map involving 7,294 reflexions and data from seven derivatives was computed in sections of constant *z* at intervals of *x*/60, *y*/88 and *z*/112—these intervals were

so chosen that the printed output had a scale of 1 cm to an angstrom. No *F*(000) term was added to the series and the contours were drawn on an arbitrary scale of 3 units, with the first contour corresponding to 3. In this scale the disulphide bridges had a density in the range 22–17—the only other peak which had a value of more than 17 in the present map was near the main chain at a region close to the residue 46, and this region had a peak value of 19. The region between the molecules rarely rises to more than contour level of 3 and there are no regions of large negative density.

A schematic drawing of the main chain as deduced from the 2 Å contour map appears in Fig. 2. Starting with the amino end, which was quite easily seen even in the 3 Å map, it is possible to proceed along a ribbon of high density corresponding to the main chain by counting along it at appropriate residue distances and using the positions of bulky side groups as a check. In this way it was possible to proceed up to the carboxyl end of the chain, and the four disulphide bridges and their known positions in the amino-acid sequence in Fig. 1 acted as a good check in case any adjustments were needed. At the scale (1 cm=1 Å) to which the map was drawn it was not easy to recognize all the side groups purely from the shape of the contours, but most of the bigger side chains could be identified by their bulk. It is hoped that the larger scale model now under construction will make it possible to recognize independently many of the side groups by their shapes and bulk. The present map has only been used to trace the course of the α-carbon atoms of the main chain assuming the correctness of the covalent structure of Fig. 1. No attempt has been made at present to check the correctness of the chemical sequence by identifying the side groups from X-ray data alone.

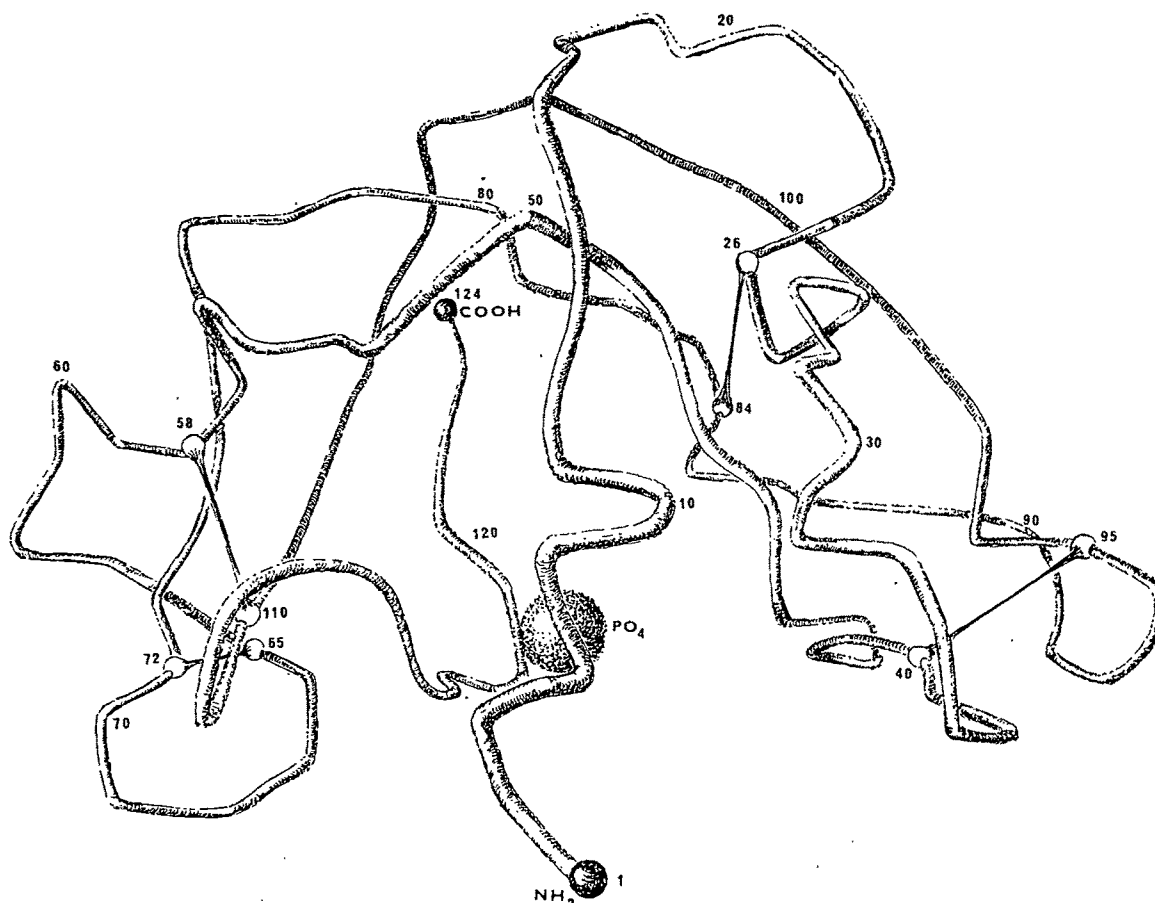


Fig. 2. Schematic diagram of the main chain folding in the ribonuclease molecule. (Thanks are due to Mr. John C. Wallace, who drew from the model.)

### Conformation of the Main Chain

The molecule is roughly kidney shaped with approximate dimensions of  $38 \times 28 \times 22$  Å with a deep depression in the middle of one side. Two of the disulphide bridges (26-84) and (40-95) are on one side of this constriction and the other two (58-110) and (65-72) are close together and on the opposite end. Between these two ends the main chains run in three roughly antiparallel sections; the sections between residues 40-58 and 98-110 running in one direction and region 75-90 running in the opposite direction. Quite clearly the molecule has comparatively low helical content. The only obvious helical segment is about two turns in the region of 5-12 near the amino end and possibly two turns each in the regions 28-35 and 51-58, even though the latter are not quite obvious. There are a couple of other regions where there is a suggestion of helical conformation.

The molecule clearly has a much more exposed structure than myoglobin; the smallest of the dimensions is about 20 Å, and no part of the molecule is shielded from the surrounding medium by more than one layer of main chain. This makes it necessary for some of the hydrophobic side chains which would otherwise be shielded from the solvent to lie near the surface. As a general rule, however, most of the segments which consist predominantly of polar side groups—as, for example, residues 66-71 and 85-91—do show up clearly at the outside of the molecule with their side groups pointing outwards into the solution.

### Location of the Active Site

From an examination of the position of the phosphate ion in crystalline ribonuclease, we now have indirect information about the location of the site of enzymatic activity of the molecule. The position of the phosphate ion was first established by crystallizing ribonuclease from a solution containing, not the usual phosphate group, but the electrostatically very similar arsenate group. The resulting crystal was very closely isomorphous with the phosphate crystal. An electron density difference map computed between the phosphate and arsenate crystals at a resolution of 4 Å, and using the known protein phases, resulted in a single peak per asymmetric unit on a very clear background. The position of the arsenate group was also established independently of any knowledge of the protein phases from a three dimensional difference Patterson map using data from the phosphate and arsenate crystals. The location of this region with respect to the protein molecule showed that the phosphate is embedded in the depression of the kidney-shaped surface of the molecule.

In fact, the isomorphism between the phosphate and the arsenate crystals was so good that, despite the fact that the actual electron density difference between an arsenate and phosphate group is not very large, complete three dimensional X-ray data were collected for the arsenate crystals, and these data were used as heavy atom derivative crystal data for protein phase angle evaluation.

### Comparison with Chemical Evidence

Assuming that the active site is indeed near the location of the phosphate ion—an assumption for which there is much chemical evidence and which we can use as a plausible hypothesis—we can locate the regions of the main chain and the amino-acid residues surrounding it. These residues are shaded in Fig. 1. These regions occur at different parts of the main chain, and detailed examination of the characteristic arrangement creating the required charge distribution, and other side chain interactions in this region, is likely to throw some light on the nature of the active site. Even though in our map we are dealing with an arrangement which results when a phosphate ion is bound at the active site, it is possible that the mechanism of attachment and the conformation

around this region will not be very different when other substrate analogues are bound there.

It is seen that the residues closest to the phosphate are residue 119 near the carboxyl end and residue 12 near the amino end. Both of these are histidines, and much chemical evidence<sup>13,14</sup> indicates their close relationship with the activity of the molecule<sup>15</sup>. Other residues further out, but which might be of importance, are lysine 7, lysine 41, histidine 48, all of which are reasonably close to the phosphate site. It is also seen from the present map that the amino end residues up to 21 or 22 stand out clearly apart from the rest of the molecule, except for the region near about histidine 12 which comes close to the active site forming its third side. This agrees well with the possibility of cleaving off of this part of the chain by the enzyme subtilisin, leaving the rest of the molecule basically undisturbed.

A fuller description of the ribonuclease molecule and a comparison of the intramolecular relationships of the functional group with chemical evidence for active site groups, intermolecular contacts, and solvent interactions are at present being prepared. Furthermore, we are also preparing a fuller description of the heavy atom "dyes", their preparation and mode of use as well as X-ray diffraction techniques used in the solution of the structure.

We thank the Dean Langmuir Foundation, the Rockefeller Foundation, and the Damon Runyon Foundation for providing the initial support of this project, and the National Science Foundation and the National Institutes of Health for continuing support during recent years. The Roswell Park Memorial Institute and the New York State Department of Health, as well as the Roswell Park Division of Health Research Incorporated, all contributed to the success of this venture by providing space and computing facilities.

In the early days of the work on the structure of ribonuclease, important contributions were made by several scientists no longer connected with this project; among them the following deserve especial mention and thanks: Dr. B. Magdoff, Dr. V. Luzzati, Dr. M. V. King, Dr. A. Tulinsky, Dr. E. von Sydow, Dr. F. H. C. Crick, Dr. T. C. Furnas, jun., Dr. R. Worthington, Dr. A. DeVries, Dr. D. Harris, Dr. H. H. Mills, Dr. R. Parthasarathy and Dr. R. Davis.

We also thank the following assistants: Miss F. Elaine DeJarnette for mounting protein crystals and for collecting most of the X-ray diffraction data during the past six years; Mrs. C. Vincent and Miss K. Go for assisting in data handling and in drawing electron density maps and constructing models; Mrs. Theresa Falzone for excellent assistance in the preparation and for handling the crystals and heavy atom dyes; Misses Elsa Swyers and Sylvia Scapa for preparing some heavy atom dyes; Mrs. Edith Pignataro for collecting data while the project was at the Polytechnic Institute of Brooklyn; Mr. W. G. Weber for constructing, and to some extent designing, the mechanical parts of the single crystal counter X-ray diffractometer.

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## BOOK REVIEWS

### KILMISTER ON EDDINGTON

Sir Arthur Eddington

By C. W. Kilmister. The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Selected Readings in Physics.) Pp. vi+279. (London and New York: Pergamon Press, Ltd., 1966.) 21s. net.

SIR ARTHUR EDDINGTON was born in 1882 and died in 1944. Thus, he lies in the middle distance historically, a man of the age of relativity and quantum theory, but unaware of modern developments in those fields. For him there were two elementary particles, the proton and the electron. How are we to classify him, as ancient or modern? Has the acceptable part of his contribution been absorbed into the stream of science, and the unacceptable part put away in storage for historians of science to mull over as the freakish product of a freakish mind? If Eddington had been dead for a hundred years, one might attempt some such dogmatic classification, but he is too recent for that, and so one welcomes this book by Professor Kilmister as of great assistance in the assessment of Eddington.

The general title of the series to which this book belongs is *Selected Readings in Physics*, and so it is appropriate that more than two-thirds of the book consists of excerpts from Eddington's writings, selected to cover his wide range of interests. There are four extracts from *Internal Constitution of the Stars*, one from *Mathematical Theory of Relativity* (a long extract giving Eddington's generalized theory), one from *Relativity Theory of Protons and Electrons* (here the Riemann-Christoffel tensor plays a central part), and one from *Fundamental Theory*. Three papers are reproduced: *On the Derivation of Planck's Law from Einstein's Equation*, *The Factorization of E-Numbers*, and *The Pressure of a Degenerate Electron Gas and Related Problems*. Finally, there is a *Manuscript on the Transfer Problem*.

Most of these extracts are prefaced with explanatory remarks by Professor Kilmister, but his real task of explanation is undertaken in the first third of the book. This consists of a number of chapters referring to the extracts already mentioned, but tied rather loosely to them. This provides a very happy arrangement. Had Professor Kilmister tied his remarks closely to the text of Eddington, elucidating this point or that, the result might well have been extremely dull. He elected, however, to dig a little deeper and lay the foundations in his own way. As his ideas about groups and algebra are clearer and more sophisticated than Eddington's, he is able to illustrate complicated situations by simple but pertinent examples. Further, he rivals Eddington in directness and vividness of style. If I were a student, coerced into reading this book for the good of my (scientific) soul, I would spend my time on Kilmister and skim through Eddington. It is not that what Kilmister says is always transparently clear. One feels, however, that there is bedrock somewhere, and from that bedrock argument can be maintained. With Eddington, one is in danger of being swept into a whirlpool of confusion with no rational bottom, or, if there is one, my toes are unable to reach it.

In thinking about Eddington, my mind goes back to the two occasions on which I met him. The first was in Dublin in 1929 or 1930, when we were guests at dinner in the house of Professor John Joly. In the course of conversation, I expressed contempt for Williamson's *Differential Calculus*, long the text-book recommended to students in Trinity

College, Dublin, but sadly lacking in mathematical rigour. Eddington disagreed; he had been reared on Williamson and found no fault with it. I understand better now what he meant. He was an intuitionist. He felt things in his bones, and was impatient of delta and epsilon. Unfortunately, tedious as logically precise discourse may seem, it is the only universal language, and it may be that Williamson is to blame for those obscurities which, as Professor Kilmister very readily admits, render some of Eddington's ideas almost impossible to understand.

A chapter is devoted to *The Gulf between Relativity Theory and Quantum Theory*. Having shown how Eddington proposed to bridge the gulf by means of the Riemann tensor, the 256-fold algebra and linear tensor identities, Professor Kilmister ends the chapter as follows: "The next task was to find a problem which could be treated by these methods. The Einstein universe was selected because it was simple in both theories. The puzzling thing for the reader, however, is that Eddington never gives any straightforward prescription of exactly how the analysis is to be carried out. Even when we have progressed as far as this, we have still not formulated any comprehensive theory in terms of which to do the calculations. These calculations still rely on the art and intuition of the theorist, and it is not easy for others than Eddington to provide this intuition".

The second occasion on which I saw Eddington was some years later in Toronto. He gave a popular lecture on cosmology to a large audience. It was, of course, an excellent lecture, but I was puzzled by the fact that he lectured with one hand behind his back. Then, at the appropriate moment, the hand came forward and raised into the air an orange, illustrating excellently a manifold of constant mean curvature but with many minor corrugations.

The only criticism I have of the extracts selected by Professor Kilmister is that there is none to illustrate the playboy in Eddington. The cover of this book shows a grim-faced Eddington and inside there is reproduced a chalk drawing by Augustus John, less grim but sadder. In the Kingdom of Heaven there is no laughter, and little among scientists, for laughter is caused by the juxtaposition of irreconcilables. In Eddington we find the irreconcilables—the showman's flair on the one hand, as evinced by the orange, or, more generally, by his popular writing, and, on the other hand, his lonely quest into the deepest secrets of nature. I like to think of him now resting on some cloud and shaking with quiet laughter. Eddington was a man who could talk low and talk high, but eschewed the middle-talk which is the currency of most scientists.

Professor Kilmister is to be congratulated on a most interesting book. It contains, I should add, a bibliography of works by Eddington and about him. J. L. SYNGE

### EARLY TYPE STARS

The Early Type Stars

By Anne B. Underhill. (Astrophysics and Space Science Library, Vol. 6.) Pp. xiii+282. (Dordrecht: D. Reidel Publishing Company, 1966.) 52 D. florins.

As a comprehensive review of the present state of knowledge regarding early type stars, both observationally and theoretically, this volume will be essential for many more astronomers than those working directly in the field. Indeed, there need be no restriction to astronomers only, for Miss Underhill briefly but efficiently sketches in the basis of each aspect of the study of these stars without presupposing expert knowledge of astronomy, before going into great detail concerning the observational material in particular.

The study of early type stars is very important because, as the most massive ordinary stars known, they are at the



same time the most luminous and the shortest-lived. Consequently, they are ideal for the study of the spiral arm structure of our galaxy; their great luminosity makes them important as distance indicators for other star systems and in studies of interstellar absorption; while their short lifetimes make them sensitive tests of theories of star formation and evolution. In addition, recent work on quasars and blue stellar objects has revealed the depth of our ignorance concerning the distribution of blue stars in the outer parts of our galaxy.

Chapters on spectral classification, photometry, binary stars and spectral analysis are relevant to all types of stars, while that concerning the details of the helium spectrum illustrates the atomic theory of the formation of spectral lines in stellar atmospheres. In the later chapters, several special varieties of early type stars are considered, such as Wolf-Rayet and shell stars, which are as yet little understood theoretically.

With a writer who has herself done so much fundamental research in the field, it is inevitable that personal opinions are sometimes apparent. These, however, only serve to add interest to the book, and to emphasize how much work remains to be done before we have a reasonably complete understanding of the subject. Many of the relevant basic data have been collected and reproduced in tables throughout the book, greatly enhancing its value to the non-expert, and the quality of the diagrams and reproductions of spectra is very high. Only the rather flimsy binding must be criticized, particularly in what should become a standard work. A very thorough list of references completes a book which, in bringing together much diverse information on one topic, should lead to much fruitful research.

R. D. CANNON

## OCEAN WAVES AND TURBULENCE

### The Dynamics of the Upper Ocean

By O. M. Phillips. (Cambridge Monographs on Mechanics and Applied Mathematics.) Pp. vii+261. (London: Cambridge University Press, 1966.) 60s. net.

THIS elegant book—more elegant than its subject—points to the progress since Ursell's devastating survey appeared in 1960. Many of us will find Phillips's coherent account a very useful guide to some recent work of Longuet-Higgins, Benjamin, Miles and to his own very considerable contributions.

The first three chapters develop the required hydrothermodynamics, allowing for stratification (heat and salt) and rotation. The underlying theory of surface waves includes the simpler non-linear solutions, yielding certain results concerning surface and bottom streaming, the depression of the mean water level over a sloping shelf, and bottom pressure fluctuations in deep water (pertinent to microseisms). The way is now clear for the three main topics: ocean surface waves (eighty-two pages), internal waves (thirty-two pages), and oceanic turbulence (forty-five pages).

The chapter on ocean surface waves, after some preliminary definitions and discussion of various spectral estimates, gives a systematic and combined treatment of the wave generation theories by Miles and Phillips. The apparatus needed here is substantial, yet I am not convinced that the age-old problem of how the wind generates waves is now essentially solved. In comparison, the subsequent discussion of the saturated frequency spectrum ( $\sim n^{-5}$ ), a concept introduced by Phillips, is far simpler and far more rewarding. The role of ripples is emphasized, and the effect of the waves on the wind (profile and drag) receives attention. The chapter closes with a discussion of departure from Gaussian statistics.

The chapter on internal waves introduces into the study of higher modes some of the statistical considerations which have been so rewarding in the study of the lowest (or

surface) mode. Approximate solutions are derived for the cases that the wave lengths are long or short as compared with the characteristic thickness of the thermocline. In the short wave limit the presence of a weak shear will twist the wave so as to produce an equilibrium spectrum  $\sim n^{-3}$ . In the long wave limit the self shear and stratification conspire to produce spectral densities  $\sim n^{-1}$  or  $n^{-3}$  according to whether the thermocline depth is small or large relative to wave length. The arguments that lead to these relations are fascinating, and the results can be reconciled with observations, but far more definitive observations are required to put these developments on a sure footing. The last section gives a very nice compact survey of the combined effects of stratification and rotation. The co-ordinates in figure 5.10 reveal the author's native hemisphere and discriminate against his northern hemisphere readers.

The final chapter on oceanic turbulence reviews the fundamentals of statistical turbulence theory. It is pleasant that the best confirmation of the Kolmogorov prediction derives from oceanographic measurements (Grant, Stewart, and Moilliet). A principal task here is an assessment of the limitations and modifications imposed by stratification on the isotropic theory. The Monin-Obukhov and Ekman depths define various pertinent layers, and the wave number  $\kappa_b \sim N^{3/2} \epsilon_0^{-1/2}$  separates the inertial from the buoyancy sub-range in the spectrum. Oceanic measurements bearing on these central concepts simply do not exist. In the closing discussion of entrainment and thermocline formation the lack of definitive measurements is even more limiting.

The author's choice of words is delightful: he speaks of "parasitic capillaries", of "foam patches marking the demise of crests that had previously broken", of "the strong, promiscuous interactions" of the turbulent Fourier components, contrasting with "the weak, selective interactions" of the internal gravity waves.

The book emphasizes how much has been accomplished in developing a formalism appropriate to the messy, symbiotic processes in the oceans; yet how little in measuring the pertinent parameters with adequate spacial and temporal resolution. I find the title "The Dynamics of the Upper Ocean" to be at once too specific and too general: too specific because so much of the last two chapters is pertinent to the abyssal oceans; too general because the dynamics of upwelling, of the general circulation, boundary currents, planetary waves is lacking. There is virtually no overlap with Stommel's book on the Gulf Stream, which also deals with ocean dynamics. Phillips's emphasis is on perturbation methods pertinent to ocean processes, and here his original and coherent account is a most welcome contribution.

W. MUNK

## RADIATION BELTS REVIEWED

### Radiation Trapped in the Earth's Magnetic Field

Edited by Billy M. McCormac. (Proceedings of the Advanced Study Institute held at Chr. Michelsen Institute, Bergen, Norway, August 16–September 3, 1965.) Pp. xiv+901. (Dordrecht: D. Reidel Publishing Company; New York: Gordon and Breach, 1966.) \$45.

THIS Advanced Study Institute was held seven years after Van Allen's discovery of the radiation belts, and the Proceedings disclose and describe the vast amount of information concerning the belts that has been obtained in that time. Particle measurements already represent a substantial survey, covering essentially the whole spatial region of trapping. In energy there is one important gap for protons below 100 KeV. It is expected that such protons are the most important source of the "ring current", which is jargon for the current causing the main phase of storms. The important temporal variation is that of the solar cycle and, whereas the original dis-

covery was made at the time of the strongest ever solar maximum, the great majority of measurements are recent and therefore at solar minimum.

Nevertheless, one important feature of the solar cycle variation is revealed in one of the few European contributions. Comparison of results from *Ariel I* and *Explorer IV* shows that the intensity of the inner belt increases with solar activity and, since it is known that the lifetime of trapped particles decreases, the source must increase substantially. On the other hand, the earliest suggested source, neutron albedo from cosmic rays, should not vary. Other aspects discussed by theorists led to one of the principal conclusions of the institute—that neutron albedo is a very minor source and that most of the particles come from the solar wind.

It was also appreciated that stochastic acceleration was of outstanding importance and this was emphasized in the discussions. It was seen that future progress in the comprehension of the radiation requires much greater effort in measuring disturbances in the electromagnetic field, which has been neglected in comparison with the particles. Furthermore, it is important to separate space and time variations and this requires multiple satellites, as emphasized in the excellent summary at the end of the Proceedings. One paper on plasma instabilities was included, and at the sequel at Munich this year (1967) much more of the programme will be given to waves, for it has been realized that the radiation belts provide a splendid opportunity for observing plasma behaviour. The last one-third of the Proceedings under review is concerned with radiation belts created by nuclear explosions and with the effects of trapped radiation on electronic devices and humans.

J. W. DUNGEY

## MATHEMATICAL PHYSICS

### Problems of Mathematical Physics

By N. N. Lebedev, I. P. Skalskaya and Y. S. Uflyand. Revised, enlarged and corrected English edition translated and edited by Richard A. Silverman. With a Supplement by Edward L. Reiss. (Selected Russian Publications in the Mathematical Sciences.) Pp. xi+429. (Englewood Cliffs, N.J., and London: Prentice-Hall, 1965.) 96s.

THIS is a collection of more than 600 exercises on methods of mathematical physics, with equal emphasis on both parts of the term. Solutions, covering 105 pages, are supplied for a representative fraction of the problems. The subject matter is mathematical physics of the strictly classical kind, with problems on elasticity, electromagnetism, fluid dynamics, heat conduction and diffraction of waves; there are none on quantum mechanics. The problems are classified mostly according to the mathematical methods rather than the field of physical application, with chapters on hyperbolic and elliptic equations, discrete and continuous Fourier and eigenfunction expansions, and a brief chapter on integral equations. Each chapter starts with a brief introduction, reviewing the key points to be used in the problems, and closes with a bibliography for further reading which is both carefully chosen and sufficiently short to be useful. (The translator says that the references in the original Russian version have been revised and brought up to date for the English edition.) There is a helpful supplement by Reiss on variational and related methods, contrasting in its emphasis with the original text, which concentrates on exact solutions.

A preparation not far short of degree level in mathematics with a large share of physics would be necessary to gain much benefit from the book, but anyone at about that stage who has worked through the first volume of Courant-Hilbert and wishes to develop his ability to set up problems and his dexterity to solve them could scarcely

do better than to settle down with the book for a vacation. The book should also serve as a useful source of examples for lecture courses. The production and printing are excellent.

A. HERZENBERG

## VACUUM SEALS

### Vacuum Sealing Techniques

By A. Roth. Pp. xiv+845. (London and New York: Pergamon Press, Ltd., 1966.) 240s. net.

THIS relatively large book supported by nearly 1,700 references represents a collection of a large number of items dealing with vacuum technology that have been reported previously. In making such a collection, the author has necessarily had to cover a number of aspects in insufficient detail for the book to serve in itself as an immediate reference for the reader wishing to construct anything but the simplest piece of vacuum equipment. For example, the section devoted to brazing, which is an important vacuum sealing technique, is restricted to some twenty pages of the chapter devoted to permanent seals. This chapter deals with welded joints in a similar manner, but glass seals, to both glass and metal, are dealt with more fully, and some information on simple glass working is included. There are also some details of metal ceramic sealing techniques and, surprisingly in this chapter, a short section on elastomers and plastic pipes. Appropriately the chapter closes with sections on the techniques for the sealing off of glass, quartz and metal tubulations.

The next chapter is of similar length and concerns semi-permanent and demountable seals; it covers the use of waxes, epoxy resins, soft solders, solder glasses, ground glass joints, liquid seals and gaskets. A large number of the techniques described, however, would be obvious to anyone with normal laboratory experience. Also included is a considerable amount of information on the types of elastomer, metal gaskets and seatings available commercially. The task of choosing from this information could be somewhat bewildering to someone starting in the vacuum field, while it would be superfluous to the practising engineer or physicist, who will have his own preferred designs selected from components readily available to him. Chapters devoted to the transmission of electricity and mechanical motion through vacuum seals give a number of methods that can be used when limited facilities are available, together with commercially available items.

The use of metal ceramic seals for electrical lead-throughs receives only passing mention, which is unfortunate in such a book in view of their ruggedness and increasing use in the vacuum field. Transfer of materials into vacuum is almost entirely restricted to gaseous matter using components from simple laboratory apparatus to commercially available valves. The final chapter is devoted to some of the seals used in the transmitting of radiations other than radio frequency radiation.

F. BROOK

## FERTILE SOIL

### The Control of Soil Fertility

By G. W. Cooke. (Agricultural and Horticultural Series.) Pp. xvii+526. (London: Crosby Lockwood and Son, Ltd., 1967.) 70s. net.

IN writing this book, Dr. Cooke has made a most valuable and balanced contribution to the literature of soil science. It is not competitive with Russell's *Soil Conditions and Plant Growth* but complementary, because it bridges so effectively the gap between the more fundamental aspects of soil science and farm practices. As a consequence of this and also of the way that it is written, this book will

be of value to progressive farmers as well as to students of agricultural science who will use it as a standard text.

One of its most impressive features is the masterful way that Dr. Cooke has married the lessons of the long-term classical soil fertility experiments, especially those at Rothamsted, with the results of more recent experimentation not only in Britain but also abroad. A huge volume of literature, much of it of very recent origin, has been consulted and the essential features of it have been combined under appropriate chapter headings. This has resulted in some overlapping between chapters, but the small amount of repetition is not detrimental because each chapter can be read as one of a series of complete essays. After all, it is unlikely that anyone, except a conscientious reviewer, will want to read the book from cover to cover within the space of a few days. For most it will be an authoritative book of reference to be consulted in respect of its separate parts, and good indexing adds to its value in this respect.

The book is strongest in those parts concerned with arable crop production, and this is understandable because the end products are so easily measurable in contrast with those of grassland, where there is the complication of the grazing animal as a middleman in the production process. It is not enough to talk about fertilizer responses in terms of additional grass dry matter, for one must also have some account of the value of these in terms of milk or meat. The present situation is complicated by the convention, when assessing the value of grass which is fed in association with concentrates, of making it the residuary legatee of any errors of calculation which are based on the attainment of theoretical feeding standards. Dr. Cooke cannot be blamed for this because he can do no more than make the most of available information. In doing this he has drawn attention to the considerable deficiencies of knowledge in fertilizer practice for pasture and the need for more critical methods of assessing pasture productivity.

The penultimate chapter, dealing with relationships between organic matter and soil productivity, is particularly valuable because it is such an objective analysis of knowledge on a topic which so often creates emotional rather than factual argument. It is the best balanced discussion of this subject that has been published, but this can also be said of many of the chapter headings. It is a really authoritative book and its author has just cause to be proud of it.

M. M. COOPER

## PRESERVATION BY IRRADIATION

### Food Irradiation

(Proceedings of the International Symposium on Food Irradiation jointly organized by the International Atomic Energy Agency and the FAO of the U.N., and held in Karlsruhe, June 6-10, 1966.) Pp. 956. (Vienna: IAEA; London: H.M.S.O., 1966.) 517 schillings; 141s. 2d.; \$20; 80 D.M.

THIS symposium reviewed developments in the whole field of food irradiation which have taken place since the similar meeting in 1958. The symposium was optimistic, for developments have on the whole been favourable.

The fundamental difficulties of the process of food irradiation have still not been overcome. The chief fundamental advance has been the demonstration that irradiation at temperatures below  $-20^{\circ}\text{C}$  reduces undesirable side effects to about one-third, which made possible the U.S. Army's plans for the sterilization of meats.

Nevertheless, the limitations are now better understood and, even accepting them, there is an already large list of potentially useful treatments. Furthermore, it is increasingly understood that feasibility must be regarded against alternative practices and, where this has been done, it seems that several irradiation processes might

be economically viable. Above all, the vast American programme of feeding irradiated foods to mammals has not yielded evidence of harmful effects, other than loss of vitamins comparable with canning; and a similar smaller programme in Britain gave similar results. The U.S. and Canadian authorities have permitted irradiation of particular foods, with more in prospect; and legislators in other countries are actively considering how to deal with the situation. While there have been substantial international exchanges, such as this symposium, where uniformity of view on technical matters has been sought, the conference noted the lack of similar attempts on the legislative plane, though this seems necessary to facilitate potential international trading in irradiated foods.

Several outstanding points of technical difficulty are evident in these proceedings, besides the obvious need to diminish the side effects and increase the microbicidal power of irradiation. These difficulties are the especially poor control by irradiation of viruses and enzymes; the significance of conceivable mutations in micro-organisms causing food poisoning; the discrepancy between the toxic effects of irradiated nutrients on single cells and the absence of corresponding effects in animal feeding trials; and the difficulty of detecting whether a food has been irradiated. The general impression is, however, that many possible processes would not be seriously affected by these difficulties, and that applications are likely in many countries as soon as the laws are regularized.

M. INGRAM

## EARLY PREGNANCY

### Pre-implantation Stages of Pregnancy

Edited by G. E. W. Wolstenholme and Maeve O'Connor. (Ciba Foundation Symposium.) Pp. xii + 430. (London: J. and A. Churchill, Ltd., 1965.) 65s.

THIS book is an account of the proceedings of one of the many small international conferences on a variety of topics of biological and medical research, which have been convened by the Ciba Foundation. This particular symposium was held in April 1965, under the distinguished chairmanship of Professor C. H. Waddington, and the resulting volume contains sixteen contributions on many different aspects of the pre-implantation stages of mammalian development.

The first two papers deal with the ultrastructural changes which occur in the egg during fertilization, and with the fine structure of the blastocysts of selected mammalian species. Others deal with energy metabolism, nucleic acid and protein synthesis, genetic aspects and the influence of various maternal factors on early embryonic development.

The short discussions which follow each paper that was read and the more extensive general discussions are quite as interesting as the papers themselves, and contribute substantially to the value of this interesting book.

B. MORRIS

## OBITUARIES

### Professor J. W. Heslop Harrison

PROFESSOR JOHN WILLIAM HESLOP HARRISON died at his home in Birtley, Co. Durham, on January 23, 1967, the day after his eighty-sixth birthday. Harrison was emeritus professor of botany of the Universities of both Durham and Newcastle upon Tyne.

He received his early education at Rutherford College, Newcastle upon Tyne, and in 1903 graduated B.Sc. from

Armstrong College in the University of Durham, with distinction in chemistry. He was science master at Middlesbrough High School between 1905 and 1917. During this time he obtained his M.Sc. and D.Sc. for research on the biology and systematics of species and hybrids, mainly of the Geometrid sub-family Bistoninae. He then joined the staff of Armstrong College and in 1920 was appointed lecturer in zoology. In 1926 he became the first reader in genetics in the University of Durham at Newcastle, and from 1927 until his retirement in 1946 he was professor of botany in Armstrong College (later King's College). From 1940-50 he was very active as secretary of the University of Durham School Examinations Board; he built up a relationship between the examining board and the grammar schools of the four northern counties which has been widely acclaimed by the heads and teachers of the schools.

In 1921 he was elected a Fellow of the Royal Society of Edinburgh and he became a Fellow of the Royal Society of London in 1928. He served on the Council of the Edinburgh Society for several periods and he was vice-president from 1945-48. He was on the editorial committee of the *Vasculum* from its foundation in 1915, and he was the sole editor of the *Vasculum Substitute* from 1942 until his death. He was also one of the original founders of the Northern Naturalists Union in 1924, and he held continuous office in this society, also until his death. After his formal retirement in 1946 Harrison held a senior research fellowship until 1949 in the agricultural faculty of King's College, Newcastle, and he was a member of the board of the faculty of agriculture until he died. Even after 1949 he continued to give courses of lectures, mainly on genetics, to agricultural and other students.

Harrison was a gifted biologist and field naturalist. His memory for biological facts was prodigious and his knowledge of flowering plants and insects was of a most unusual depth and understanding. Harrison published more than 500 papers and many thousands of biological records in the *Vasculum*, the *Entomologist* and similar journals. His chief research centred on evolutionary topics and he was particularly active in the years when Darwinists and Lamarckists fought almost pitched battles. He contributed to both sides and he was also a staunch mutationist. His work in these fields helped to lay the foundations for much future research when the processes of organic evolution were becoming better understood. In particular Harrison, by his work on the occurrence, significance and induction of industrial melanism in moths, set the pace for the future understanding of these phenomena. Harrison published his chief papers in studies of the Bistoninae, on the effects of chemicals on the insects *Selenia bilunaria* and *Tephrosia bistortata* and on the egg-laying insects of the sawfly, *Pontania salicis*. On *Selenia* he worked at first with F. C. Garrett, and he claimed to have induced mutations; he was thus among the first to induce mutations. He claimed that his work on *Pontania* showed a Lamarckian effect, and although this work has not been repeated neither has it been disproved. Harrison also worked on the systematics, cytology and genetics of the genera *Rosa* and *Salix*, and, to a lesser extent, *Rubus* in conjunction with Dr. Kathleen B. Blackburn.

In 1934 Harrison visited the Isle of Raasey with Professor A. D. Peacock of University College, Dundee, and the following year he initiated his own expeditions to many islands of the Inner and Outer Hebrides. Official parties from King's College, Newcastle, under Harrison visited these islands once or twice a year from 1935 to 1946. After 1946 Harrison continued his visits until about 1962. During these expeditions a thorough and systematic study was made of the flora and fauna (chiefly insects) of the Hebrides, and many finds and new records were made. Most of the results of this work were published in a series of papers in the *Proceedings of the University of Durham Philosophical Society*.

Harrison will be remembered for his scientific work which has become part of the history of evolutionary biology, but he will be particularly remembered for his wonderful gift of teaching.

P. G. FOTHERGILL

### Professor F. C. Ormerod

FRANK ORMEROD, emeritus professor of laryngology and otology in the University of London, died at his home in London on January 25 at the age of 72.

Educated at Manchester Grammar School, he graduated M.B., Ch.B., at the University of Manchester in 1916; then followed three years of service with the R.A.M.C. in Mesopotamia and Afghanistan. He graduated M.D. in 1920, became F.R.C.S.Ed. the following year and F.R.C.S. in 1926. In 1921 he joined the staff of the Hospital for Diseases of the Throat at Golden Square in London, and he was also consulting surgeon to the ear, nose and throat department at the Westminster Hospital and consultant to the Brompton Hospital and King Edward VII Sanatorium, Midhurst. Despite an extensive private practice, Ormerod was always primarily interested in the academic side of his specialty, and after the formation of the Royal National Throat, Nose and Ear Hospital (in which he played a leading part) he was largely responsible for the creation and inauguration of the Institute of Laryngology and Otology of the British Postgraduate Medical Federation.

When the first, and still the only, chair of laryngology and otology in the United Kingdom was established by the University of London in 1949, he was the natural choice for the appointment. It was fortunate for otolaryngology that Ormerod was willing to relinquish his consultant appointments and private practice to accept this challenge. He built up at the institute a highly successful clinical and research unit. At various times he occupied official positions in the sections of otology and laryngology in the Royal Society of Medicine. Not only was he a co-founder, but also served as secretary of the British Association of Otolaryngologists, and just before his appointment to the professorship he became scientific secretary to the fourth International Congress of Otolaryngology held in London. As a member of the Court of Examiners of the Royal College of Surgeons of England, he was able to influence the training of young otolaryngologists, and the establishment of organized training programmes for these surgeons occupied much of his time. His knowledge of European otolaryngologists, both past and present, made him an invaluable source of reference for intending travellers overseas. In 1953 he gave the Semon Lecture of the University of London, and in 1965 the James Yearsley Lecture. At one time or another he had lectured in most countries of the world, and although not gifted with natural eloquence these communications were so well prepared and constructed that he enjoyed an international reputation as a speaker.

His book *Tuberculosis of the Upper Respiratory Tract* was published in 1939, but his main interest lay in the management of malignant diseases of the head and neck. With his retirement from clinical work in 1959, he was appointed director of research and this permitted him to develop interests in the physiology of the endolymph and pathology of congenital deafness. Several important papers were written on these subjects. When he retired from the Institute of Laryngology and Otology in 1962, he continued to assemble a historic collection of instruments and photographs illustrating the development of otorhinolaryngology. This was dear to his heart and there was nobody living more fitted to accomplish the task successfully. Unfortunately he died before it was completed, but it will be continued by others and will serve as a unique memorial to an exceptional man.

D. F. N. HARRISON

# Helium Deficiency in Old Halo B Stars

by

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Calculations show that in old halo B stars most helium will have diffused below the photosphere and therefore will not be detected spectroscopically. This may account for the low abundances of helium observed in these stars.

THERE is a growing body of evidence<sup>1-4</sup>, based on a weakness or absence of helium absorption lines in the spectra, for an abnormally low abundance of helium in the atmospheres of old halo B stars. A possible conclusion is that these stars contain little or no helium. If so, these are the only objects we know in which the abundance of helium differs significantly from a more or less universal value of about 25 to 30 per cent by mass<sup>5</sup>.

The large magnitude of this universal abundance is difficult to obtain on the basis of the conventional theory of stellar nucleosynthesis<sup>6,7</sup>. The galaxy would have to have been far brighter for long periods of time than it is now, or than we believe it could have been in the past. Furthermore this abundance also appears to be characteristic of other galaxies which have had significantly different evolutionary histories. One possible explanation for this difficulty is that helium has been produced as a natural consequence of the high temperature-high density phase of an expanding cosmology (ref. 7 and personal communication, R. V. Wagoner, W. A. Fowler, and F. Hoyle). We have attempted to account for both the value of the helium abundance and the likelihood that it is a universal abundance on this basis. The recent discovery of the isotropic microwave background radiation by Penzias and Wilson<sup>8</sup> and others provides direct evidence that the universe was at one time in such a highly compressed state.

The low abundance of helium in the surfaces of old halo B stars appears to be the only evidence which contradicts this hypothesis. In this note we suggest that these stars have a normal abundance of helium in their interiors, and that the surface helium has diffused below the photosphere.

The equilibrium structure of an ionized stellar atmosphere has been discussed by Parker<sup>9</sup>. For the case of an atmosphere consisting of singly ionized hydrogen and helium a lower layer of helium and an upper layer of hydrogen are formed. The abundances of the two constituents decay exponentially on either side of the boundary between them.

This ultimate equilibrium configuration is almost never reached. In most cases the slow diffusion of helium into the interior of a star is opposed by far more rapid motions which return it to the surface. In most main sequence stars these motions take the form of an outer convection zone, the upper boundary of which lies just below the photosphere, but the halo stars with which we are concerned are B stars, which have no such zones. Main

sequence B stars are rapid rotators<sup>10</sup> ( $\Omega \approx 10\Omega_\odot$ ), and this rotation can generate relatively rapid large scale meridional circulation within them which tends to return helium to their surfaces. The halo stars, however, are horizontal branch B stars, which are known to be slow rotators<sup>4,10</sup> except for a possible exception mentioned in ref. 3. Their slow rotation gives rise to internal currents with velocities which are less than the diffusion velocity of helium by several orders of magnitude.

Sweet<sup>11</sup> has analysed the currents generated by the uniform rotation of a star. He finds

$$v_{\text{circ}} = v(z)LR^5\Omega^2/M^3 \quad \text{cm/sec}$$

Here  $v_{\text{circ}}$  is the circulation velocity, and  $L, R, \Omega$  and  $M$  are the luminosity, radius, angular velocity and mass of the star, measured in units of those of the Sun. Defining

$$z = R(2.5 \times 10^{-15} \rho_c / T_c)^{1/2}$$

where  $\rho_c$  is the central density and  $T_c$  is the central temperature, he finds

$z$	2	5	7
$\frac{v(z)}{(\text{cm/sec})}$	$7 \times 10^{-11}$	$1.5 \times 10^{-9}$	$6.7 \times 10^{-9}$

The diffusion velocity of helium downwards is given approximately by  $v_d = \frac{1}{2}gt_{\text{coll}}$ ;  $g$  is the acceleration of gravity and  $t_{\text{coll}}$  is the mean time between collisions with other particles. Spitzer<sup>12</sup> has analysed the collision problem for a variety of circumstances: for the cases with which we are dealing

$$t_{\text{coll}} = 7T^{3/2}n_H \text{ sec}$$

Here  $T$  is the temperature in °K and  $n_H$  the number density of hydrogen ions.

In the Sun we have<sup>10</sup>  $T = 6,000$  °K and  $g = 3 \times 10^4$  cm/sec<sup>2</sup>. From this the hydrogen scale height is  $H = 160$  km. We estimate the hydrogen density  $n_H$  at the photosphere by putting the optical depth there equal to unity: that is,  $\tau = (\text{opacity}) (\text{scale height}) (\text{density at photosphere}) = 1$ . The opacity is a function of  $T$  and  $n_H$  (ref. 13); a consistent choice is  $n_H = 10^{17}$  particles/cm<sup>3</sup>. From this we find the diffusion velocity of helium to be  $v_{\text{diff}} = 5 \times 10^{-8}$  cm/sec. The central density and temperature of the Sun are given in Allen<sup>10</sup>; we find  $z = 9$  is that



the circulation velocity caused by the Sun's rotation  $v_{\text{circ}} = 8 \times 10^{-9}$  cm/sec. This is slower than the diffusion velocity. The Sun, however, has an outer convection zone, with large scale motions of the order of magnitude of kilometres per second, which keeps the helium from settling below the photosphere.

In a main sequence *B* star there is no outer convection zone, so the only motion that would oppose the diffusion is a rotation-induced circulation. If we adopt a model where  $M=10$ ,  $L=10^4$ ,  $R=10$ ,  $\Omega=10$  and  $T=3 \times 10^4$  °K at the surface<sup>10</sup>, we find the acceleration of gravity at the surface to be  $g=GM/R^2=3 \times 10^3$  cm/sec<sup>2</sup>. The scale height  $H$  is then 8,000 km and a surface density of  $10^{15}$  particles/cm<sup>3</sup> satisfies the surface opacity condition. We then find that the diffusion velocity is  $5 \times 10^{-5}$  cm/sec. Estimates<sup>10</sup> of central conditions are  $\rho_c=(1/10)\rho_{\odot}$ ,  $T_c=2T_{\odot}$  so that  $z=20$ . Sweet's calculations do not include such large values of  $z$ , but we may estimate an order of magnitude of  $v(z)=10^{-7 \pm 2}$  cm/sec. The circulation velocity then would be  $v_{\text{circ}}=10^{1 \pm 2}$  cm/sec, which is far larger than  $v_{\text{diff}}$ . With velocities of this magnitude, however, material is transported from the centre to the surface of the star in a time short compared with its time scale for evolution. In these circumstances Sweet's results are not applicable. Roxburgh<sup>14</sup> has obtained stable models of such rapidly rotating stars with no meridional circulation at all. These models correspond to a particular distribution of angular rotation with depth within the star: any other velocity field gives rise to circulation. No circulating models have been obtained, however, and it is possible that they are all unstable and evolve into circulation-free states. At present we are unable to say whether a particular rapidly rotating main sequence *B* star possesses meridional circulation or not. We may calculate, however, the minimum circulation which would oppose the helium diffusion:  $v_{\text{circ}}=5 \times 10^{-5}$  cm/sec. This circulation moves material from the centre to the surface of the star in  $7 \times 10^8$  years, which is long compared with the lifetime of the star on the main sequence.

It has recently been found (ref. 15 and T. S. Deeming and G. A. H. Walker, personal communication) that there is a correlation between the strength of helium absorption lines in main sequence *O* and *B* stars and their projected angular velocity. The same effect is observed in *Be* stars which are all presumably rotating at the verge of equatorial rotational instability. Such stars presumably bring helium to the surface near the equator, and diffusion may be responsible for weakening the helium lines near the poles.

The halo stars of interest here are horizontal branch *B* stars. They have no outer convection zones. They are thought to have evolved from stars similar to the Sun, through a red giant stage, to their present state. During the red giant stage they would have lost some fraction of their masses and a large fraction of their angular momentum. Thus if we take  $M=1$ ,  $L=10^3$ ,  $\Omega=1$  and  $T=3 \times 10^4$  °K we over-estimate their circulation velocities. Sargent and Searle<sup>3</sup> measure the surface gravity of their peculiar stars to be about  $g=5 \times 10^5$  cm/sec<sup>2</sup>. Setting  $g=GM/R^2$  we find  $R=0.25 R_{\odot}$ . As before we find the scale height=50 km, the surface density to be  $10^{16}$  particles/cm<sup>3</sup> and the diffusion velocity of helium to be  $v_d=10^{-3}$  cm/sec. The helium diffuses downwards one scale height in a time far less than the time during which the star remains on the horizontal branch.

We encounter a difficulty in applying Sweet's results directly to this star, because his calculations refer to a point-convective stellar model. Horizontal branch stars possess a dense helium core containing perhaps three-quarters of the star's mass but occupying a small fraction of its volume. Within this core  $\rho_c=10^4$  g/cm<sup>3</sup>,  $T_c=10^8$  °K. If we calculate  $z$  from these parameters we find  $z=2 \times 10^4$ —a ridiculously large value—but the helium core does not participate in the circulation currents, and should properly be excluded in some way from the problem.

We may approximate the actual situation by estimating the conditions just outside the core.

We estimate  $\frac{T_c}{T_{\odot}} = \frac{R_{\odot}}{R}$  since for homologous contrac-

tion of a star,  $TR=\text{const}$ . Approximately  $4\pi\rho_c R^3/3=\text{mass}$ ; we find  $\rho_c R^3 M_{\odot}/M = \rho_{\odot} R_{\odot}^3$ . If we assume that the mass outside the core is quarter the star's mass, and it is distributed through a volume comparable with that of the star, we find

$$\rho_c = \frac{\rho_{\odot}}{4} \left( \frac{R_{\odot}}{R} \right)^3$$

From these  $z=z_{\odot}/2$  and  $v_{\text{circ}}=2 \times 10^{-10}$  cm/sec. This circulation velocity must be increased near the stellar surface to take into account continuity of flow. Mestel<sup>16</sup> has pointed out that Sweet's results need modification; in a uniformly rotating star a critical level surface is formed at which the meridional circulation vanishes, and outside which the meridional circulation is greater than that in the interior by a factor  $\rho^*/\rho$ , where  $\rho^*$  is the density at the critical surface. According to Mestel,

$$\frac{\rho^*}{\rho} = \frac{2}{3} \frac{\Omega^2 R^3}{GM}$$

where  $\bar{\rho}$  is the mean density and c.g.s. units are employed throughout the above expression. With the parameters assumed above for the horizontal branch *B* star, we find finally that  $v_{\text{circ}} \sim 10^{-5}$  to  $10^{-6}$  cm/sec. This is several orders of magnitude slower than the downward diffusion velocity.

The only other motion which could counteract the downward diffusion of helium would be the presence of a stellar wind, in which the atmospheric layers would be subject to hydrodynamic expansion away from the star. In general such expansion would require the presence of a hot corona, and the heating of the corona requires the generation of waves by turbulence or vibration in the outer convection zone. Because these motions appear not to be present in the horizontal branch *B* stars, no significant stellar wind should be present.

Supergiant *B* stars appear to be losing mass at a rapid rate (D. C. Morton, personal communication). It has been proposed (L. Lucy and P. M. Solomon, personal communication) that this loss of mass arises from the radiation pressure on certain ions in certain stages of ionization, where strong resonance lines lie near the peak of the Planck spectrum. Main sequence *B* stars do not show this effect (D. C. Morton, personal communication), and the lower luminosity to gravity ratio in the horizontal branch *B* stars should assure that the process will not be important in them.

From these considerations we see that the horizontal branch *B* stars are the only general class of star in which it can be expected that helium will gravitationally settle out of the photosphere. If the main sequence *B* stars were to rotate more slowly, we would expect some gravitational settling to take place; we have noted that some sharp-lined main sequence *B* stars have weak helium lines<sup>15</sup>. Thus we conclude that the horizontal branch *B* stars should not be considered evidence against the cosmological synthesis of helium.

Similar diffusion arguments hold with respect to other ionic species. Most of the abundant lighter elements will be singly or doubly ionized in *B* star photospheres. The diffusion velocity of an ion of mass  $m$  and charge  $Z_{\text{eff}}$  is proportional to the quantity  $m^{1/2}/Z_{\text{eff}}^2 [\Phi(x) - G(x)]$ , where the function  $[\Phi(x) - G(x)]$  is tabulated in ref. 12. In horizontal branch *B* stars typical light and medium elements diffuse downwards in the range 1–10 times faster than helium. In this connexion it may be noted that the star Feige 65 shows abnormally weak lines of oxygen

II, carbon II, nitrogen II, silicon II (absent), and silicon III (ref. 3). If, however, some of these elements possess very strong absorption lines near the peak of the Planck spectrum (L. Lucy and P. M. Solomon, personal communication), then radiation pressure forces may be stronger than gravity, and these elements can then diffuse away from the star.

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## Uranium and Thorium Abundances in Carbonaceous Chondrites

by

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Determinations of uranium and thorium in carbonaceous chondrites by neutron activation show that both uranium and thorium are inhomogeneously distributed within Type I and are generally homogeneously distributed within Type II carbonaceous chondrites. If Type I carbonaceous chondrites represent primitive solar material, then for the experimentally determined values of the ratio  $^{232}\text{Th}/^{238}\text{U}$  either a continuous or sudden model of nucleosynthesis is plausible. The value of this ratio does, however, raise some problems.

UNTIL quite recently it was considered that the chondritic abundances of uranium<sup>1</sup> and thorium<sup>2</sup> were constant at about 0.01 p.p.m. and 0.04 p.p.m. respectively. This conclusion was based on the analysis of specimens from the two "ordinary" chondrite classes. More recently, analyses have been reported<sup>3-5</sup> for uranium and thorium in a few samples of Type I and Type II carbonaceous chondrites, the results of which are summarized in Table 1. The carbonaceous chondrites, though rare in occurrence, are generally thought to be of considerable importance because they appear to be more representative of the chemical composition of primitive solar material than any other chondritic class<sup>6,7</sup>. Except for one duplicate analysis of Orgueil<sup>3</sup>, the abundances shown in Table 1 for uranium and thorium are considerably higher than those accepted for the ordinary chondrites. If this difference were real it could be of great significance, because solar abundances calculated from nucleosynthetic theory<sup>8,9</sup> are also much higher than the ordinary chondrite values.

It can be seen from Table 1 that the only case in which it is possible to derive a thorium/uranium weight ratio yields a value of 2.68, equivalent to a  $^{232}\text{Th}/^{238}\text{U}$  atomic ratio of 2.77. Fowler and Hoyle<sup>10</sup> estimated a  $^{232}\text{Th}/^{238}\text{U}$  ratio of 3.8 for primitive solar material, and used this value in conjunction with calculated uranium and thorium nucleosynthetic production rate ratios to show that nucleosynthesis was a continuous process. If, however, the experimentally derived ratio of  $^{232}\text{Th}/^{238}\text{U}$  for Orgueil is used for the same calculation, production of uranium and thorium by a single event some  $6 \times 10^9$  yr ago is indicated<sup>11</sup>. It was therefore of considerable importance that careful measurements should be made for uranium and thorium on as many carbonaceous chondrites as possible.

For this work we accumulated a collection of nine samples of the four known Type I carbonaceous chondrite falls and eight samples of six Type II carbonaceous chondrites. These samples are listed in Table 2. The results for two of the samples (Orgueil IIIc and Renazzo I) were rejected because of suspected contamination of the original samples.

Analyses were made for uranium and thorium by neutron activation. Samples and standards were each sealed in silica ampoules and irradiated together in a graphite facility in the HIFAR reactor at the Australian Atomic Energy Commission Research Establishment at Lucas Heights. The duration of irradiation was usually one week, in a nominal thermal flux of  $9 \times 10^{12}$  neutrons.  $\text{cm}^{-2}.\text{sec}^{-1}$ . Determinations were based on the activities of protactinium-233 and neptunium-239, which were produced by neutron capture followed by beta decay from thorium-232 and uranium-238 respectively. Details of the analytical procedure and discussion of possible sources of interferences have been published previously<sup>12</sup>.

Table 1. PREVIOUS DETERMINATIONS OF URANIUM AND THORIUM IN CARBONACEOUS CHONDRITES

Meteorite	Uranium (p.p.m.)	Thorium (p.p.m.)
Type I		
Orgueil <sup>3</sup>	0.008	—
	0.008	—
Orgueil <sup>4</sup>	0.020	0.060
	0.029	0.070
Type II		
Mighei <sup>3</sup>	0.016	—
Mighei <sup>4</sup>	0.021	—
	0.008	—
Murray <sup>4</sup>	0.018	—
	0.028	—
	0.015	—

Table 2. SOURCE AND SAMPLING DETAILS OF TYPE I AND TYPE II CARBONACEOUS CHONDRITE SPECIMENS

Meteorite	Sampling details	Weight sample crushed (g)	Source
Type I			
Alais	Small fragments, hand picked	0.176	Prof. J. Orcel, Natural History Museum, Paris
Ivuna I	Coarse powder	2	Dr. L. P. Greenland, U.S. Geological Survey, Washington, D.C.
Ivuna II	Sawn slice from interior	0.311	The Commissioner, Mineral Resources Division, Tanzania
Ivuna III	One small fragment	0.125	Dr. B. Mason, American Museum of Natural History, New York
Orgueil I A	Numerous large fragments	10	Mr. E. O. Chalmers, Australian Museum (specimen No. DR 883), Sydney—originally from Natural History Museum, Paris
Orgueil I B	Three large fragments	1	Prof. W. D. Ehmann, University of Kentucky—originally from British Museum (Natural History) (No. 1960, 331)
Orgueil II	Powder	0.531	Kyancutta Museum, South Australia—originally from Natural History Museum, Paris
Orgueil III C	Small fragments, hand picked	0.294	Dr. L. P. Greenland, U.S. Geological Survey, Washington, D.C.
Orgueil IV	Small fragments, hand picked	0.114	Dr. M. V. N. Murthy, Geological Survey of India, Calcutta (specimen No. 255)
Tonk	Small fragments (total 0.281 g), hand picked		
Type II			
Cold Bokkeveld	Several fragments	0.5	Kyancutta Museum, South Australia—originally from Geological Survey of India, Calcutta (specimen No. 141)
Mighei	Fragments broken off sawn slice	0.5	Kyancutta Museum, South Australia—originally from British Museum (specimen No. 1920, 322)
Murray	Fragments broken off larger pieces (6.6 g)	0.5	Dr. E. P. Henderson, National Museum, Washington, D.C. (specimen No. 1769-73)
Nawapali	Small fragments (total 0.5 g)	0.193	Dr. M. V. N. Murthy, Geological Survey of India, Calcutta
Renazzo I	Small fragments (mainly chondrules)	0.2	Dr. B. Mason, American Museum of Natural History, New York
Renazzo II	One fragment	0.630	Dr. E. P. Henderson, U.S. National Museum, Washington, D.C.
Renazzo III	Sawn slice, cleaned	0.337	Prof. P. Gallitelli, University of Bologna, Italy
Staroe Boriskino	Several fragments (total 0.40 g)	0.30	Dr. E. L. Krlnov, Committee on Meteorites, U.S.S.R. Academy of Sciences, Moscow

Schmitt (personal communication) has suggested a possible source of error in the analytical procedure for neptunium-239. His chief doubt appears to be concerned with the possibility that fission products, particularly cerium, are not completely removed by the method. This seems extremely unlikely, however, because the procedures used are actually based on well established methods for the separation of neptunium from fission product mixtures. The decontamination factor for cerium is particularly good, probably between  $10^6$  and  $10^8$ .

The results for the analyses are shown in Table 3. Included for completeness are the results for Orgueil I A which have been published previously<sup>5</sup>.

*Type I carbonaceous chondrites.* Comparison of the Orgueil results in Table 3 with those previously reported and summarized in Table 1 indicates that the large discrepancy in uranium abundances found in earlier work is caused by the extreme inhomogeneity of the distribution of uranium and thorium in the Orgueil fall. It is clear that the results for Orgueil I A are truly representative of this specimen of the meteorite fall, as values obtained from Orgueil I B are almost identical, even though the second sample was prepared from three large lumps from the same bottle, and was not a further split of the same powder. Similarly the Orgueil uranium values previously

reported by Reed *et al.*<sup>3</sup> are very similar to those from Orgueil IV analysed here. The results for Orgueil II show intermediate values for uranium and thorium.

It will be seen that for Ivuna also there is a large variation in uranium values, the highest being almost twice as great as the lowest. Thorium values, on the other hand, are remarkably constant. The results for the other two falls, Tonk and Alais, lie well within the range of values defined by Orgueil and Ivuna.

*Type II carbonaceous chondrites.* The uranium results obtained here for Mighei and Murray agree well with those of previous workers. The Mighei value reported by Reed *et al.*<sup>3</sup> was a single determination and is identical with the lower of the values reported here. Goles and Anders made duplicate determinations on Mighei, and the results of the present work lie well within the range of their two values. The new results for Murray are only about half as large as the average of the triplicate analysis by Goles and Anders<sup>4</sup>, but the lowest of their results does not differ significantly from the results reported here.

To discuss the abundances in different chondrite classes, and to compare them with calculated solar abundances, it is necessary to convert analytical results to atomic abundances. By convention it is customary to normalize to  $10^6$  silicon atoms. Greenland and Lovering<sup>13</sup> have suggested that it may be preferable to normalize to titanium, especially when comparing observed abundances in the Sun's photosphere with chondritic abundances. This innovation, however, has not been generally adopted and in this paper normalization will be with reference to silicon. Atomic abundances calculated in this way are shown in Table 4.

In order to calculate mean abundances as fairly as possible, each meteorite fall was given unit weight. Results for the single samples of Alais and Tonk were therefore given unit weight; each sample of Ivuna was given one third weight, and for Orgueil, samples II and IV were given one third weight and I A and I B one sixth weight. All the Type II samples were given unit weight, except for Renazzo, where each of the two samples was given one half weight.

These weighted means are shown at the bottom of Table 4. A *t* test of the two sets of means showed that they were not significantly different at the 95 per cent confidence level. The weighted data for the two groups were therefore combined to give mean abundances for all the carbonaceous chondrites.

It is interesting to compare these empirical uranium and thorium abundances with those calculated from theories of nucleosynthesis. The abundances of thorium-232, uranium-235 and uranium-238 change with time as all three nuclides are subject to radioactive decay. For

Table 3. URANIUM AND THORIUM ABUNDANCES IN TYPE I AND TYPE II CARBONACEOUS CHONDRITES

Meteorite	Uranium (p.p.m.)	Thorium (p.p.m.)	Th/U
Type I			
Alais	0.0102 } 0.0098	0.0375 } 0.0387	3.8 } 3.9
	0.0096 }	0.0398 }	4.2 }
Ivuna I	0.0114 } 0.0126	0.0284 } 0.0289	2.5 } 2.3
	0.0138 }	0.0293 }	2.1 }
Ivuna II	0.0074 } 0.0075	0.0264 } 0.0277	3.6 } 3.7
	0.0076 }	0.0290 }	3.8 }
Ivuna III	0.0082 } 0.0083	0.0292 } 0.0297	3.6 } 3.6
	0.0083 }	0.0302 }	3.6 }
Orgueil I A	0.0195 } 0.0242	0.0595 } 0.0648	3.1 } 2.7
	0.0288 }	0.0700 }	2.4 }
Orgueil I B	0.0262 } 0.0240	0.0768 } 0.0708	2.9 } 2.9
	0.0218 }	0.0648 }	3.0 }
Orgueil II	0.0172 } 0.0183	0.0407 } 0.0406	2.4 } 2.2
	0.0195 }	0.0405 }	2.1 }
Orgueil IV	0.0081 } 0.0079	0.0332 } 0.0338	4.1 } 4.3
	0.0077 }	0.0344 }	4.5 }
Tonk	0.0115 } 0.0107	0.0310 } 0.0313	2.7 } 2.9
	0.0098 }	0.0316 }	3.2 }
Type II			
Cold	0.0113 } 0.0110	0.0398 } 0.0400	3.5 } 3.6
Bokkeveld	0.0106 }	0.0401 }	3.8 }
Mighei	0.0161 } 0.0169	0.0467 } 0.0456	2.8 } 2.7
	0.0177 }	0.0455 }	2.6 }
Murray	0.0110 } 0.0113	0.0452 } 0.0454	4.1 } 4.0
	0.0116 }	0.0456 }	3.9 }
Nawapali	0.0103 } 0.0108	0.0391 } 0.0384	3.8 } 3.6
	0.0113 }	0.0377 }	3.3 }
Renazzo II	0.0118 } 0.0119	0.0403 } 0.0401	3.4 } 3.4
	0.0119 }	0.0413 }	3.5 }
Renazzo III	0.0114 } 0.0111	0.0417 } 0.0420	3.7 } 3.8
	0.0108 }	0.0423 }	3.9 }
Staroe	0.0102 } 0.0111	0.0392 } 0.0387	3.8 } 3.5
Boriskino	0.0119 }	0.0382 }	3.2 }

the calculation of solar abundances it is therefore necessary to establish a time scale for the production of these nuclei, as well as estimating relative production rates. Burbidge *et al.*<sup>14</sup> proposed a nucleosynthesis model in which uranium and thorium are formed from seed nuclei of the iron group elements by neutron capture at a rapid rate compared with intervening decays (the so-called *r* process). They were able to calculate relative production rates for uranium-235 and uranium-238, and from the known present day isotopic ratio of these two nuclides they derived several cosmochronological models based on the differential radioactive decay rate. The rate of production of thorium-232 was also calculated, but at that time the present day  $^{232}\text{Th}/^{238}\text{U}$  ratio for the solar system was considered to be very uncertain, and independent confirmation of their chronologies by using the differential decay of thorium-232 and uranium-238 was not attempted.

Fowler and Hoyle<sup>10</sup> re-examined the production of uranium and thorium by the *r* process. From terrestrial lead isotope measurements<sup>15</sup> and direct determinations of meteoritic uranium and thorium abundances<sup>1,2</sup>, they estimated a value of  $3.8 \pm 0.3$  for the present day solar  $^{232}\text{Th}/^{238}\text{U}$  ratio. Using the differential decay rates of thorium-232 and uranium-235 relative to uranium-238, it was found that a concordant solution suggested a model in which nucleosynthesis was continuous. In this model *r* process nucleosynthesis began  $11.6 \times 10^9$  yr ago, and has decreased exponentially until at present it is taking place at only 26 per cent of its original rate. The solar system was isolated from nucleosynthetic sources about  $4.7 \times 10^9$  yr ago.

Table 4. ATOMIC ABUNDANCES OF URANIUM AND THORIUM IN TYPE I AND TYPE II CARBONACEOUS CHONDRITES

Meteorite	Silicon (per cent)	Atoms per $10^6$ silicon atoms Uranium	Atoms per $10^6$ silicon atoms Thorium	Atomic ratio Th/U
Type I				
Alais	9.71	0.0120	0.0482	4.0
Ivuna I	10.60	0.0141	0.0330	2.3
Ivuna II	10.60	0.0084	0.0316	3.8
Ivuna III	10.60	0.0092	0.0339	3.7
Ivuna mean		0.0105 $\pm$ 0.0029	0.0328 $\pm$ 0.0015	
Orgueil LA	10.55	0.0270	0.0743	2.7
Orgueil LB	10.78	0.0263	0.0787	3.0
Orgueil II	10.78	0.0201	0.0456	2.3
Orgueil IV	10.78	0.0087	0.0379	4.4
Orgueil mean		0.0184 $\pm$ 0.0086	0.0533 $\pm$ 0.0188	
Tonk	10.47	0.0120	0.0362	3.0
Type II				
Cold Bokkeveld	12.76	0.0102	0.0379	3.7
Mighei	13.00	0.0154	0.0425	2.8
Murray	13.89	0.0100	0.0411	4.1
Nawapali	12.64	0.0101	0.0368	3.7
Renazzo II	15.81	0.0089	0.0313	3.5
Renazzo III	15.81	0.0083	0.0322	3.9
Renazzo mean		0.0086 $\pm$ 0.0004	0.0317 $\pm$ 0.0006	
Staroe Boriskino	12.89	0.0101	0.0364	
Type I, weighted mean		0.0132 $\pm$ 0.0056	0.0426 $\pm$ 0.0129	3.2 $\pm$ 0.7
Type II, weighted mean		0.0107 $\pm$ 0.0023	0.0377 $\pm$ 0.0037	3.5 $\pm$ 0.5
Type I and Type II, weighted mean		0.0117 $\pm$ 0.0040	0.0396 $\pm$ 0.0087	3.4 $\pm$ 0.7

If the  $^{232}\text{Th}/^{238}\text{U}$  ratio in the carbonaceous chondrites is representative of that of primitive solar material, the results of the present work are not in conflict with the Fowler-Hoyle chronology. Unfortunately the uncertainty of the experimental ratio is so large as to permit also a concordant solution for sudden synthesis. The continuous model of nucleosynthesis is so widely accepted for abundance calculations, however, that it will be used in the following discussion. Hoyle and Fowler<sup>8</sup> used their own cosmochronological model to calculate the atomic abundances of uranium and thorium in primitive solar material. In order to relate abundances to silicon (which is not formed by the *r* process), it was necessary to estimate a normalizing factor,  $r'$ , for uranium, thorium and their progenitors. Because they believed that values for this factor of  $> 0.4$  and  $< 0.2$  were unlikely, Hoyle and Fowler adopted  $r' = 0.3 \pm 0.1$ .

The atomic abundances which they calculated using this factor are shown in Table 5, together with the experimental results of the present study.

Table 5. COMPARISON OF CALCULATED AND EXPERIMENTAL SOLAR ABUNDANCES OF URANIUM AND THORIUM

	$r'$	Atomic abundances per $10^6$ silicon atoms	
		Uranium	Thorium
Calculated			
Hoyle and Fowler <sup>8</sup>	0.3 $\pm$ 0.1	0.034 $\pm$ 0.011	0.130 $\pm$ 0.043
Clayton <sup>9</sup> Lead = $2.5 \pm 1.0$	0.42 $\pm$ 0.17	0.048 $\pm$ 0.019	0.184 $\pm$ 0.074
Lead = 1.35	0.23	0.026	0.099
Experimental			
This work	$\sim 0.1$	0.012 $\pm$ 0.004	0.040 $\pm$ 0.009

Clayton<sup>9</sup> has also considered the problem of the abundances of uranium and thorium in primitive solar material. From the neutron capture cross-sections of lead-206 and lead-207 he was able to estimate the  $^{206}\text{Pb}/^{207}\text{Pb}$  ratio formed by the *s* process (that is, by neutron capture on a slow time scale compared with that of intervening decays). The primordial lead isotope ratios, exclusive of georadiogenic contributions, have been measured<sup>16</sup> in the troilite phase of iron meteorites, which contain appreciable abundances of lead, but (presumably) negligible amounts of uranium and thorium. Using the Fowler-Hoyle chronology, Clayton was able to calculate the change in the  $^{206}\text{Pb}/^{207}\text{Pb}$  ratio by cosmoradiogenic decay and obtained an expression for the normalization factor,  $r'$ , in terms of the solar abundance of lead.

Helliwell<sup>17</sup> found the solar atomic abundance of lead to be  $2.5 \pm 1.0$ , relative to  $10^6$  silicon atoms. From this Clayton derived  $r' = 0.42$ .

More recently Mutschlechner<sup>18</sup> has redetermined the solar abundance of lead to be 1.35 atoms per  $10^6$  silicon atoms. If this new value is used for the calculation it is found that  $r' = 0.23$ . The uranium and thorium abundances derived from these two values of  $r'$  are shown in Table 5.

It is clear from a comparison of the calculated and experimental uranium and thorium values that a very large and significant discrepancy exists. It can therefore be concluded that either the uranium and thorium abundances in the carbonaceous chondrites do not represent those of primitive solar material; or the calculated abundances are too high, and the normalization factor  $r'$  should be around 0.1, corresponding to a solar lead abundance of about 0.6 atoms per  $10^6$  silicon atoms. It should be noted, however, that the few measurements of lead abundances in Type I and Type II carbonaceous chondrites<sup>3,19</sup> are more in agreement with  $0.2 < r' < 0.4$ .

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# Haemoglobin New York

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A haemoglobin variant has been found in which a glutamic acid amino-acid residue is substituted at position 113 in the  $\beta$  chain for the usual neutral valine. This haemoglobin is called after the city in which it was found and is Hb- $\beta$ 113 Glu.

MOST of the abnormalities of human haemoglobin (Hb) have been identified on the basis of alterations in electrophoretic mobility of the haemoglobin variants. The present article is concerned with a haemoglobin which was difficult to separate from normal haemoglobin (Hb A) on electrophoresis in starch gels although more clear cut separation was obtained with paper electrophoresis (Figs. 1A and B). The variant haemoglobin, designated haemoglobin New York, migrated slightly further toward the anode at pH 8.6 than did Hb A, but the difference in mobility was less than would be expected from a net difference of two charges which is commonly found in abnormal haemoglobins.

Haemoglobin New York (Hb N.Y.) was encountered in a Chinese-American family in which three individuals were heterozygous for the new variant and two were doubly heterozygous for Hb N.Y. and high  $A_2$  thalassaemia. The interaction with high  $A_2$  thalassaemia, which resulted in the synthesis of large amounts of Hb N.Y. and little or no Hb A, together with the absence of a minor component which would correspond to the  $\alpha_2$  abnormal  $\delta_2^A$  component expected in an  $\alpha$ -chain variant, suggested that Hb N.Y. represented a substitution of the  $\beta$  chain. Hybridization studies were not satisfactory but on electrophoresis of globin on urea gels by the method of Chernoff and Pettit<sup>1</sup> the  $\beta$  chains of Hb N.Y. migrated farther toward the anode than did the globin of Hb  $\beta^A$ , while  $\alpha^{N.Y.}$  chains did not differ in mobility from the normal  $\alpha$  chains.

For structural studies, haemolysates were prepared from the blood of an individual most of whose haemoglobin was of the variant type. Haemoglobin N.Y. was separated from Hb F and from non-haem proteins by column chromatography on 'IRC-50' with developer No. 2 of Allen *et al.*<sup>2</sup>, and was later isolated by starch granule electrophoresis in veronal buffer pH 8.6. To minimize possible contamination with Hb A, only the more rapidly migrating portion of the main component was eluted from the starch granule separation. Peptide maps prepared by the method of Baglioni<sup>3</sup> from the tryptic digest of globin of Hb N.Y. did not reveal significant differences from maps of globin of Hb A.

The  $\beta$  chains of Hb N.Y. and of Hb A were isolated by a modification of the method of Bucci and Fronticelli<sup>4</sup> in which the haemoglobin was exposed to parahydroxymercuribenzoate (PMB) at pH 5.8 overnight and  $\beta$  polypeptide chains (with —SH groups blocked by PMB) of Hb N.Y. and of Hb A were isolated by electrophoresis on starch granules in barbital buffer at pH 8.6. The  $\beta$  chain component was eluted, concentrated by ultra-filtration and the respective globins were prepared by precipitation with acid-acetone. A sample of each lyophilized globin was treated with mercaptoethanol, urea and, after dialysis,

with ethylenimine by the method of Clegg, Naughton and Weatherall<sup>5</sup>. The conversion by ethylenimine of cysteine residues to *S*-aminoethyl-cysteine results in a modified

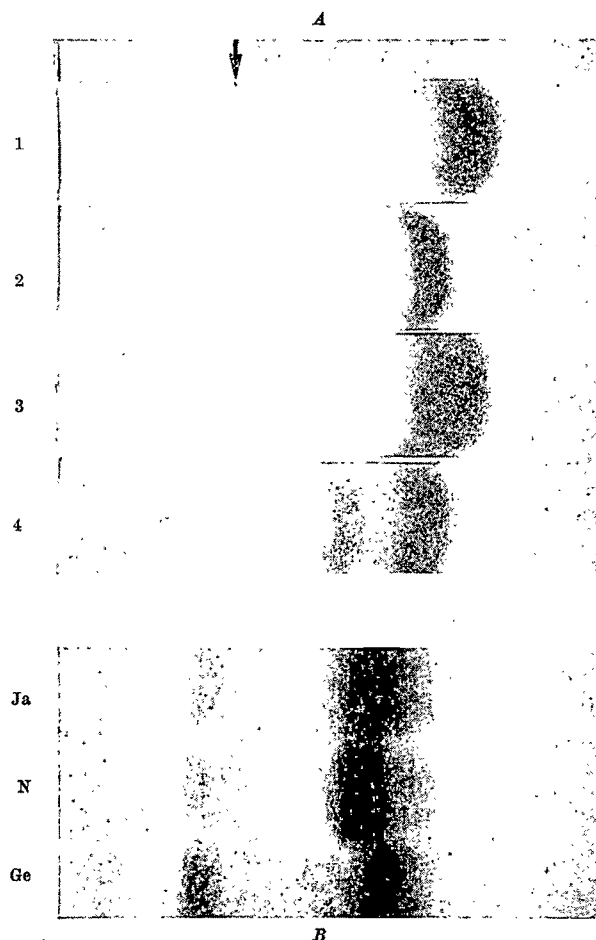


Fig. 1. A, Filter paper electrophoretic pattern. Tris-EDTA-borate-veronal buffer pH 8.6. Anode on right. Unstained. Arrow indicates site of application of sample. (1) Hb N.Y. (from individual doubly heterozygous for Hb N.Y. and high  $A_2$  thalassaemia); (2) normal Hb A; (3) Hb A and Hb N.Y. (from individual heterozygous for Hb N.Y.); (4) Hb S + Hb A (from unrelated individual with sickle cell trait). B, Starch gel electrophoresis of haemoglobin from individuals with Hb N.Y. and from a normal individual. Tris-borate buffer pH 8.6. Anode on right. Benzidine stain. Benzidine stain diffused somewhat; consequently resolution was sharper in unstained gels; addition of ethylenediamine tetraacetic acid did not improve the resolution. Sample Ja contained Hbs  $A_2$ , A and N.Y.; sample N from a normal individual contained Hbs  $A_2$  and A; sample Ge (from individual doubly heterozygous for Hb N.Y. and high  $A_2$  thalassaemia) contained increased proportions of Hbs  $A_2$  and F together with Hb N.Y.



globin which no longer contains a trypsin resistant "core"<sup>6,7</sup>. Peptide maps of tryptic digest of aminoethylated (AE)  $\beta^A$  and  $\beta^{N.Y.}$  chains are shown in Fig. 2. Aminoethylated globin New York contained a peptide which differed chromatographically and migrated farther toward the anode than did the corresponding peptide of AE $\beta$  globin A. The differing peptides were eluted by the method of Sanger and Tuppy<sup>8</sup>, hydrolysed in 6 normal hydrochloric acid for 22 h at 105° C, and applied directly<sup>5</sup> to the short and long columns of an automatic amino-acid analyser (Beckman 120B).

In Table 1 are given the results of the amino-acid analysis and the composition of the peptides from AE $\beta^{N.Y.}$  and AE $\beta^A$ .

Table 1. AMINO-ACID ANALYSIS AND COMPOSITION OF PEPTIDES  $\beta$ T-12b FROM AE $\beta$  CHAINS OF Hb N.Y. AND OF Hb A

Amino-acid	AE $\beta^{N.Y.}$			AE $\beta^A$		
	Found ( $\mu$ moles)	Molar ratio	Integral value	Found ( $\mu$ moles)	Molar ratio	Integral value
Lysine	0.139	1.0	1	0.070	0.8	1
Histidine	0.280	2.1	2	0.159	1.9	2
Glutamic acid	0.113	0.8	1	—	—	—
Glycine	0.152	1.1	1	0.107	1.3	1
Alanine	0.120	0.9	1	0.092	1.1	1
Valine	—	—	—	0.083	1.0	1
Leucine	0.134	1.0	1	0.069	0.8	1
Phenylalanine	0.128	1.0	1	0.088	1.1	1

The core peptide of the  $\beta$  chain of composition indicated for the peptide derived from Hb A in Table 1 corresponds to residues 113 to 120 in the  $\beta$  chain sequence<sup>9</sup>:

112 113 114 115 116 117 118 119 120  
Cys - Val - Leu - Ala - His - His - Phe - Gly - Lys

This sequence is the carboxyl terminal part of  $\beta$ T-12: it results from the trypsin attack of the bond between  $\beta$ 113 and  $\beta$ 112 (an *S*-aminoethyl-cysteine residue in AE $\beta$  globin). This basic peptide was found by Jones<sup>7</sup> in AE $\beta$  globin and was designated  $\beta$ T-12b. In Hb N.Y.,  $\beta$ T-12b peptide lacked a valine residue present in Hb A and contained a mole of glutamic acid which was absent from

Hb A. Because the  $\beta$ T-12b peptide of  $\beta^A$  contains only one valyl ( $\beta$ 113) residue in Hb N.Y., valine  $\beta$ 113 appeared to be replaced by glutamic acid. The difference in electrophoretic mobility in the peptides  $\beta$ T-12b of Hb N.Y. and of Hb A strongly suggested that the substituted residue in Hb N.Y. was glutamic acid rather than glutamine, although the absence of glutamine in  $\beta$ T-12b from Hb N.Y. has not been directly demonstrated. Furthermore the substitution of glutamic acid, but not of glutamine, for valine could result from a change in a single base of the triplet code of messenger RNA (ref. 10). Because the substitution of glutamic acid for valine appeared to be at the N-terminal position in the peptide  $\beta$ T-12b, confirmatory evidence for the substitution was obtained by treating these peptides from Hb N.Y. and from Hb A with fluorodinitrobenzene by a modification of the method of Sanger<sup>11</sup>. The peptides were eluted from four peptide maps, exposed to fluorodinitrobenzene, extracted with ether, and the aqueous solutions hydrolysed at 105° C for 12 h. In peptide  $\beta$ T-12b from Hb A, DNP-valine was demonstrated by thin layer chromatography of the ether soluble fraction of the hydrolysate. Direct demonstration of DNP-glutamic acid in  $\beta$ T-12b from Hb N.Y. was not achieved in two attempts; however, on each occasion, the molar ratio of glutamic acid had decreased markedly (to level encountered from contaminants on analyses of paper elutions) on amino-acid analysis of the aqueous phase of the hydrolysate.

The properties conferred on Hb N.Y. by this core substitution are of some interest. The  $\beta$ 113 residue corresponds to the helical residue G15 in the model of Perutz<sup>12</sup>. Residue G15 is apparently at the surface or in a surface crevice of the polypeptide chain<sup>13</sup>; the corresponding residue of the  $\alpha$  chain is threonine, a polar residue. Although G15 is not itself involved in sub-unit contact in tetrameric haemoglobin, nearby residues (G17, G18) are, according to Perutz<sup>12</sup>, possible non-polar contacts between residues in the  $\alpha_1$  and  $\beta_1$  sub-units of haemoglobin. In

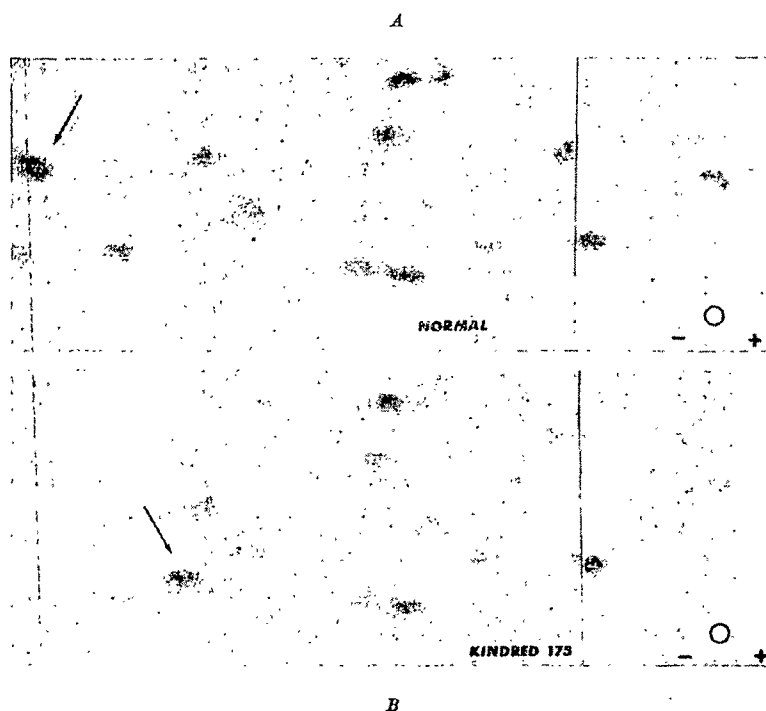


Fig. 2. A and B, Peptide maps of AE $\beta$  chains of Hb A (normal) and of Hb N.Y. (kindred 175). Circle indicates site of application. Arrows indicate peptide ( $\beta$ T-12b) in normal Hb which was more negatively charged and differed chromatographically from Hb N.Y. Electrophoresis at 3,000 V for 105 min in 1.25 per cent acetic acid, 1.25 per cent pyridine, pH 4.7. Ascending chromatography for 16 h in pyridine: isoamyl alcohol: water (35:35:27). Papers were cut vertically after electrophoresis (vertical line) in order to fit chromatography tank.

Fig. 1, the electrophoretic mobility of haemoglobin S (in which valine replaces glutamic acid at  $\beta 6$ ) is compared with that of Hb N.Y. (glutamic acid for valine at  $\beta 113$ ). The substituted glutamic residue of Hb N.Y. does not confer the expected charge difference, perhaps because of its location in the tetrameric haemoglobin. The electrophoretic mobilities of isolated  $\beta^{N.Y.}$  globin and  $\beta^A$  globin, which lack the tetrameric conformation, were consistent with an additional 2 net negative charges in Hb N.Y.

The difficulty in demonstrating the heterozygous state for Hb N.Y. on electrophoresis using starch gels or horizontal paper methods makes uncertain the incidence of this variant haemoglobin. Limitations on interpretations of the electrophoretic mobilities of haemoglobins are suggested by the studies of Hb Köln in which substitution of a neutral for a neutral amino-acid residue yielded a haemoglobin with a definite alteration in its electrophoretic mobility<sup>14</sup> and by the present investigation of Hb N.Y. in which the tetrameric haemoglobin containing substitution of an acidic for a neutral amino-acid showed only modest electrophoretic differences from Hb A.

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## Low Molecular Weight RNA Components from KB Cells

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Simple oligonucleotide maps can be obtained by two-dimensional paper electrophoresis from enzyme digests of 5S RNA from KB carcinoma ribosomes. These maps can be distinguished from those obtained from 5S RNA derived from *E. coli* and from RNA components of low molecular weight derived from KB cells with and without adenovirus infection.

A low molecular weight component of ribonucleic acid (5S RNA) is present, bound to ribosomes, in a number of different cell species: *Escherichia coli*<sup>1,2</sup>, yeast<sup>3</sup>, the aquatic fungus *Blastocladiella emersonii*<sup>4,5</sup>, sea urchins<sup>6</sup>, rat liver cells<sup>6</sup>, KB cells (human epidermoid carcinoma line)<sup>6,7</sup>, and rabbit reticulocytes. Adenovirus 2 infection causes the appearance in KB cells of a somewhat similar RNA component (VA-RNA)<sup>7</sup> which is synthesized at a much greater rate than is 5S RNA in uninfected KB cells. Both VA-RNA and 5S RNA share sedimentation coefficients in sucrose gradients and base compositions which are similar to those of sRNA (soluble or transfer RNA); both can be separated from sRNA by chromatography on columns of methylated albumin kieselguhr (MAK) or on long columns of 'Sephadex G-100'; both differ from sRNA in their inability to accept amino-acids (ref. 6 and Forget, B. G., and Weissman, S. M., unpublished results), and in their lack of methylated bases and pseudouridine. VA-RNA differs from 5S RNA of uninfected KB cells only by small differences in base composition, its rate of synthesis, and its cellular localization predominantly in the 100,000g supernatant fraction of disrupted KB cells. For a lack of more specific distinguishing characteristics these two RNA components were not easily differentiated from each other.

Recently Sanger *et al.*<sup>8</sup> have introduced an improved two dimensional, paper electrophoresis, oligonucleotide mapping technique for the study of enzymatic digests of phosphorus-32 labelled RNA. After electrophoresis, the total number, relative yields, position, and base sequences of the oligonucleotides in an RNA digest give an indication of the size and molecular homogeneity of this RNA

component and can provide a specific "fingerprint" for its identification.

This procedure has enabled us to differentiate definitively from each other: VA-RNA, KB cell 5S RNA, *E. coli* 5S RNA and another 5S-like RNA which we have isolated from ribosomes and nuclei of uninfected KB cells. It has also enabled us to establish the relatively homogeneous nature of these RNA components.

The KB cells, adenovirus 2 stock and techniques of cell culture, inoculation with virus, radioactive labelling, cell fractionation, RNA preparation, fractionation and analysis were the same as previously described<sup>7</sup>, except where otherwise indicated. The KB cell line used (originally obtained from Dr. M. Green) was shown to be free of mycoplasma by culture. *E. coli* cells, type B and strain RNase 19 (RNase deficient)<sup>9</sup>, were obtained from Dr. S. Pestka of the National Institutes of Health; they were grown and labelled as described by Sanger *et al.*<sup>8</sup>.

Between 5 and 10 mc. of (<sup>32</sup>P) phosphoric acid (carrier free, obtained from International Chemical and Nuclear Corporation) was added to 600–1,200 ml. of KB cell suspension cultures: adenovirus 2 infected cells were labelled for 4 h from the 16th to 20th hour after inoculation with virus; uninfected KB cells in a concentration of 2 × 10<sup>6</sup> cells/ml. were labelled for 24–48 h. After disruption of the cells in a homogenizer, the nuclei were removed by centrifugation at 800g and treated as described by Steele *et al.*<sup>10</sup>, to obtain the "nucleolar fraction". The 800g supernatant fraction of the homogenate was made 0.5 per cent with sodium deoxycholate, spun at 10,000g to remove heavier sediment, then the ribosomes and 100,000g supernatant fractions were separated by ultra-

centrifugation for 2 h. The fractionation steps were monitored by light microscopy to ensure (more than 95 per cent) disruption of the cells, removal of cytoplasmic fragments from nuclei, and complete disruption of nuclei with preservation of intact nucleoli. Low molecular weight RNA was prepared from *E. coli* as described by Schleich and Goldstein<sup>11</sup>; after 'Sephadex' gel filtration, the fractions containing 5S RNA ('region or peak 3') were pooled and re-chromatographed on columns of MAK.

After MAK chromatography and analysis for radioactivity, the appropriate RNA fractions were pooled and dialysed for 2 h at 4° C against 2 per cent potassium acetate to remove phosphate. After addition of 0.5 mg of carrier commercial *E. coli* sRNA (Nutritional Biochemicals Corporation) the RNA was precipitated by addition of two volumes of 95 per cent ethanol. Enzyme digestion and oligonucleotide mapping of the phosphorus-32 labelled RNA components were carried out as described by Sanger *et al.*<sup>8</sup>. We used the same materials except for the following: pancreatic ribonuclease, five times crystallized, was obtained from the Sigma Chemical Corp.; ribonuclease *T*<sub>1</sub> from Calbiochem; cellulose acetate strips were obtained from Gelman Instrument Co. ('Cellogel') and from Colab Laboratories, Inc. ('Sephaphore No. 3'). Ethylenediamine tetraacetic acid (EDTA) was added, to a final concentration of 0.01 moles/l., to the buffer in which the strips were soaked before the RNA digest was applied. Gilson model D electrophorators were used for both ionophoresis steps: the temperature of the tanks was maintained at 70° F.

Chromatography on MAK columns of radioactive RNA prepared from *KB* cell ribosomes gave somewhat different results when different extraction procedures were used: when the hot phenol sodium dodecyl sulphate (SDS) technique<sup>12</sup> was used, three radioactive RNA components eluted after sRNA (Fig. 1). These will be referred to here as ribosomal components A, B and C. Occasionally components A and B were not well separated from each other, and formed a single somewhat broader peak. When the cold phenol-SDS technique<sup>13</sup> was used a single main radioactive component, 5S RNA, eluted after sRNA. It was chromatographically similar to the 5S RNA obtained by others<sup>2,6</sup>, and in experiments involving dual label co-chromatography, it occupied the same position as ribosomal component A obtained after extraction with hot phenol-SDS. Much less radioactive material eluted in the positions of ribosomal components B and C when cold phenol-SDS was used to extract the RNA from ribosomes. These patterns were obtained repeatedly from different cell preparations, if the cells were labelled for a relatively long period of time (more than 12 h); the same chromatographic patterns were obtained when the cells were labelled, in non-phosphate deficient medium, with tritiated or carbon-14 uridine. The buffer used for the hot phenol-SDS RNA extractions contained 0.05 per cent bentonite instead of PVS, as used by Scherrer and Darnell<sup>12</sup>.

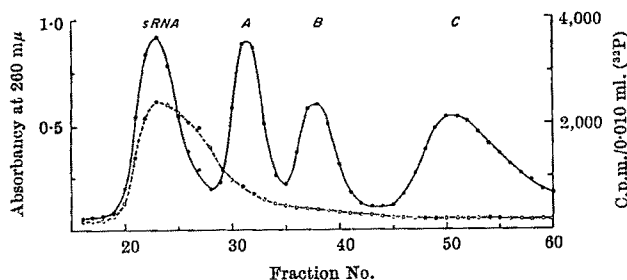


Fig. 1. MAK column chromatogram of RNA prepared by the hot phenol-SDS method from ribosomes of uninfected *KB* cells labelled for 48 h with 5 mc. of phosphorus-32. Two milligrams of non-radioactive *E. coli* sRNA were added to the RNA preparation before it was loaded on to the column. ○, Absorbance at 260 mμ. ●, C.p.m. phosphorus-32/0.010 ml. of each fraction eluted.

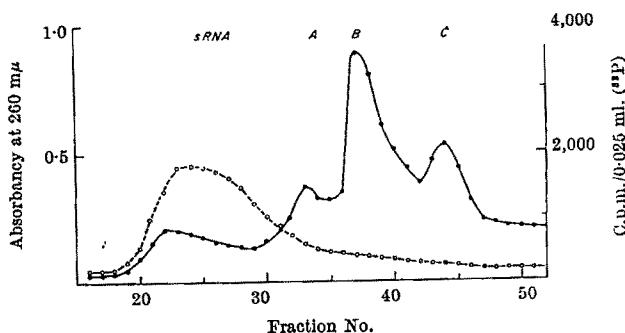


Fig. 2. MAK column chromatogram of RNA prepared by the hot phenol-SDS method from the 'nucleolar fraction' of uninfected *KB* cells labelled for 48 h with 5 mc. of phosphorus-32. Two milligrams of non-radioactive *E. coli* sRNA were added to the RNA preparation before it was loaded on to the column. ○, Absorbance at 260 mμ. ●, C.p.m. phosphorus-32/0.025 ml. of each fraction eluted.

Because 5S RNA ('peak X') has been described in nucleoli of *B. emersonii*<sup>4</sup>, the RNA from the 'nucleolar fraction'<sup>10</sup> of *KB* cells was studied by MAK chromatography after both hot and cold phenol RNA extraction. Chromatography of the RNA gave the same result after either extraction procedure: as with the RNA extracted from ribosomes, three radioactive components (nucleolar components A, B and C) eluted after sRNA (Fig. 2), but the relative amount of radioactivity in each peak was quite different from that obtained with RNA from hot phenol-SDS extracted ribosomes. Table 1 lists the results of alkaline digest base analyses performed on these various RNA components by the method of Sebring and Salzman<sup>14</sup>.

Oligonucleotide mapping was performed on pancreatic ribonuclease digests of the various radioactive RNA components of low molecular weight obtained from ribosomes and nuclei of *KB* cells. Phosphorus-32 labelled 5S RNA extracted from *KB* cell ribosomes with cold phenol-SDS provided, after digestion with pancreatic ribonuclease, a simplified and distinctive oligonucleotide map. A diagram of this map (Fig. 3) is presented with the oligonucleotides numbered according to the scheme of Sanger *et al.*<sup>8</sup>.

Table 1. BASE COMPOSITION OF PHOSPHORUS-32 LABELLED RNA COMPONENTS

RNA species	Cytidylic acid (per cent)	Adenylic acid (per cent)	Guanidylic acid (per cent)	Uridylic acid (per cent)
<i>KB</i> cell				
sRNA	29.9	19.2	30.8	20.1
Ribosomal 16S RNA	27.7	20.6	31.1	20.6
Ribosomal 28S RNA	31.4	18.4	35.4	16.7
5S RNA	27.4	18.8	31.3	22.3
Ribosomal component A	27.3	18.4	32.1	22.2
Ribosomal component B	27.4	18.5	31.9	22.2
Ribosomal component C	27.3	19.6	29.4	23.6
Nucleolar component A	27.4	21.6	29.8	21.2
Nucleolar component B	26.8	20.1	28.1	24.9
Nucleolar component C	24.4	20.1	29.5	25.9
VA-RNA	29.1	18.1	34.4	20.5
<i>E. coli</i> B				
sRNA	27.1	19.2	30.1	23.6
5S RNA	29.3	19.1	34.7	17.0

The radioactive RNA components were isolated by MAK column chromatography (or sucrose density-gradients for ribosomal 16S RNA and 28S RNA), and analysed as described by Sebring and Salzman<sup>14</sup>.

Oligonucleotide maps prepared from pancreatic ribonuclease digests of phosphorus-32 labelled *KB* cell ribosomal RNA components A, B and C extracted with hot phenol-SDS gave the following results: components A and B yielded maps indistinguishable from that of the 5S RNA (Fig. 3). Component A is therefore 5S RNA and component B may be an aggregate of 5S RNA. On the other hand, ribosomal component C yielded a map (Fig. 4) which, although simple, differs from the 5S RNA map in a number of areas: Spots 10, 17, 23 and 29 present in 5S RNA are absent in component C, while spots 8, 22, 25 and 45 are present in component C but absent in 5S RNA; spots 9 and 11, very faint in 5S RNA, are very dark in component C, and the larger oligonucleotides also differ.

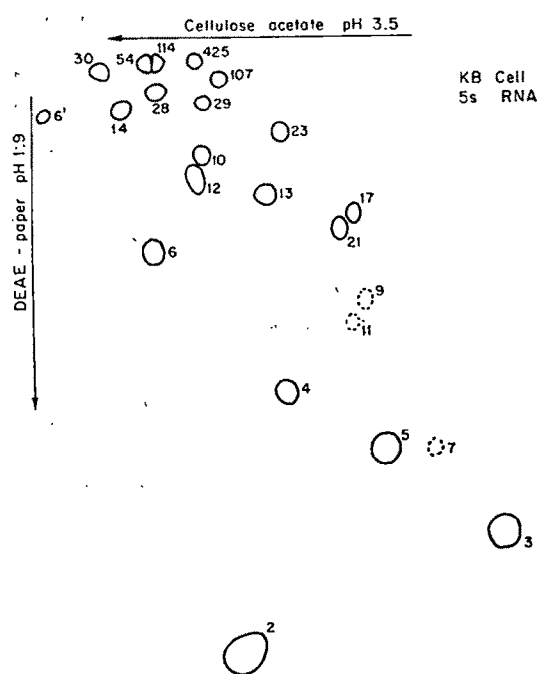


Fig. 3. Diagram of the autoradiograph of a two-dimensional fractionation of a pancreatic ribonuclease digest of phosphorus-32 labelled *KB* cell 5S RNA. The oligonucleotides are numbered according to the scheme of Sanger *et al.*<sup>8</sup> and are identified in Table 2. Cyclic nucleotides and trace spots thought to result from random nuclease activity have been omitted from the diagram.

Oligonucleotide maps obtained from pancreatic ribonuclease digests of the nucleolar 5S-like RNA components gave the following results: nucleolar component *A* gave a map indistinguishable from that of 5S RNA, but nucleolar components *B* and *C* both gave maps which closely resembled that of ribosomal component *C*. The latter maps differ from that of ribosomal component *C* by the presence of additional spots: spots 10, 16 and a few other faint spots are present in the nucleolar maps, but not in the ribosomal component *C* maps. On the basis of oligonucleotide maps, therefore, other homogeneous 5S-like RNA components, present in RNA from both ribosomes and the nucleolar fraction of *KB* cells, exist in addition to the principal 5S RNA component of *KB* cell ribosomes.

Oligonucleotide mapping also provided a tool for comparing 5S RNA of *KB* cells with that obtained from bacteria. 5S RNA from *E. coli* cells labelled with phosphorus-32 was digested with pancreatic ribonuclease and mapped in the same fashion as the *KB* cell 5S RNA. The resulting maps (Fig. 5) were identical for both the *B* and RNase 19 (RNase deficient) strains of *E. coli*, but differed from the maps of *KB* cell 5S RNA. The map of *E. coli* 5S RNA reveals a simple pattern, but many of the oligonucleotides present are different from those of *KB* cell 5S RNA: spots 17, 28, 29 and 30 present in *KB* 5S RNA are absent in *E. coli* 5S RNA; spots 35 and 62 prominent in *E. coli* 5S RNA are absent in *KB* 5S RNA. In addition, the larger oligonucleotides differ and spots 2' and 9' representing the ends of the molecule of *E. coli* 5S RNA<sup>15</sup> are absent from *KB* 5S RNA. Spot number 6' probably represents the 5' end of *KB* cell 5S RNA. There are therefore differences in the primary structure of the chief 5S RNA components of these two species, and the presence of 5S RNA in the RNase deficient strain of *E. coli* makes it less likely that 5S RNA results from RNase action on heavier molecular weight RNA components.

Finally, the oligonucleotide maps obtained from pancreatic ribonuclease digests of phosphorus-32 labelled VA-RNA (Fig. 6) were quite different from those of the major *KB* cell or *E. coli* 5S RNA components, or those

of the other *KB* cell 5S-like RNA components. The map was slightly more complex than the 5S RNA maps. It contained more trace spots and these spots were darker than the very faint trace spots obtained on 5S RNA maps. These could result from small amounts of other ribonucleic acids contaminating the VA-RNA preparations or could indicate that VA-RNA is larger and/or less homogeneous than 5S RNA. The VA-RNA map was still simple and distinctive, however, when compared with maps which we obtained from pancreatic ribonuclease digests of *KB* cell sRNA and ribosomal RNA. These maps were extremely complex and similar to those presented by Sanger *et al.*<sup>8</sup> for *E. coli* sRNA and ribosomal RNA. The VA-RNA map differed from the map of the major *KB* cell 5S RNA (Fig. 3) in the following areas: spots 16, 19, 26, 27, 37 and 62 present in VA-RNA are entirely absent in *KB* cell 5S RNA maps; while spots 17, 23 and 24 present in 5S RNA are absent from VA-RNA; spot 11, prominent in VA-RNA, is present only in trace amounts in 5S RNA, whereas spot 10, prominent in 5S RNA, is faint in VA-RNA. Finally, the larger oligonucleotides present in VA-RNA are different from those in the 5S RNA map.

These differences in the composition and proportional yields of oligonucleotides obtained from pancreatic ribonuclease digests of VA-RNA and 5S RNA indicate that these two RNA components contain many regions in their molecules where there are no common base sequences. VA-RNA and 5S RNA are therefore different molecules easily distinguishable one from another by oligonucleotide mapping. Ribonuclease *T*<sub>1</sub> digests of these RNA components also provide distinctive oligonucleotide maps with many differences between VA-RNA, *KB* cell 5S RNA, *E. coli* 5S RNA and the other *KB* cell 5S-like RNA components.

Table 2 lists the base sequences and relative yields of the various oligonucleotides obtained from pancreatic RNase digestion of VA-RNA, *KB* cell 5S RNA, *E. coli* 5S RNA and *KB* cell ribosomal component *C*. There are only twenty-one chief and nine trace components in digests of VA-RNA; twenty-one chief and three trace components

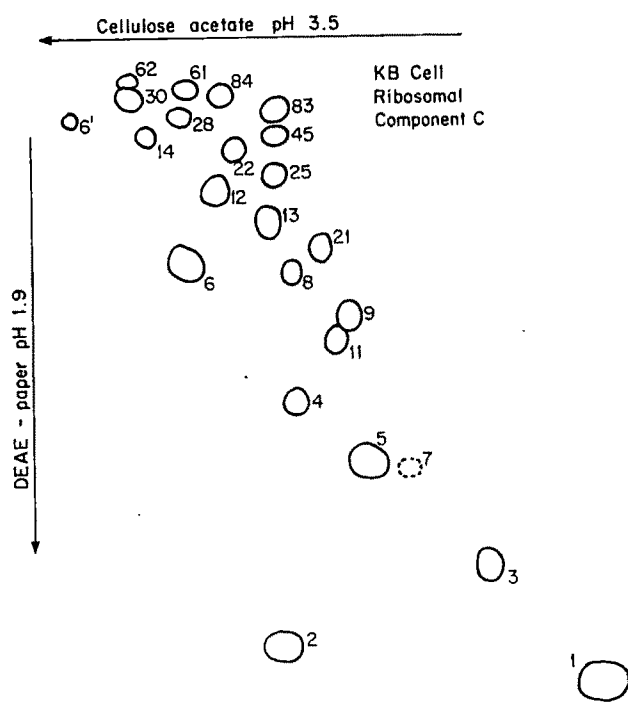


Fig. 4. Diagram of the autoradiograph of a two-dimensional fractionation of a pancreatic ribonuclease digest of phosphorus-32 labelled *KB* cell ribosomal component *C*. The oligonucleotides are numbered according to the scheme of Sanger *et al.*<sup>8</sup> and are identified in Table 2. Cyclic nucleotides and trace spots thought to result from random nuclease activity have been omitted from the diagram.

Table 2. NUCLEOTIDES FROM PANCREATIC RIBONUCLEASE DIGESTS OF RNA COMPONENTS OF LOW MOLECULAR WEIGHT

Spot No.	Nucleotide sequence	VA-RNA	KB cell 5S RNA	<i>E. coli</i> 5S RNA	KB cell component C
1	C	+	+	+	+
2	U	+	+	+	+
3	pUp	0	0	+	0
4	AC	+	+	+	+
5	AU	+	+	+	+
6	ApU	0	0	+	0
7	GC	+	+	+	+
8	GU	+	+	+	+
9	pGpU	+	+	0	+
10	AAU	Tr	Tr	Tr	Tr
11	AAU	0	0	0	0
12	AGC	Tr	Tr	+	+
13	AGU	Tr	Tr	+	+
14	GAC	+	Tr	+	+
15	GAU	+	+	+	+
16	GAC	+	+	+	+
17	GGU	+	+	+	+
18	AAA	+	0	0	0
19	AAGC	0	+	0	0
20	AGAC	+	0	0	0
21	GAAC	+	+	+	+
22	GAAU	0	0	0	+
23	AGGC	0	+	+	+
24	GAGC	0	0	0	+
25	GAGU	+	0	0	0
26	GGAC	+	0	0	0
27	GGAU	+	+	0	+
28	GGGC	+	+	0	+
29	GGGU	Tr	+	0	+
30	AGAAC	Tr	0	0	0
31	GAAAC	0	0	+	0
32	AGGAC	Tr	0	0	+
33	GGAAC	Tr	0	0	0
34	GAGGU	0	+	0	0
35	GGGGC	0	0	0	+
36	GGGGU	+	0	+	+
37	AA(G,A)GU	0	0	+	0
38	AGGAAC	0	0	0	+
39	AGGAAU	0	0	0	+
40	AGGGAC	Tr	0	0	0
41	G <sub>1-2</sub> AAGGU	+	0	0	0
42	GGAAGC	Tr	+	0	0
43	GGAAGU	0	+	+	0
44	GGGAAU	0	+	0	0
45	G <sub>1-2</sub> (A,G)GGU	+	0	0	0
46	AGGGAAC	0	+	0	0
47	GGGAGAC	0	+	0	0

The oligonucleotides have been numbered according to the scheme of Sanger *et al.*<sup>8</sup>. The 5' end of the oligonucleotide is always given first in the sequence. The exact sequence of the nucleotides between parentheses is not determined. The position of the oligonucleotides is given in Figs. 3-6. C, Cytidylic acid; A, adenylic acid; G, guanylic acid; U, uridylic acid; pUp, uridine di (3'-5') phosphate; ApU, adenylic acid-uridine; pGpU, guanosine di (3'-5') phosphate-uridylic acid; Tr, nucleotides present only in trace amounts.

in digests of KB cell 5S RNA; twenty-one chief and one trace component in digests of *E. coli* 5S RNA; twenty-four chief and one trace component in digests of KB cell ribosomal component C. Certain oligonucleotides are conspicuously absent. The two mononucleotides and four dinucleotides expected are present in substantial amounts in all four components; of the eight trinucleotides possible, one, spot 8 (AAU), is entirely absent from

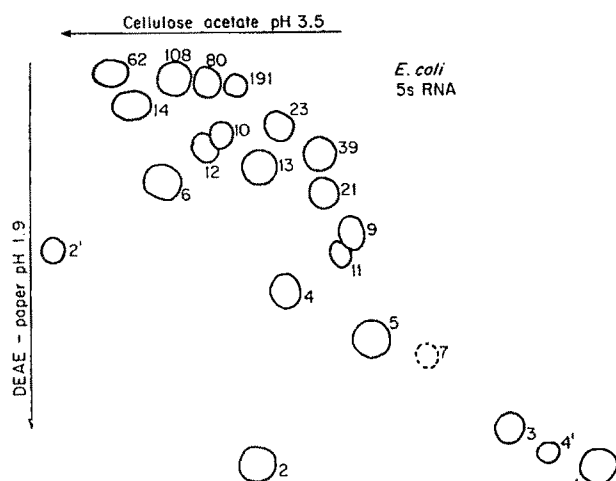


Fig. 5. Diagram of the autoradiograph of a two-dimensional fractionation of a pancreatic ribonuclease digest of phosphorus-32 labelled *E. coli* 5S RNA. The oligonucleotides are numbered according to the scheme of Sanger *et al.*<sup>8</sup> and are identified in Table 2. Cyclic nucleotides and trace spots thought to result from random nuclease activity have been omitted from the diagram.

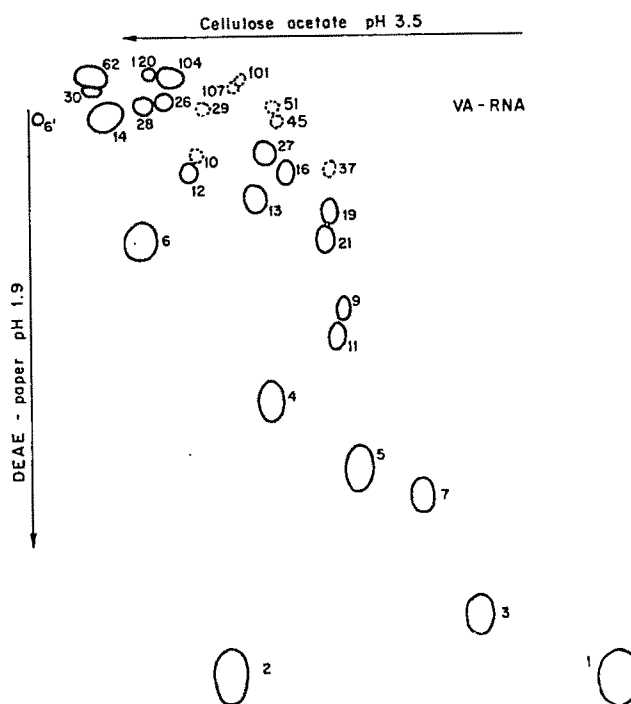


Fig. 6. Diagram of the autoradiograph of a two-dimensional fractionation of a pancreatic ribonuclease digest of phosphorus-32 labelled VA-RNA<sup>7</sup>. The oligonucleotides are numbered according to the scheme of Sanger *et al.*<sup>8</sup> and are identified in Table 2. Cyclic nucleotides and trace spots thought to result from random nuclease activity have been omitted from the diagram.

VA-RNA, KB cell and *E. coli* 5S ribonucleic acids but is present in component C which, however, entirely lacks spot 10 (AGU). Several other trinucleotides are present only in trace amounts in one or the other RNA components. Of the sixteen possible tetranucleotides, only two are present in *E. coli* 5S RNA, six in KB cell ribosomal component C, six in KB cell 5S RNA, and seven in VA-RNA. Four possible tetranucleotides (15, 18, 20 and 24) (ref. 8) are entirely absent from all four components. These results have been reproducible in repeated maps of these RNA components obtained from different *E. coli* and KB cell cultures; they characterize VA-RNA, 5S RNA and component C as distinct RNA components and virtually rule out the possibility that they are precursors or random degradation products of ribosomal or messenger RNA or that they result from random aggregation of light weight polynucleotides. The relatively small number of oligonucleotides present constitutes good evidence for the relative homogeneity of these RNA molecules, yet proves that they do not consist of a single repeating sequence.

Although the biological or biochemical functions of VA-RNA and 5S RNA are still unknown, more knowledge of the chemical structure of these low molecular weight components has been obtained by the technique of oligonucleotide mapping. It has demonstrated the relatively homogeneous nature of these molecules, permitted positive differentiation of VA-RNA from 5S RNA and provided evidence for the occurrence of at least two distinct low molecular weight RNA molecules, other than sRNA, in extracts of uninfected KB cells.

After this work was performed, we learned through Information Exchange Group No. 7 (ref. 15) of the work of J. Hindley, M.R.C. Laboratory of Molecular Biology, Cambridge, England. With pancreatic ribonuclease digests of phosphorus-32 labelled 5S RNA from *E. coli* strain MRE-600, he obtained oligonucleotide maps very similar to those which we obtained from 5S RNA of *E. coli* type B and RNase I<sup>9</sup>. By means of acrylamide gel electrophoresis, he was able to separate his chief 5S RNA component into two bands which gave identical maps,



and in addition he was able to isolate from crude preparations of *E. coli* sRNA two other RNA components which, after digestion with enzymes, yielded simple oligonucleotide maps, distinct from that of the chief 5S RNA band. Maps prepared from enzyme digests of yeast 5S RNA also gave a simple pattern, but different from that of *E. coli* 5S RNA and from any of those which we have presented in this article.

We thank Miss Carol Hybner for technical assistance in this work.

Photographs of the autoradiographs can be obtained from us.

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## Genetics of Phenylketonuria

by

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The first article deals with the problem of using the response to injected phenylalanine to determine whether or not the subject is heterozygous for phenylketonuria. The second article suggests a third allele on the phenylketonuria locus, the corresponding enzyme having a higher affinity for phenylalanine than has the "normal" enzyme.

### Heterozygosity for Phenylketonuria

HETEROZYGOTES for phenylketonuria have only one active gene at the relevant locus, and they may therefore have less phenylalanine hydroxylase in their liver cells than normal people (homozygotes for the wild-type gene). Orally administered loads of phenylalanine are, in fact, metabolized more slowly by heterozygotes than by normals<sup>1-3</sup> and the rise in the concentration of tyrosine in the blood after an oral phenylalanine load is lower in heterozygotes than in normals<sup>4</sup>. These differences are statistically highly significant, but because the absorption of phenylalanine from the gut is a lengthy and variable process it is impossible to calculate rate constants for the metabolism of phenylalanine. Nelson<sup>5</sup> has pointed out that the results obtained could equally well be explained by differences in the distribution of phenylalanine in the various body compartments. The discriminating power of the tests is not very high, and allows a considerable proportion of individuals to be wrongly classified, a most important defect from the practical standpoint. Intravenous loads of phenylalanine have been used (refs. 6-8, and Perry, T. L., personal communication) in efforts to decrease the percentage of people misclassified and produce clearer evidence on enzyme levels. Our experiments and results are described here.

Thirty-one heterozygotes (the parents of children with phenylketonuria) and thirty-four normals (adults unrelated to a phenylketonuric, so far as could be determined) were given phenylalanine as a single dose by intravenous injection of the buffered sodium salt in sterile aqueous solution after an overnight fast. The dose was about 80 mg phenylalanine/kg body weight; 10 mg phenazone/kg body weight was added to the solution for injection. Full details of the mode of administration, etc., will be published elsewhere. A specimen of blood was obtained before the injection and, having allowed

50 min for equilibration after the injection, a second blood specimen was taken; further blood specimens were taken at timed intervals for a total of about 225 min. The plasma was analysed for phenylalanine<sup>9</sup>, tyrosine<sup>9,10</sup> and phenazone<sup>11</sup>, using several replicates for each determination.

In all but four of the sixty-five subjects the rate of return of plasma phenylalanine concentration to the fasting level was proportional to the difference between them, that is,

$$\frac{d\phi}{dt} = -k(\phi - \phi_0)$$

where  $\phi$  is phenylalanine concentration at time  $t$ ,  $\phi_0$  is the fasting level and  $k$  is a rate constant varying from individual to individual. The linearity of the relation of  $\log(\phi - \phi_0)$  to  $t$  was established both graphically and from the sums of squares of deviations<sup>12</sup>. By extrapolating back to the concentration at zero time, and from the amount of phenylalanine injected, the apparent volume through which the injected phenylalanine was distributed (the "phenylalanine space",  $v_\phi$ ) was obtained. The rate at which phenylalanine was being metabolized is

$$v_\phi \cdot \frac{d\phi}{dt} = -k(\phi - \phi_0) \cdot v_\phi$$

Urinary loss of phenylalanine and of its metabolites phenylpyruvic acid and *o*-hydroxyphenylacetic acid was found to be negligible. Although tyrosine hydroxylase can act on phenylalanine, the reaction is slow. Hence, to a close approximation, all the phenylalanine administered was converted to tyrosine by the action of phenylalanine hydroxylase and the parameter  $k$  should be related to the amount of this enzyme in the liver. Taking the liver mass as proportional to body weight or, more accurately, to total body water,  $v_w$  (determined as the phenazone space<sup>11</sup>)  $kv_\phi/v_w$  should be proportional to the amount of

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phenylalanine hydroxylase in each liver cell. The value of  $kv_p/v_w$  for the thirty-four normal individuals was  $1.035 \pm 0.21 \text{ min}^{-1}$  (mean  $\pm$  standard deviation) and for the thirty-one heterozygotes it was  $0.530 \pm 0.15 \text{ min}^{-1}$ . It would appear from these figures that homozygotes for the wild-type gene have almost exactly twice as much enzyme in each liver cell as heterozygotes.

The phenylalanine space,  $v_p$ , was not in general equal to the phenazone space,  $v_w$ . The value of  $(v_p - v_w)/v_w$  was  $0.297 \pm 0.12$  for normal males ( $n = 17$ ),  $0.171 \pm 0.24$  for normal females ( $n = 17$ ),  $0.041 \pm 0.096$  for male heterozygotes ( $n = 15$ ) and  $-0.078 \pm 0.11$  for female heterozygotes ( $n = 16$ ) (mean  $\pm$  standard deviation in each case). The differences between all these values are statistically significant. A positive value of  $(v_p - v_w)/v_w$  implies that phenylalanine is being concentrated in some tissue or tissues, possibly all, and a negative value implies that the intracellular concentration of phenylalanine is lower than the extracellular concentration for some or all tissues. The transport of phenylalanine into cells is probably independent of transport out of cells, and both are probably carrier-mediated, active processes in the cell membrane. It is not necessary to postulate an effect of genes at the phenylketonuria locus on carrier mechanisms in the cell membrane, independent of the effect on phenylalanine hydroxylase; changes in the cell membrane may be a slowly adaptive response to the higher concentrations of phenylalanine in the plasma of heterozygotes<sup>13</sup>. Preliminary investigations on homozygotes for phenylketonuria suggested that levels of  $(v_p - v_w)/v_w$  are lower than in heterozygotes; there appeared to be a tendency for this ratio to be higher where the value of  $\phi_0$  was lower. The magnitude of the negative values of  $(v_p - v_w)/v_w$  in some heterozygotes and phenylketonurics requires that many tissues, perhaps all, should be involved.

If all cells in the body concentrate or exclude phenylalanine to an equal extent, then, since intracellular water is about 75 per cent of total body water for males and 73 per cent for females, the intracellular concentration of phenylalanine in normal males would be 1.40 times as high as the extracellular concentration. This factor would be 1.23 in normal females, 1.05 in male heterozygotes and 0.89 in female heterozygotes. The apparent values of  $k$  would therefore be too high or too low by these factors (compare ref. 5); the corrected mean values of  $kv_p/v_w$  would be 0.739 for normal males, 0.841 for normal females, 0.502 for male heterozygotes, and 0.592 for female heterozygotes.

For distinguishing between heterozygotes and normals, the discriminatory power<sup>14</sup>,  $D/\bar{S}$ , of  $kv_p/v_w$  (uncorrected) is 2.8; using this test alone, 92 per cent of normals and heterozygotes would be classified correctly. The discriminatory power of  $(v_p - v_w)/v_w$  is 2.37 for males and 1.42 for females. The concentration of phenylalanine in the blood after an overnight fast ( $\phi_0$ ) was in our series (including some additional subjects)  $0.818 \pm 0.19$  ( $n = 40$ ) for normals and  $1.133 \pm 0.23$  ( $n = 39$ ) for heterozygotes, giving a discriminatory power of 1.50. The concentration of tyrosine in the plasma after an overnight fast ( $T_0$ ) in normals was  $1.127 \pm 0.19$  ( $n = 39$ ) and  $1.042 \pm 0.23$  ( $n = 34$ ) in heterozygotes. The rate and extent of the rise in blood tyrosine concentration after phenylalanine injection were significantly different in normals and heterozygotes; we have found the best discriminant on this basis to be

$$[\int_0^t (T - T_0) \cdot dt / \int_0^t (\phi - \phi_0) dt]^{\frac{1}{2}}$$

where  $T$  is the concentration of tyrosine in the plasma at time  $t$ . The discriminatory power between heterozygotes and normals of this function is 4.8 for females and 2.7 for males. Only 0.8 per cent of females would be misclassified by this criterion.

With the help of Dr. A. Barr and Dr. C. E. Phelps, discriminatory analysis is being applied to various func-

tions of  $k$ ,  $v_p$ ,  $v_w$ ,  $\phi_0$  and  $T$  with the object of finding a better discriminant incorporating them all.

We thank the heterozygotes and normals who volunteered to act as subjects. Dr. B. Kirman, Dr. V. A. Cowie, Dr. F. P. Hudson, Professor O. Wolff, Dr. G. Woods, Dr. C. Ounsted, Dr. G. E. Roberts, Dr. V. Smallpeice, Dr. A. C. Fairburn and Dr. W. L. Walker very kindly allowed us to investigate the relatives of patients under their care. We also thank Dr. D. Wade for administering the phenylalanine to many of the subjects and collecting blood specimens, and Dr. J. Stern for help with the many tests carried out at the Fountain Hospital and Queen Mary's Hospital, Carshalton.

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### Third Allele at the Phenylalanine Hydroxylase Locus in Man

PHENYLALANINE, administered as an intravenous load, is metabolized by a first order reaction in the majority of heterozygotes for phenylketonuria and homozygotes for the wild-type gene (see preceding article). If  $\phi$  is the phenylalanine concentration in the plasma at time  $t$  and  $\phi_0$  is the fasting level, in thirty-three out of thirty-four normal individuals and in twenty-seven out of thirty-one heterozygotes investigated,  $\log(\phi - \phi_0)$  plotted against  $t$  gave a straight line and  $\phi$  plotted against  $t$  gave a curve convex towards the origin (Figs. 1 and 2) in accordance with first order kinetics. In one heterozygote (case 1),

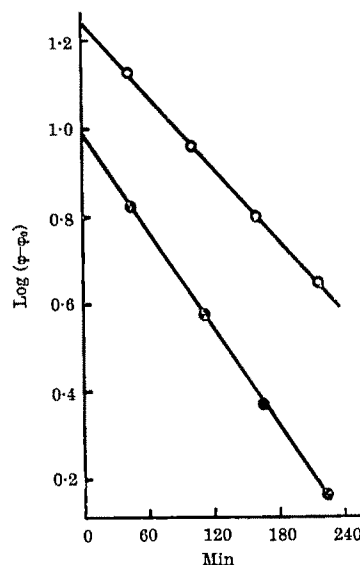


Fig. 1. Logarithm of the concentration of phenylalanine in the blood plasma, minus the pre-injection concentration, plotted against time after injection for a typical heterozygote (○) and a typical normal homozygote (●) for the wild-type gene.

however, the rate of disappearance of phenylalanine from the blood was linear with time, that is, independent of phenylalanine concentration within the limits studied and obeying zero order kinetics (Figs. 3 and 4).

The mean square deviations from linear regression were calculated for most of the subjects, both normal and heterozygote, and compared with the variance (mean square) due to random error of the determinations (Tables 1 and 2). From Tables 1 and 2 the plot of  $\phi$  against time for case 1 does not depart significantly from linearity, but the plot of  $\log(\phi - \phi_0)$  against time is non-linear. Similarly, in cases 2 and 3, the plot of  $\phi$  against time is more nearly linear than the plot of  $\log(\phi - \phi_0)$  against time (Figs. 3 and 4); in case 2 the deviation from linearity of the arithmetic plot was largely caused by the scattering of points alternately on either side of the best straight line, probably the result of experimental error. In one heterozygote (case 4) it was impossible to decide, either graphically or from the mean square deviations from linear regression, whether first order or zero order kinetics were being followed.

Table 1. DEVIATIONS FROM LINEAR REGRESSION:  $\phi$  PLOTTED AGAINST TIME

Geno- type*	Deviation from linearity		Random error of determination		Ratio† of vari- ances	P‡
	Sum of squares	D.F.	Sum of squares	D.F.		
H	1.3556	2	0.3436	8	11.8	0.01 > P > 0.001
N	2.5707	2	0.2151	8	43.8	$\sim 5 \times 10^{-5}$
Case 1	0.2426	2	0.2261	8	0.31	$50.3 > P > 0.2$
„ 2	0.5088	2	0.1593	8	8.69	$\sim 0.01$
„ 3	0.4951	2	0.3683	12	2.07	$\sim 0.2$
„ 4	0.2765	1	0.2070	6	2.01	$\sim 0.2$
„ 5	2.3632	2	0.0890	8	102	$\sim 2 \times 10^{-4}$

Table 2. DEVIATIONS FROM LINEAR REGRESSIONS:  $\log(\phi - \phi_0)$  PLOTTED AGAINST TIME

Geno- type*	Deviation from linearity		Random error of determination		Ratio† of vari- ances	P‡
	Sum of squares	D.F.	Sum of squares	D.F.		
H	0.001026	2	0.0009105	8	0.51	$50.4 > P > 0.3$
N	0.007414	2	0.004774	8	2.21	$50.2 > P > 0.1$
Case 1	0.002696	2	0.0003069	8	31.2	$2 \times 10^{-4} > P > 10^{-4}$
„ 2	0.006639	2	0.0002175	8	118	$\sim 10^{-5}$
„ 3	0.001253	2	0.000965	12	1.49	$\sim 0.3$
„ 4	0.008376	1	0.004520	6	5.11	$0.1 > P > 0.05$
„ 5	0.004019	2	0.001280	8	8.56	$0.02 > P > 0.01$

\*H, heterozygote for wild-type and phenylketonuric genes; N, a homozygote for the wild-type gene.

† Variance due to deviation divided by variance due to random error, that is Snedecor's F or 1/F as appropriate.

‡ Probability that the observed deviation from rectilinearity occurred by chance.

§ Deviation from linearity not significant.

There was no detectable qualitative difference between the metabolism of phenylalanine in cases 1-4, the other heterozygotes and normals. The concentration of tyrosine in the blood,  $T$ , rose after injecting phenylalanine into these four heterozygotes, the rate and extent of the rise,

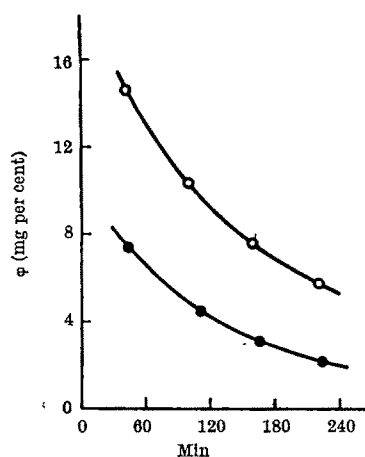


Fig. 2. Concentration of phenylalanine in the blood plotted against time for the same two individuals as in Fig. 1. O, Heterozygote; ●, normal.

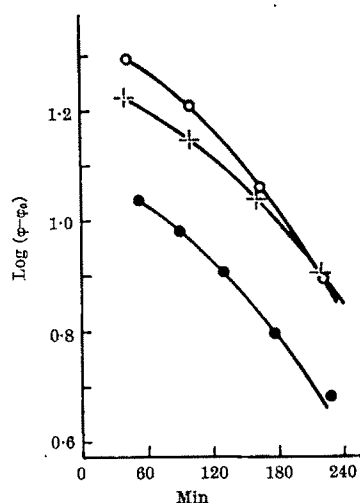


Fig. 3. As Fig. 1; cases 1 (+), 2 (O) and 3 (●).

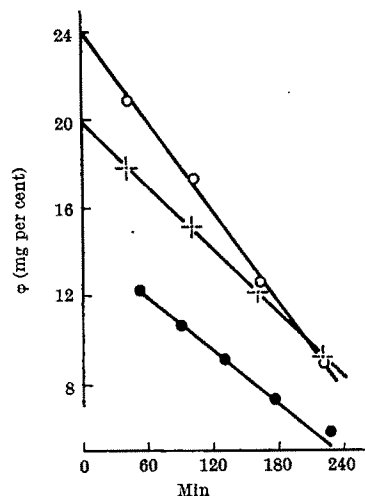


Fig. 4. As Fig. 2; cases 1 (+), 2 (O) and 3 (●).

at the dose of phenylalanine used, being about the same as for the other heterozygotes and

$$\left[ \int_0^t (T - T_0) \cdot dt / \int_0^t (\phi - \phi_0) \cdot dt \right]^{\frac{1}{2}}$$

being within one standard deviation from the mean of all heterozygotes. Urinary excretion of phenylalanine and its metabolites was very similar, in cases 1-4, to that found in the other heterozygotes and in normals.

It is generally assumed, and our results support this view, that the rate-limiting step in the disappearance of phenylalanine from the blood is the conversion of phenylalanine to tyrosine in the liver. Phenylalanine hydroxylase behaves like other enzyme systems in obeying Michaelis-Menten kinetics, that is

$$-\frac{d\phi}{dt} = \frac{\phi V}{K_m + \phi}$$

where  $V$  is the limiting reaction velocity at high substrate concentration and  $K_m$  is the Michaelis constant of the enzyme. In most of the cases we studied, the reaction velocity was proportional to  $\phi - \phi_0$ , that is, the value of  $K_m$  was apparently far higher than  $\phi$ . However, in case 1 and probably in cases 2 and 3, although  $\phi$  was of the same order of magnitude as in the other cases, the reaction velocity was independent of  $\phi$ , that is, the value of  $K_m$  appeared to be well below  $\phi$ .

If, in cases 1-3, the liver cell concentrated phenylalanine to an abnormal extent, so that its concentration in the cytoplasm greatly exceeded  $K_m$ , this might explain our results without invoking an abnormal phenylalanine

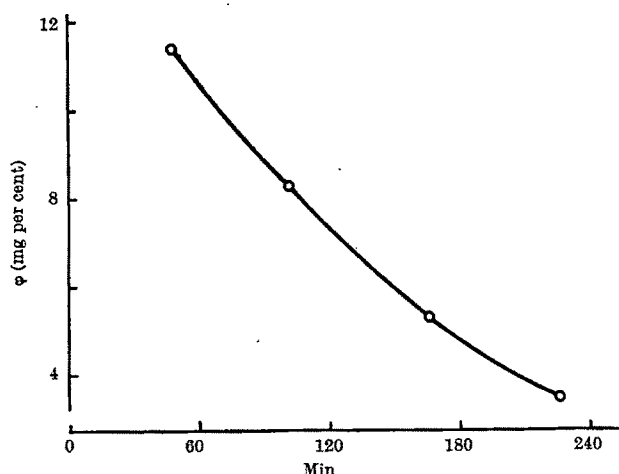


Fig. 5. As Fig. 2; case 5.

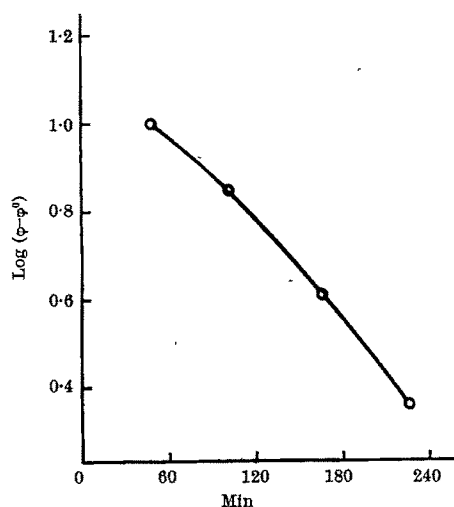


Fig. 6. As Fig. 1; case 5.

hydroxylase. On the other hand, the distribution of phenylalanine in total body water made any such concentration of phenylalanine by the liver unlikely. The values of  $(v_0 - v_w)/v_w$ , in cases 1-3, who were female, were 0.01, -0.04 and -0.04; these are within one standard

deviation from the mean for female heterozygotes (see preceding article). Equilibration of phenylalanine in the blood with that in tissue fluids was in all cases virtually complete before the first specimen was taken; if equilibration had not been achieved the first point would have been unduly high, exaggerating any convexity towards the origin and giving a result the opposite of that observed in cases 1-3.

These observations could be explained if, in case 1 and perhaps cases 2-4, the rate of transport of phenylalanine into the cells of the liver was the rate-determining step and a zero order process. This requires several additional hypotheses for which there is no evidence.

We conclude that a qualitatively atypical enzyme was probably acting in case 1 and, perhaps, in cases 2 and 3; we further conclude that the single active gene at the relevant locus was, in each case, allelic to both the typical gene and the gene for phenylketonuria.

One "normal" individual gave results different from all the others (Tables 1 and 2, case 5). The plot of  $\phi$  against time gave a smooth curve convex to the origin, but the plot of  $\log \phi$  against time gave a smooth curve concave to the origin (Figs. 5 and 6). The results resemble a combination of those in case 1 and those in a "typical" heterozygote; the deviations from linearity are significantly higher than the random errors of determination on either a logarithmic or arithmetic plot. It seems possible that case 5 possesses both "typical" and "atypical" enzymes and is heterozygous for the wild-type and the third allele described here.

In case 3 a fifth blood sample was taken 51 min after the last of the four required for analysis. The phenylalanine concentration in this specimen was considerably higher than would be predicted by extrapolation of either the logarithmic or the arithmetic curve (Figs. 3 and 4). This could be explained if, as the concentration of phenylalanine fell, it approached  $K_m$  and initially pseudo-zero order kinetics changed towards pseudo-first order kinetics.

The lower  $K_m$  value of the atypical enzyme indicates that phenylalanine has a higher affinity for the atypical than the typical enzyme; the relatively low frequency of the "atypical" allele, at most four out of thirty-one, suggests that possession of an enzyme with increased affinity for phenylalanine does not lead to increased survival fitness.

We thank Dr. M. C. Pike, of the Medical Research Council Statistical Research Unit, for details of the method of calculation of deviations from linearity.

## Fractionation of Beef Liver Ribosomes with Specific Antiserum

by

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Ribosomes may be specific in their role in protein biosynthesis. A preliminary study suggests that antisera against specific beef liver proteins selectively precipitate ribosomes to which the specific proteins are bound.

A NUMBER of studies have made it possible to identify the specific proteins associated with bacterial and mammalian ribosomes<sup>1-5</sup>. Some of the ribosomes have been shown to be associated with messenger RNA which appears to bear the nascent proteins<sup>6,7</sup>. The messenger RNA need not, however, be present, as the nascent proteins have been

shown to remain attached to the 70S particles under conditions where most of the messenger RNA is free of the ribosomes<sup>8,9</sup>. Warren and Goldthwait<sup>9</sup> have shown by serological methods that triose phosphate dehydrogenase is associated with a fraction of the total ribosomal population. Similar results have been presented for albumin<sup>5</sup>

and  $\beta$ -galactosidase<sup>8</sup>. The protein RNase does, however, appear to be closely associated with essentially all the ribosomal particles from the pancreas<sup>4</sup>.

In the work presented here antisera against beef liver catalase, albumin and glutamate dehydrogenase were prepared in an attempt to determine whether or not these proteins are bound to the same ribosomes or separate groups of ribosomes.

Beef liver was obtained from a local slaughterhouse. A portion of liver was removed as quickly as possible, sliced and placed directly on ice. The liver was homogenized in five volumes (w/v) of 0.01 molar phosphate buffer, pH 7.4, containing 0.01 molar magnesium chloride and 0.15 normal sodium chloride. The microsomes and ribosomes were prepared according to the procedure of Palade and Siekevitz<sup>9</sup>.

Antisera to catalase, glutamate dehydrogenase and albumin were prepared by immunizing white New Zealand rabbits. The  $\gamma$ -globulin fractions were obtained by ammonium sulphate precipitation and further purified by DEAE-cellulose chromatography<sup>10</sup>.

Catalase was assayed by the method of Beers and Sizer<sup>11</sup> and glutamate dehydrogenase by the methods described by Olson and Anfinsen<sup>12</sup>.

The precipitin curve obtained with varying amounts of catalase and a constant amount of anti-catalase (7.4 mg) is shown in Fig. 1. The amount of  $\gamma$ -globulin necessary for complete precipitation of catalase was calculated to be 43  $\mu$ g of catalase per mg  $\gamma$ -globulin. The catalase-antibody complex retained 100 per cent of the activity of the original enzyme.

The precipitin curve obtained with varying amounts of glutamate dehydrogenase and a constant amount of anti-glutamate dehydrogenase (11 mg) is shown in Fig. 2. Enzyme appeared in the supernatant fluid before the equivalence point was reached. Using the point at which no enzyme was found in the supernatant fluid, the amount of antiserum necessary for complete precipitation was calculated at 17  $\mu$ g of glutamate dehydrogenase for each mg of  $\gamma$ -globulin. The enzyme-antibody complex retained only 15 per cent of the original activity at the point just before the appearance of enzyme in the supernatant fluid. This inhibition appears to be of the steric type, as inhibition decreased from 95 per cent when antibody was present in excess to 75 per cent in antigen excess.

A typical precipitin curve was obtained with varying amounts of albumin and a constant amount of anti-albumin. The amount of  $\gamma$ -globulin necessary for complete precipitation of albumin was 16  $\mu$ g albumin for each mg of anti-albumin.

The beef liver microsomal fraction contained measurable amounts of catalase and glutamate dehydrogenase (Table 1). Treatment with deoxycholate (0.5 per cent w/v) produced particles which were considerably richer

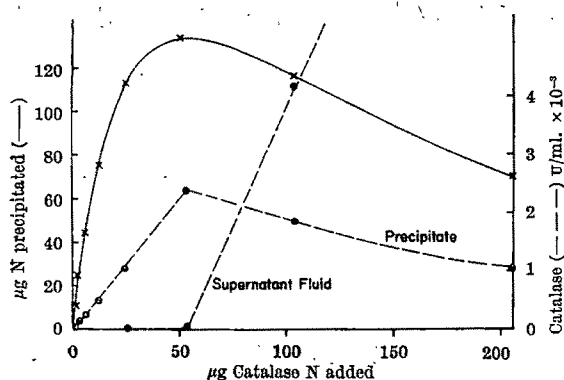


Fig. 1. Quantitative precipitin curve of the reaction between varying amounts of bovine catalase and a constant amount of anti-catalase (7.4 mg). The dashed lines refer to amounts of enzyme recovered in the precipitates and the supernatant fluids.

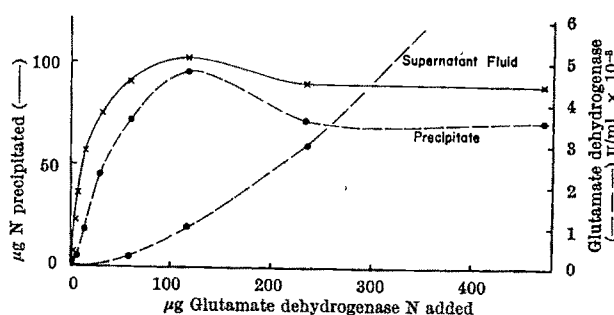


Fig. 2. Quantitative precipitin curve of the reaction between varying amounts of bovine glutamate dehydrogenase and a constant amount of anti-glutamate dehydrogenase (11 mg). The dashed lines refer to amounts of enzyme recovered in the precipitates and supernatant fluids.

in RNA. After four successive washes, the ribosomes still contained measurable amounts of both enzymes. Crystalline catalase or glutamate dehydrogenase added to the microsomes before treatment with deoxycholate did not affect the enzyme activity of the ribosomes after the second wash.

Table 1. CATALASE AND GLUTAMATE DEHYDROGENASE ASSOCIATED WITH VARIOUS FRACTIONS AFTER SUCCESSIVE CENTRIFUGATION CYCLES

Fraction	Protein: RNA	Catalase (units/mg protein)	Glutamate dehydrogenase (units/mg protein)
Soluble	81	450	89
Microsomal	7.1	27	50
Ribosomal (crude)	1.3	57	21
1 wash	1.4	12	20
2 wash	1.2	10	17
3 wash	1.2	7	16
4 wash	1.2	6	10

Varying amounts of the different antibodies were added to a constant amount of washed ribosomes. The tubes were allowed to stand for 24 h at 4° C. To facilitate the precipitation of the ribosome-antibody complex, anti-rabbit  $\gamma$ -globulin prepared in sheep was added to each tube. The precipitates were collected by centrifugation for 15 min at 3,000g, washed twice, and assayed for RNA, protein and enzyme activity. The amount of anti-catalase or anti-glutamate dehydrogenase necessary for complete precipitation of all catalase or glutamate dehydrogenase activity associated with the ribosomes is given in Table 2. The value listed for anti-albumin is based on the point at which no further increase in RNA precipitate was noted (equivalence zone).

Table 2. PRECIPITATION OF RIBOSOMES\* WITH SPECIFIC ANTIBODIES

$\gamma$ -Globulin fraction	Amount required† (mg)	Protein precipitate (mg)	RNA precipitate (mg)	RNA (%)
Anti-GDH	22	0.66	0.14	0.53
Anti-albumin	11.6	0.49	0.10	0.4
Anti-catalase	7.4	0.54	0.12	0.47
Normal	20	0.16	0.04	0.08

\* 10 ml. ribosomes (26 mg ribosomal RNA) added to each tube.

† Antiserum required for complete precipitation of corresponding antigen.

The amount of ribosomes precipitated with anti-glutamate dehydrogenase was 0.53 per cent of the total RNA. The amount of glutamate dehydrogenase associated with the precipitate was 3–5 units/mg. 90–95 per cent of the catalase activity could be recovered from ribosomes remaining in suspension, and only trace amounts (<1 unit) were associated with the precipitate.

With anti-albumin it was possible to precipitate about 0.4 per cent of the total ribosomal RNA. Detectable amounts of catalase were associated with this precipitate;



on the other hand, 90–95 per cent of both catalase and glutamate dehydrogenase remained associated with the ribosomes remaining in suspension.

The amount of RNA precipitated with anti-catalase was 0.47 per cent of the total added. The amount of enzyme associated with the precipitate was 50–56 units/mg protein; an enrichment of eight to twelve times. The actual number of ribosomes with catalase attached would be somewhat less than 0.47 per cent, because 0.1 per cent of the ribosomes precipitated non-specifically. This non-specific precipitation should not contribute more than 0.1 per cent of the total glutamate dehydrogenase or albumin associated with the total ribosomal population unless these proteins were bound to the same ribosomes. Such a small amount of glutamate dehydrogenase could not be detected in the assay system used, however, because 95–100 per cent of the glutamate dehydrogenase activity was recovered from the ribosomes remaining in suspension, and this enzyme was therefore not bound to the antibody-ribosome complex. The ribosomes remaining in suspension could not be tested directly for albumin, but further addition of anti-albumin after removal of the ribosome-catalase-anti-catalase complex resulted in the precipitation of additional ribosomes.

If the enzymes associated with the ribosomes were the result of contamination with soluble enzymes, no RNA should precipitate unless ribosomes were trapped within the enzyme-antibody-anti-antibody complex. No appreciable increase in the amount of RNA precipitated was found when crystalline catalase or glutamate dehydrogenase, in the amount already present, was added to the

ribosomal suspension before the addition of antibody. Contamination with cytoplasmic elements therefore seems to be negligible as a source of enzyme. Although we cannot rule out the possibility that the protein is adsorbed on to the ribosome, there is evidence that pure enzyme added after homogenization does not increase the enzyme activity associated with the ribosomal fraction after several centrifugation cycles.

In view of these findings, it might be concluded that particular ribosomes are quite specific in their role in protein biosynthesis. Proof of the formation of specific enzymes by specific ribosomes is, however, lacking, in that the ribosomes present at any one time may contain only that enzyme produced by the attachment of specific messenger RNA and only that enzyme which was produced at that particular time would precipitate along with the ribosome.

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## Activity of Ribosomal Phosphodiesterase in a Protozoan

by

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In *Tetrahymena pyriformis* GL a phosphodiesterase bound to ribosomes has a requirement for divalent cations and is stimulated by potassium ions.

THE intracellular ribonuclease of *Tetrahymena* hydrolyses both purine and pyrimidine internucleotide bonds and produces cyclic 2':3'-nucleotides<sup>1</sup> which are slowly converted to nucleotide 3'-phosphates<sup>2</sup>. Three ribonuclease components have been purified several hundred-fold and characterized by their activity on synthetic polyribonucleotides<sup>3,4</sup>. In addition to nuclease activity in the soluble phase of homogenized preparations we found a hitherto undescribed phosphodiesterase activity exclusive to the ribosomal fraction. This phosphodiesterase differs from the soluble ribonuclease in its optimum pH, the effect of cations, and its action on *bis-p*-nitrophenyl phosphate, which is readily hydrolysed by the former but not by the soluble ribonuclease. We report here the distribution of the phosphodiesterase in four cell fractions, the effect of mono- and di-valent cations, and chelating agents on the activity of partially purified enzyme preparation.

*Tetrahymena pyriformis* GL was grown in sterile conditions in a medium containing 1 per cent proteose-peptone, 0.25 per cent yeast extract and 0.1 per cent dextrose. All cultures were aerated and incubated at 29° C. During late log phase the cells were collected,

washed twice in 0.4 per cent sodium chloride and used either immediately or frozen in a bath of alcohol and dry ice and stored at -22° C. All later steps were carried out at the temperature of the ice bath. The washed cells were homogenized by passage through a French press ('Aminco') at 12,000 to 15,000 lb./in.<sup>2</sup>. The homogenate was made 0.05 molar with *tris* hydrochloric acid buffer (pH 7.45) and centrifuged at 2,000g for 10 min. The pellet was washed five times with equal volumes of the *tris* buffer; it will be referred to as "residue". The combined supernatants were centrifuged at 10,000g for 10 min. The second pellet represents the mitochondrial fraction. The supernatant was centrifuged at 105,000g for 90 min. The resulting ribosomal pellet was washed once in *tris* buffer.

The distribution of phosphodiesterase in the *Tetrahymena* fractions is shown in Table 1. Enzyme activity is associated only with particulate fractions, especially the ribosomes. No phosphodiesterase activity was observed in the 105,000g supernatant with incubation of the assay mixture for periods up to 24 h. Although washing of the ribosome fraction in 0.05 molar *tris* hydrochloric acid buffer, pH 7.45, slightly reduced the activity

of phosphodiesterase, washing three times with 0.2 molar *tris* hydrochloric acid buffer, pH 7.45, was sufficient to abolish almost all activity. Using data on total protein in the fractions analysed, the distribution of enzyme activity was calculated, assuming the activity in the crude homogenate to be 100 per cent (right hand column, Table 1). More than 95 per cent of the activity is found in the ribosomal fraction. The ribosome fraction showed a distinct maximum in activity at pH 10.3, but the preparations were also active at lower pH. For example, at pH 5.0 the activity was 30 per cent to 60 per cent of the values at pH 10.3.

Table 1. DISTRIBUTION OF PHOSPHODIESTERASE ACTIVITY IN *Tetrahymena* CELLS

Fraction	Phosphodiesterase activity ( $\mu$ moles/mg/h)	Per cent
Crude homogenate	33.2	100
Residue	0	0
Mitochondria (unwashed)	6.52	4.9
Ribosomes		
Unwashed	139	—
Washed once	125	95.1
Ribosomal wash	0	0
105,000 g supernatant	0	0

The determination of phosphodiesterase activity was carried out in a 1.0 ml. reaction mixture containing 25  $\mu$ moles of diethylamine buffer, pH 10.3, 1  $\mu$ mole magnesium chloride and 1  $\mu$ mole of calcium *bis-p*-nitrophenyl phosphate. The reaction was started by addition of the sample containing 0.5–2.0 mg of protein and terminated by addition of 100  $\mu$ l. of cold 50 per cent trichloroacetic acid after incubation at 29° C for 80 min. After 15 min on ice, the suspension was centrifuged and 0.5 ml. of the supernatant was added to an equal volume of 0.5 normal sodium hydroxide. The absorbancy was determined at 400 m $\mu$ . Each sample was assayed in duplicate and corrected for reaction mixtures without substrate and without protein. The substrate was stable under these assay conditions for several days. The phosphodiesterase activity is expressed in  $\mu$ moles of *p*-nitrophenol formed/mg of protein in the sample.

Using a washed ribosomal fraction in the assay system described in the legend to Table 1, we determined the effect of cations on phosphodiesterase activity in the absence of magnesium. Potassium chloride from 0.2 to 0.8 molar maximally stimulated phosphodiesterase activity on the average of approximately 25 per cent; half as effective (at the same concentrations) were the chloride salts of sodium, as well as lithium and caesium. Ammonium chloride at concentrations of 0.5 moles/l. and 1.0 moles/l. inhibited phosphodiesterase activity 18 per cent and 28 per cent, respectively. An equal concentration of potassium ions completely reversed the inhibition of 0.5 molar ammonium ions.

The divalent cations calcium and magnesium increased phosphodiesterase activity 20 per cent and 7 per cent, respectively, at concentrations of 0.05 moles/l. Calcium was slightly inhibitory at concentrations above 0.5 moles/l. All other cations we tested were inhibitory at a concentration of 0.05 moles/l.; copper ions (13 per cent), ferric ions (95 per cent), zinc ions (100 per cent), cobalt ions (100 per cent) and mercury ions (100 per cent). This effect is caused by cations, because we failed to observe any difference between chloride and sulphate salts using potassium or sodium as the cation. The chelating agents sodium citrate and ethylenediamine tetraacetic acid (EDTA), as well as L-tryptophan<sup>5</sup>, inhibited phosphodiesterase activity (Table 2). The effect of tryptophan was partially reversed by an equimolar concentration of calcium chloride, but not by an excess of potassium chloride (Table 2). This clearly demonstrates that *Tetrahymena* phosphodiesterase requires a divalent cation for activity.

Phosphodiesterase activity has been found in ribosomal fractions prepared from wheat<sup>6</sup>, mammalian tissues<sup>8,9</sup>, milk<sup>10</sup>, *E. coli*<sup>11</sup> and *Lactobacillus casei*<sup>12</sup>. The occurrence of ribosome-bound enzymes *in vivo* has been questioned and the possibility for adsorption artefacts during isolation has been mentioned<sup>13,14</sup>. The inability to dissociate phosphodiesterase activity from *Tetrahymena* ribosomes,

unless the ribosomal structure is disrupted by detergents, indicates that the enzyme is a constituent of the ribosome<sup>4</sup>. This ribosome-bound enzyme with its high pH optimum activity is uniquely distinct from other nucleases in *Tetrahymena*, because both its DNase<sup>15,16</sup> and RNase<sup>3,4,16</sup> have acid pH optima. Furthermore, the highly purified *Tetrahymena* RNase does not hydrolyse *bis-p*-nitrophenyl phosphate<sup>4</sup>.

Table 2. EFFECT OF CHELATING AGENTS ON PHOSPHODIESTERASE ACTIVITY

Additions	$\mu$ moles	Phosphodiesterase activity $\mu$ moles <i>p</i> -nitrophenol	Percentage stimulation	Percentage inhibition
None	—	244	0	0
Sodium citrate	10.0	193	—	19
EDTA	10.0	0	—	100
None	—	137	0	0
Plus potassium chloride	250.0	173	31	—
Plus calcium chloride	10.0	152	10	—
Plus L-(—)-tryptophan	1.0	74.1	—	46
Plus L-(—)-tryptophan	10.0	69.6	—	49
Plus potassium chloride	250.0	86.6	—	50
Plus calcium chloride	10.0	111	—	27

The assay system contained 25  $\mu$ moles of diethylamine buffer, pH 10.3, 2.5  $\mu$ moles of calcium *bis-p*-nitrophenyl phosphate in 1.0 ml. and was carried out as described in the legend to Table 1. The percentage inhibition was calculated using the control for that specific experimental condition, for example, tryptophan plus potassium chloride relative to plus potassium chloride alone.

Monovalent cations are known to stimulate the activity of the phosphodiesterase in *E. coli*<sup>11,17–19</sup>, *L. casei*<sup>12</sup> and snake venom<sup>20</sup>. The stimulation of phosphodiesterase activity by potassium ions and sodium ions could be a non-specific effect of the ionic strength in the reaction mixture. The monovalent cations potassium, lithium, caesium and sodium produced a similar activation of phosphodiesterase independent of the degree of hydration of these ions<sup>21</sup>, and so the observed stimulation suggests some type of direct interaction. The activators may play a part in (a) anchoring the substrate to the enzyme, or (b) stabilizing the enzyme molecule, or (c) increasing the rate of hydrolysis of the enzyme-substrate complex<sup>21</sup>. Because there is an increase in the apparent maximum velocity of the enzyme reaction at a given substrate concentration, possibilities (b) and (c) could apply to a description of the effect of potassium ions on phosphodiesterase activity. The failure to reverse the tryptophan inhibition with potassium ions indicates that this ion is not involved in the binding of enzyme and substrate; the divalent cations of calcium and magnesium could have such a function, because calcium ions partially reverse the inhibition induced by tryptophan (Table 2).

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# Origin of Kaposi's Sarcoma from the Reticulo-endothelial System

by

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The histological appearance of Kaposi's sarcoma tissue stained by silver impregnation suggests that the sarcoma originates from the reticulo-endothelial tissue. Like Burkitt's lymphoma, the disease occurs in small areas of Africa, and may be linked with oncogenic viruses.

THE disease variously called "Kaposi's sarcoma" or "multiple idiopathic haemorrhagic sarcoma of Kaposi" has posed many problems to pathologists. Although often regarded as a neoplasm, possibly spreading by metastasis, or perhaps of multicentric origin, it has also been thought of as an infective granuloma and a benign vascular proliferation. There are more than fifty reports of its common occurrence in patients who have, or who later developed, disorders of the reticulo-endothelial system—for example, lymphosarcoma, Hodgkin's disease and leukaemia<sup>1,2</sup>. Because such an association has been noted more often than seems possible for a purely chance occurrence, several authors have suggested that Kaposi's sarcoma is a disease of the reticulo-endothelial system (RES). The only other support for this hypothesis has come from the rather weak circumstantial evidence of the histochemical detection of enzymes in the tumour cells which are commonly found in the RES<sup>3</sup> and the apparent histological continuity of lesions of Kaposi's sarcoma and Hodgkin's disease noted in two patients by Lothe and Murray<sup>4</sup> and Uys and Bennett<sup>5</sup>.

The experimental studies of Marshall<sup>6</sup> showed that certain silver stains used in neuropathology specifically to impregnate microglial phagocytic cells of the central nervous system could be adapted to demonstrate with equal selectivity the majority of cells belonging to the RES elsewhere in the body. We have used Marshall's modification of the Weil-Davenport technique to study the nature of the cells in lesions of Kaposi's sarcoma in five west European patients.

At the London Hospital, from 1908 to 1965, biopsies were taken from five patients with Kaposi's sarcoma, and the histological blocks were still available embedded in paraffin. Sections 20 $\mu$  thick were cut, de-waxed with xylol, hydrated and impregnated with silver by Marshall's<sup>6</sup> modification of the Weil-Davenport technique.

The five patients examined were the only ones with both a clinical and pathological diagnosis of Kaposi's sarcoma who had had skin biopsies and whose paraffin blocks were available. One patient, who had concurrent Hodgkin's disease and Kaposi's sarcoma, had had a biopsy of both a lymph node and a cutaneous lesion. The node showed contiguity of both types of abnormal tissue. This patient is discussed in more detail elsewhere<sup>7</sup>.

The silver impregnation was successful in four of the five cases as shown in Fig. 1 by the characteristic elongated appearance of selectively impregnated normal RES cells scattered throughout the dermis. These cells were far more numerous around Kaposi lesions. In the nodules of Kaposi's sarcoma, many selectively impregnated tumour cells were found wherever conventionally stained sections had shown the characteristic spindle cells massed around proliferating capillaries. The dark brown to black metal-  
lophilic cells measured about 15–20 $\mu$  long by 5–10 $\mu$  in diameter (Fig. 2). They had relatively compact cell bodies from the corners of which short processes could

sometimes be seen to arise (Figs. 3 and 4). The region of the nucleus appeared as a rounded swelling of the cell. They were usually very closely packed together and the crowding prevented full examination of their morphology. Not all cells in the lesions were impregnated, because clear areas were found around foci of inflammatory cells. As reported elsewhere<sup>2</sup>, in our one case where Hodgkin's disease and Kaposi's sarcoma tissue lay next to each other in a lymph node, impregnation of characteristic cells had occurred in both lesions.

Deposition of silver had also occurred on many elongated branching cells in the basal and Malpighian layers of the

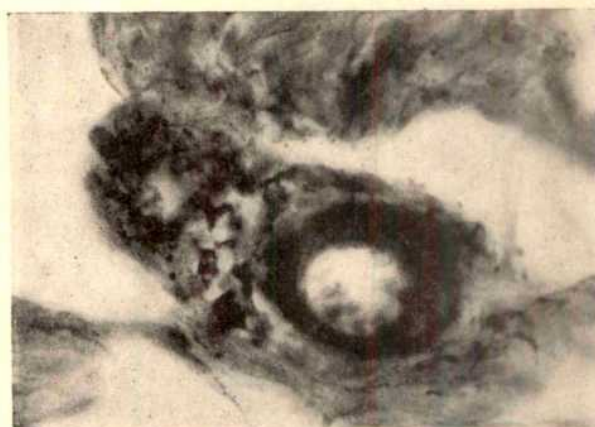


Fig. 1. Typically metallophilic cells of the reticulo-endothelial system, probably macrophages, lying near a small blood vessel in the dermis (Weil-Davenport,  $\times c. 120$ ).

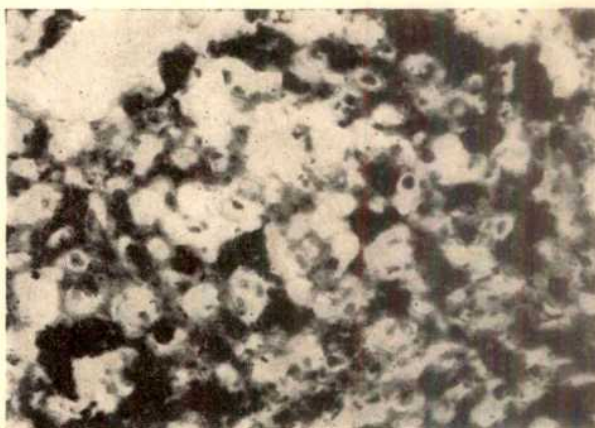


Fig. 2. Pyramidal and club-shaped metallophilic cells in Kaposi's sarcoma (Weil-Davenport,  $\times c. 200$ ).



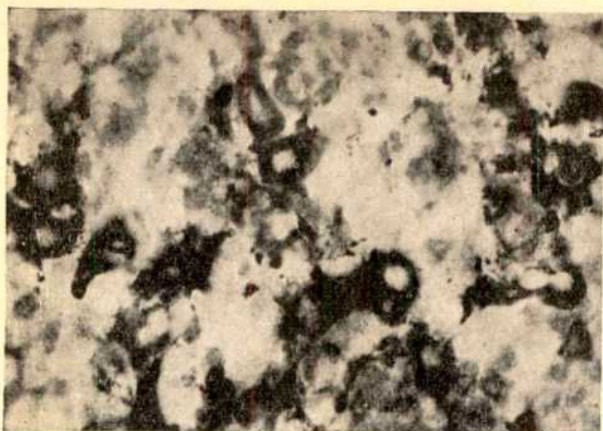


Fig. 3. Selective impregnation of branching cells in Kaposi's sarcoma (Weil-Davenport,  $\times c. 200$ ).



Fig. 4. Metallophilic spindle cells of Kaposi's sarcoma showing their fine processes (Weil-Davenport,  $\times c. 200$ ).

epidermis. On morphological grounds they were identified as melanocytes.

The appearance of the selectively metallophilic cells in lesions of Kaposi's sarcoma is comparable with the results obtained by applying the same method to other normal tissues of the RES. In the present series, normal macrophages were demonstrated in four of the five specimens, and so were the abnormal reticulum cells in the lymph node tissue containing Hodgkin's disease<sup>2</sup>. Published photographs of metallophilic cells in accepted examples of RES disease<sup>6</sup> closely resemble our findings. Marshall's results have been confirmed and extended by several authors in, for example, microgliomata of the brain<sup>7-9</sup>.

Their illustrations are very similar to ours. It must be emphasized that our results are based on wax-embedded material up to 30 years old, and that confirmation is required using freshly fixed tissues for optimal conditions.

We consider that the present results provide strong evidence in favour of Kaposi's sarcoma being a disease derived from the reticulo-endothelial system. Previously, direct support for this suggestion has come only from the high frequency of its clinical association with other accepted disorders of the RES. Histochemical studies<sup>10</sup> have shown several enzymes in Kaposi tissue which are found also in macrophages. In particular, acid phosphatase was present in many tumour cells. The occurrence of this enzyme is not, however, specific for RES cells, and the more primitive types do not always contain it<sup>11</sup>. Electron microscopy of tumour cells<sup>12</sup> has shown multilaminated osmophilic structures in their cytoplasm. They were interpreted as non-myelinated nerve fibres, and this led to a suggestion that Kaposi's sarcoma originates from Schwann cells. Examination of the published photographs does not, however, reveal any axons within the structures; they are too small to be regarded as nerve fibres, and mesaxons are not shown. They probably represent scavenged debris—"myelin figures"—common to many cells under adverse conditions.

If Kaposi's sarcoma is considered as a disease derived from the RES, certain of its other peculiarities become of particular interest. The geographical distribution of the disease is unique in that its highest incidence is in small areas of Africa. In this and other ways it recalls the occurrence and clinical features of another disease of the RES—Burkitt's lymphoma<sup>13,14</sup>. In view of recent reports suggesting the occurrence of oncogenic viruses in Burkitt's lymphoma<sup>14</sup>, it would be interesting to examine Kaposi tissue for the same or related viruses.

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## Ecology and Taxonomy of the Gorilla

by  
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Multivariate analysis shows that the ecological diversity of the gorilla is reflected in the morphology of the skull and is of assistance in subspecific taxonomy.

THE gorilla is the largest living primate, and systematically one of the closest to man. It is probably this closeness as much as anything else that has stimulated a great deal of scientific interest in this animal, from Owen's<sup>1,2</sup>

early osteological studies through the taxonomic era of Matschie<sup>3-6</sup> to the very recent behaviour study of Schaller<sup>7</sup>. The general range of variability is known largely through the work of Schultz<sup>8,9</sup> and the distribution is well known as a result of the work of Coolidge<sup>10</sup> and Schaller.

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It is not clear to what extent gorillas are divided into geographical races—races with ecological determinants—partly because of the typological point of view of the earlier workers and the chance that gorillas tended to be collected in the extreme west and east of the total range of the species. The old literature tends either to describe new forms, based on very little information, or merely to contrast the mountain and lowland types as a whole. Actually, as Schaller has shown, many of the eastern gorillas are at low altitudes. Investigations of the influence of ecology on the morphology of man are only just beginning. It can be supposed, however, that gorillas, lacking the mobility of human beings, are more likely to show a comparatively uncomplex pattern of influence.

A further question which has recently been raised by several authors (Simpson, Mayr, Washburn) is the relationship of the gorilla to the chimpanzee. This question is beyond the scope of the present paper, but is indicative of a new trend of taxonomic thought.

The altitudes at which gorillas are found vary from sea-level in West Africa to 13,000 ft. on the Uganda-Rwanda-Congo border. The whole area of distribution is split into two portions—one in West Africa, approximately between the Nigerian border and the lower Congo River, and one in East-Central Africa, between the Upper Congo River and the Uganda border, being mostly (but not entirely) to the west of the Albertine Rift. These two quite separate geographic regions are generally referred to as the areas of "lowland" and "mountain" gorillas respectively. The currently recognized classification, dating from 1929 (ref. 11), allots a single sub-species to each of these two zones, the western or lowland gorilla being the typical form, *Gorilla gorilla gorilla* Savage and Wyman, 1847, and the eastern or mountain gorilla being known as *Gorilla gorilla beringei* Matschie, 1903. Vogel<sup>12</sup> among others has queried this point of view, basing his judgment on the morphology of the mandible and the frequencies of some non-metrical features.

The first immediately striking feature of this arrangement is that the western gorilla is by no means an exclusive inhabitant of low-lying country: on the plateau of the interior of Cameroun the altitude is around 2,000 ft., while on the Cross River, at the western extreme of the range, altitudes of 5,000 ft. have been recorded for gorillas. Equally, the eastern gorilla is by no means entirely a mountainous form. Schaller<sup>7</sup> shows that at least two-thirds of the eastern distribution area lies in lowland rain forest at altitudes of 1,600–2,600 ft.

For these reasons, it was felt that a new analysis of gorilla material was required, based on as large a number of specimens as possible, with a view to relating geographic variation to ecological factors. The present investigation therefore includes material from all the major collections of the world\*, as well as many minor ones. The five largest collections of the world are located as follows: British Museum (Natural History), London; Powell-Cotton Museum, Quex Park, Birchington, Kent, England; Musée Centrafricaine, Tervuren, Belgium; Institut für spezielle Zoologie und zoologisches Museum, Berlin (East); Natural Science Museum, Cleveland, Ohio, U.S.A. In several other collections there exist a fairly large number of specimens, but these are mostly of unknown locality. In all, including the smaller collections, 469

adult male and 278 adult female skulls were used in this investigation, as well as numerous juveniles which were useful for comparison. The specimens examined included the holotypes of all the many supposed species or sub-species described by Matschie and his contemporaries, with the exception of *Gorilla beringei* Matschie, 1903, and *Gorilla gorilla schwarzi* Fritze, 1912, both of which were destroyed during the war. The original co-types of Savage and Wyman were also examined.

The primary method used was the multivariate technique of generalized distance, which is well described by Trevor<sup>13</sup> and Talbot and Mulhall<sup>14</sup>; the analysis was done by the canonical variates method (see refs. 15 and 16). Although the results of the canonical analysis were of great interest, space precludes their detailed description in the present paper. A total of forty-five measurements was taken on each skull. This large number was reduced by means of correlation coefficients to sixteen, of which ten pertained to the cranium (cranial length and breadth, bi-orbital breadth, palate length and breadth, bi-canine breadth, facial height, post-orbital breadth, tooth row length and nuchal surface height), and six to the mandible (bicondylar and bi-gonial breadths, ramus height and breadth, tooth row length and jaw body breadth). The cranium and mandible were treated separately in the analysis because of a comparative lack of specimens possessing both; thus, there were four analyses to perform because males and females were also taken separately. Taking the smallest geographical units possible (one area or district if there were sufficient specimens, otherwise a fairly circumscribed area) it was possible to use nineteen different groups for the male crania and mandibles, but only eleven for female crania and ten for female mandibles. In each case four of the groups were from the eastern area; the remainder, from the western.

Ecologically the western group can be divided into four sections: (A) Coast, which includes the high rainfall area down the coastal strip in West Africa from the lower Njong River, Cameroun, the mouth of the Congo, and extending inland to the low-lying Mambili River region. (B) Plateau; the lower rainfall area of the Cameroun hinterland, above 2,000 ft.; most of the groups large enough to be used in the analysis were from districts within this area, for example, Batouri, Lomie, Abong Mbang, Ebolowa. (C) Sangha, a zone ecologically intermediate between (A) and (B); on both sides of the Sangha River, from three localities: Youkadouma (includes, for example, Ziendi), Nola and Ouesso. (D) Nigeria, the Cross River district, from 1,000 ft. to nearly 6,000 ft. in hilly country, and rather isolated from the other three. These areas are shown on the map in Fig. 1.

The four eastern groups used in the analysis were, to use Schaller's terminology: Virunga, from 8,000–13,000 ft., in the Virunga Volcanoes; Tshiaberimu, from about 5,000–8,000 ft.; the mountains west of Lake Edward; Mwenga-Fizi, of similar altitude but less precipitous topographically; it is west of the north end of Lake Tanganyika; Utu, lowland rain forest west of the mountainous region, averaging 2,000 ft. high.

The generalized distance analysis shows that this ecological diversity is reflected in morphological diversity (Table 1). At first all nineteen groups (or eleven, or ten, according to which section of the analysis was being examined) were compared, and the western ones were seen to fall into larger groups which were therefore pooled;

Table 1. MEAN GENERALIZED DISTANCES FOR GORILLA SKULLS: COMBINED DATA FOR MALES AND FEMALES, CRANIA AND MANDIBLES

	Deme A	Deme B	Deme C	Deme D	Utu	Mwenga-Fizi	Tshiaberimu	Virunga
<i>G.g.gorilla</i> : Deme A								
Deme B	3.42							
Deme C	2.36	1.87						
Deme D	3.75	3.64	2.76					
<i>G.g.manyema</i> : Utu	10.58	7.19	8.98	12.00				
Mwenga-Fizi	13.97	9.63	12.01	13.39	2.61			
Tshiaberimu	18.32	12.80	17.69	19.91	5.26	2.85		
<i>G.g.beringei</i> : Virunga	19.35	14.27	15.63	19.55	8.10	5.78	4.06	

\* For a catalogue of the gorilla material in the British Isles, see Groves and Napier<sup>15</sup>.



the larger groups corresponded exactly to the four-fold division described here, even to the extent of the coast group (A) which includes the inland Mambili skulls. Division was fairly sharp between neighbouring groups in different areas, for example, between Bipindi (on the Cameroun coast) and Ebolowa (a few miles inland, on the plateau); here it seems that gene exchange has not been sufficient to counteract the ecological difference. Likewise the uniformity of the Sangha gorillas (C) was maintained, even though the very considerable isolating barriers of the rivers Sangha and Dja separate the three groups concerned; morphology seems here to be affected more by environment than by isolation. Thus reduced in number, the remaining eight broad divisions show an interesting pattern of diversity. The Nigerian gorillas are fairly distinct from all others in the western area; the coast group is distinct from the plateau group, but the Sangha group is intermediate. For none of the canonical variates (from which the generalized distance table was calculated) do the means of these groups lie more than one standard deviation apart; no sub-specific distinction is involved.

Among eastern gorillas, the Virunga skulls stand out from the other populations more than any of the western groups differ from one another. The other three eastern groups do not differ very much from each other, but show varying degrees of resemblance to the western or the Virunga gorillas. The Utu group of skulls, it will be seen, is almost equidistant from the Virunga group and from the western groups generally; among the latter it bears rather more resemblance to the Plateau group than to the others. By reference to the original canonical variates it is possible to show that there is a significant difference at the 90 per cent level from both Virunga and the western populations, and that the Utu gorilla merits separation at the sub-specific level from both of the others. This is a not unexpected consequence of the discovery, largely pin-pointed by Schaller, that this is an eastern gorilla that is decidedly not a "mountain" gorilla. The Mwenga-Fizi and Tshiaberimu gorillas are intermediate between Utu and Virunga, but nearer to the former, that is, the cline is "stepped": they would therefore be most justifiably placed with the Utu population in the same

sub-species. Renewed examination of the canonical variates showed that it was characters of the jaws and teeth that most weighted the results.

The newly recognized race, being largely a lowland forest form, may be referred to as the eastern lowland gorilla. The two mountain-living populations have been known as *Gorilla graueri* Matschie, 1914, and *Gorilla gorilla rex-pygmaeorum* Schwarz, 1927, names given to the Mwenga-Fizi and Tshiaberimu populations respectively. It would be unfortunate were the race to be known under a name given to an atypical population. Fortunately it is possible to determine that there is a valid, earlier name attached to the Utu population. In 1908, Rothschild<sup>17</sup> referred to a skull in his possession as that of "*Gorilla gorilla manyema* Alix and Bouvier, from the south Congo region". The intention is to indicate a form described by Alix and Bouvier as *G. mayema*; the misspelling would seem to indicate that there was confusion here with Manyema, a province of the Belgian Congo on the upper Congo (Lualaba) region—an area where the eastern lowland gorilla is found. (The actual type locality of Alix and Bouvier's form was Conde, near Landana, on the lower Congo.) If this reading of the evidence is correct, then clearly Rothschild was referring to the eastern lowland gorilla, and *manyema* will be the valid name for the sub-species. Additionally, it must be stated that there is in the British Museum (Natural History) a skull, number 1939.945, from the Rothschild bequest, which bears no information on the label; but on the braincase can be discerned, in faded ink, the inscription "*Gorilla gorilla manyema*. Upper Congo". Rothschild never indicated in any of his papers that he possessed more than one skull of this form, and so B.M. No. 1939.945 is doubtless the holotype of the race. Indeed, metrically, it would be difficult to find a more typical skull of the eastern lowland gorilla.

Several skulls were available of populations whose status is in doubt, and of which large enough samples were impossible to obtain for the primary analysis. From application of the results of the analysis to these skulls, it would seem that the gorillas from the Kayonza forest, Uganda, may be *G.g.manyema*, while those from Mount Kahuzi, west of Lake Kivu, are very possibly *G.g.beringei*.

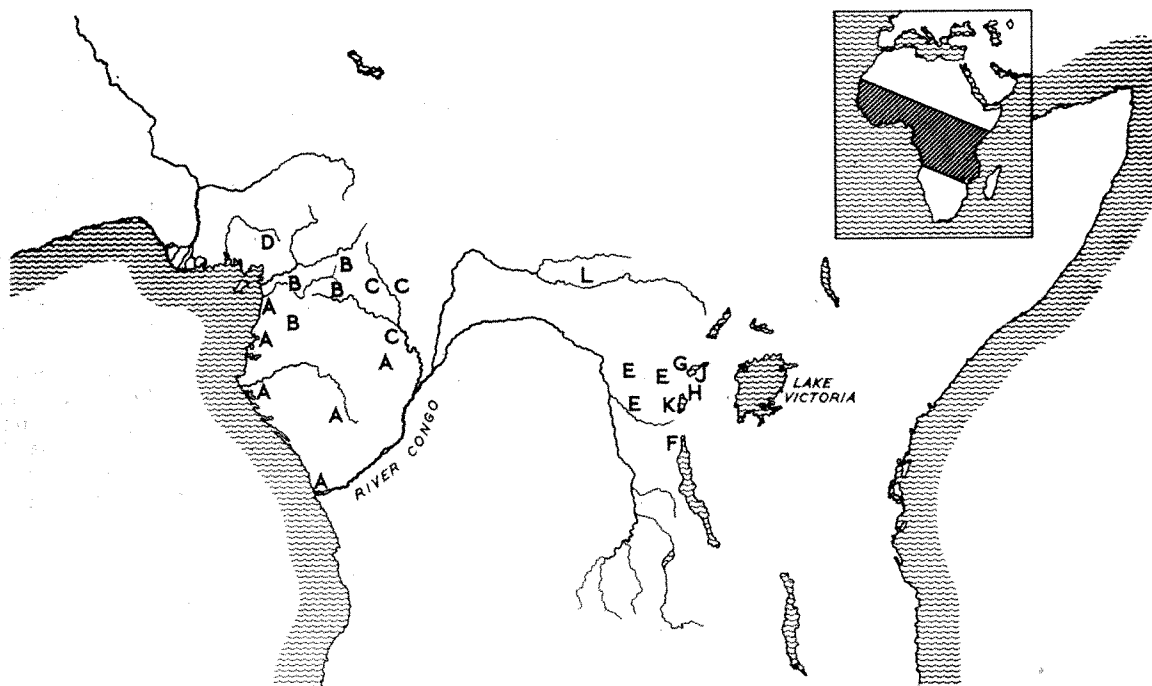


Fig. 1. Map to show distribution of gorilla demes and sub-species. (A) *G.g.gorilla*, coast; (B) *G.g.gorilla*, plateau; (C) *G.g.gorilla*, Sangha; (D) *G.g.gorilla*, Nigeria; (E) *G.g.manyema*, Utu; (F) *G.g.manyema*, Mwenga-Fizi; (G) *G.g.manyema*, Tshiaberimu; (H) *G.g.beringei*, Virunga; (J) Kayonza forest; (K) Mt. Kahuzi; (L) Bongo, Uele valley.

The skulls from Bondo, in the Uele valley (a locality intervening between the eastern and western zones<sup>10</sup>), are decidedly *G.g.gorilla*.

In conclusion, the gorilla forms but a single species, whose characters overlap in the different races. There are three valid sub-species, at least two of which have ecologically differing demes (for an earlier use of demes as quasi-taxonomic units, see ref. 18). They are as follows: *Gorilla gorilla gorilla* Savage and Wyman, 1847; western gorilla. *Gorilla gorilla manyema* Rothschild, 1908; eastern lowland gorilla. *Gorilla gorilla beringei* Matschie, 1903; eastern mountain gorilla. It may be noted that Vogel<sup>12</sup> similarly concluded that two full sub-species exist in the eastern region, but on quite different grounds. An examination of post-cranial features also supports this conclusion.

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## Polarized Light and Underwater Vision

by

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Many invertebrates are able to distinguish the plane of polarized light. Does this enable them to see further underwater? Experiments carried out in Malta show that the ability to analyse the plane of polarized light may indeed be an advantage in clear water.

It now seems to be established that many invertebrates<sup>1</sup>, including *Octopus*, are able to distinguish the plane of polarized light<sup>2</sup> while the adipose eyelids of some fishes are birefringent and may act as polarizing filters<sup>3</sup>. Underwater spacelight is polarized as a result of the scattering of sunlight, and it has been suggested that the plane of polarized light might be used as a navigational aid by some animals underwater<sup>4</sup>. In an attempt to find out whether the ability to distinguish the plane of polarized light could have any effect on the distance that objects can be seen underwater, a polarizing screen (a lens from a pair of polarizing sunglasses) was taken underwater while aqualung diving off Malta. When the screen was rotated in front of the eye it was evident that the plane of maximum polarization (e-vector) lay at right angles to the Sun's rays in the expected way<sup>4</sup>, but further, when the screen was oriented to exclude the maximum spacelight, it was observed that the apparent brightness of the small fishes present (chiefly Sparidae and an *Atherina*) was reduced less than the background spacelight, and hence fishes stood out in greater contrast against their background. Furthermore, there was a strong subjective impression that the more distant individuals, although invisible to the naked eye, became visible using the polarizing screen.

Two experiments (one photographic, one visual) were carried out to test these observations. In the photographic experiment four rectangles, 15 cm × 20 cm, were painted black, white and mixtures of the two to produce two shades of grey, using I.C.I. 'Kemobel'

chemical resistant paint. When a polarized light source is shone on the painted targets underwater the reflected light is very largely depolarized, and in the experiments to be described it is reasonable to assume that the image-forming light from the targets is essentially unpolarized. The four rectangles were bolted to a 'Perspex' sheet held in a rigid frame resting on a rocky bottom as shown in Fig. 1. A Rolleimarin camera with its standard 'Rolleipol' polarizing filter was used. The filter was mounted on the camera in such a way that the surface reflexion from a bowl of water in air was reduced to a minimum. The target display was photographed against an unobstructed water background at ranges which varied from 3 m to 8 m and at several depths between 3 m and 40 m. First a photograph was taken with the camera held upright. The camera was then rotated through 90° and another photograph taken. The photographs were taken during August 1-3, 1966, between the hours 1300 to 1500 local time, with a cloudless sky. Taking into account the orientation of the camera and target with respect to the Sun, the e-vector should be tilted at about 15° to the horizontal<sup>4</sup>. One such photographic pair is shown in Fig. 1. It appears that the background has undergone a greater brightness change than the targets, and indeed one target which was brighter than its background with the camera held upright was darker when the camera was horizontal.

It is likely that such gross differences should be reflected in the distance at which the targets became invisible when seen underwater. This was tested by fixing two polar-

izing screens to the outer surface of a diver's faceplate and orientating them to exclude the maximum underwater spacelight. Four target displays were used, each similar to those in Fig. 1 except that sixteen targets of different brightness were distributed among the four displays. The brightness of each target was measured by making an underwater brightness match against a standard range of grey ceramic tiles. The horizontal range at which each target just became invisible was measured by a technique previously used and proved reliable<sup>5</sup>. Added precautions were taken, however, to hold the target display as steady as possible in the water by means of one front and one rear guy rope extending from each corner and anchored to the bottom. The target display was moored midway between the surface and the bottom, which was a flat meadow of *Zostera* lying at a depth of 7 m. The display was sited so that it was due north of the observer, and because the sightings shown in Fig. 2 were made between 1330 and 1430 on August 25, 1966, the e-vector was very nearly horizontal. The first series was made with the polarizing screens in place; the screens were then removed and the second sighting series made. There were, however, a number of factors which reduced the precision of the measurements. Despite the guy ropes, that target display rocked slightly in the swell. The refraction of sunlight through the surface ripples caused a dappling of light on the target. Bright unfiltered light was able to reach the eye between the edges of the polarizing filters and the opaque rubber mask and this may well have reduced the range at which the targets should be seen when the polarizing filters were used. Finally, the toughened glass of which the divers' face plates are made is partially birefringent and will have reduced the effectiveness of the polarizing filters. Nevertheless, it is quite clear from Fig. 2A that the polarizing filters increased the range at which objects brighter than the water background could be seen although the visible range of darker objects decreased.

Le Grand<sup>6</sup> and Duntley<sup>7</sup> have derived equations which show that, as a horizontally seen object recedes from the eye underwater, the brightness contrast presented by the object against its water background falls exponentially at a rate determined by the attenuation coefficient of light through the water.

The contrast between the object,  $iN$ , at distance  $r$  and the water background,  $bN$ , is defined as follows

$$C_r = \frac{iN_r - bN}{bN}$$

and the contrast is reduced with horizontal distance thus<sup>7</sup>

$$C_r = C_0 e^{-\alpha r}$$

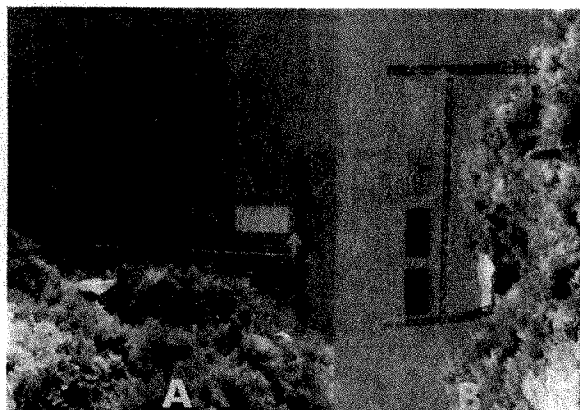


Fig. 1. The target array photographed at a depth of 3 m and at a range of 7 m through a polarizing filter. In A a photograph was taken with the camera held horizontally, and in B after it had been rotated through 90°.

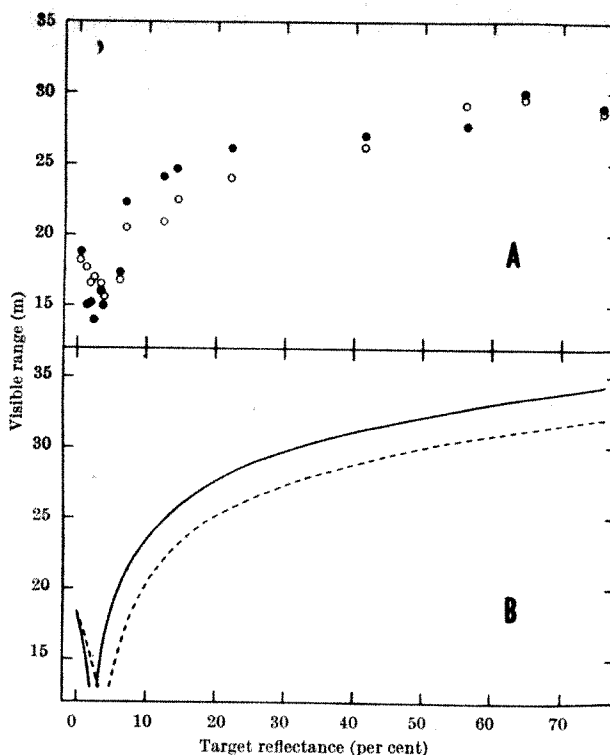


Fig. 2. A, The range at which grey targets just become invisible when viewed horizontally against the background spacelight. Filled circles, polarizing screens in place; open circles, polarizing screens absent. B, Calculated relationship between target brightness and visible range. Solid line, polarizing screens in place; broken line, no polarizing screens.

where  $\alpha$  is the total attenuation coefficient of image-forming light.

When  $C_0$  falls below about  $\pm 0.02$  for a typical observer in good light<sup>8</sup>, the object becomes invisible. In the case of a black object  $iN_0 = 0$  and  $C = -1$ . Hence  $\alpha$  can be calculated if the distance at which the object just becomes invisible is known as it is here. The relative values for  $iN_0$  for the sixteen different targets have been measured and, because the target which becomes invisible at the shortest distance is the one which most closely matches the background spacelight, the relative value of  $bN$  is known both with and without polarizing filters. Fig. 2b shows that expected relationship between  $r$  and  $iN_0$ , both using a polarizing screen when  $bN = 2.5$  and without when  $bN = 4.0$ . In both cases  $\alpha$  is 0.212. There is a reasonable fit between prediction and practice except for the brighter targets, which are not visible at as great a range as might be expected. We have as yet no adequate explanation for this.

There is no doubt that in these relatively clear waters the ability to analyse the plane of polarized light will in some circumstances confer a real visual advantage on an animal. Because the plane of maximum polarization may tilt by more than 45° at low Sun elevation<sup>4</sup>, and because dark objects are hardest to see when the polarizing screen is orientated to render bright objects most visible (and vice versa), it follows that a simple polarizing screen in the eye of fixed orientation will be less versatile than the system found in *Octopus* (common around both experimental sites), where there is the intra-ocular ability to distinguish light polarized in one plane from that polarized in another.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Unified Model for Interstellar Extinction and Polarization

THE development of improved techniques for measuring interstellar extinction and polarization has led to a revival of interest in the investigation of grain models. The interstellar extinction curve has recently been extended by Stecher<sup>1</sup>, using rocket techniques, as far as  $\sim 1200 \text{ \AA}$  in the far ultra-violet. It has already become clear that the conventional dirty ice grain model must be ruled out for several reasons. This model cannot explain the detailed shapes of the extinction curves for the visible spectral region obtained by Nandy<sup>2</sup>; nor can it reproduce even the qualitative features of the near and middle ultra-violet observations<sup>3,4</sup>.

It has been shown by Stecher and Donn<sup>5</sup> that a size-distribution of graphite particles could reproduce the structure of the extinction curve up to  $\sim 2000 \text{ \AA}$ , but again no agreement is possible for shorter wavelengths. We have now performed theoretical extinction computations for graphite grains covered with dirty ice mantles with a view to matching the entire wavelength range of the observed interstellar extinction up to about  $1200 \text{ \AA}$ . Experimentally determined values<sup>6</sup> for the refractive index of graphite were adopted for the cores, and the mantle refractive index was taken to be  $m = 1.33 - 0.5i$ . For a graphite core of radius  $r_c = 0.054 \mu$  with a dirty ice mantle of outer radius  $r_m = 0.16 \mu$  good agreement with the entire wavelength range of the extinction law is obtained (Fig. 1). In Fig. 2 the detailed agreement in the visible with Nandy's Cygnus curve is shown. Normalized extinction values are set out in Table 1.

Table 1. THEORETICAL NORMALIZED EXTINCTION VALUES FOR A GRAPHITE CORE OF RADIUS  $0.054 \mu$  SURROUNDED BY A DIRTY ICE MANTLE OF OUTER RADIUS  $0.16 \mu$

$\lambda^{-1} (\mu^{-1})$	$\Delta m$	$\lambda^{-1}$	$\Delta m$
0.00	-0.74	2.22	1.00
0.47	-0.65	2.49	1.15
0.86	-0.35	2.78	1.33
1.19	-0.03	3.01	1.47
1.22	0.00	3.85	1.91
1.56	0.35	4.54	2.22
1.80	0.61	5.00	2.52
		6.67	3.02

The present fit for grains of a single size must be interpreted to imply that there exists on the average, in the line of sight of a star, a bivariate size distribution in  $(r_c, r_m)$  such that the maximum contribution to the integrated extinction arises from particles with  $r_c \approx 0.054 \mu$ ,  $r_m \approx 0.16 \mu$ .

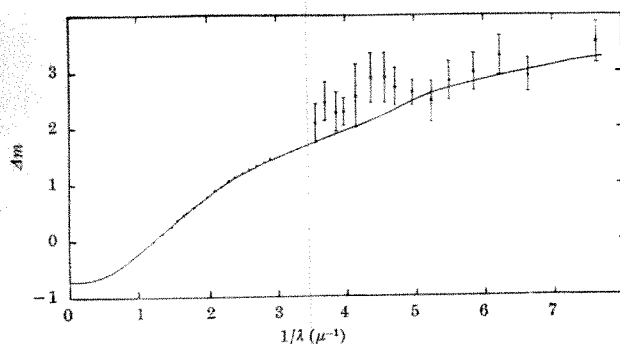


Fig. 1. Theoretical extinction curve for a graphite core of radius  $0.054 \mu$  with a dirty ice mantle of outer radius  $0.16 \mu$ . Normalization is to  $\Delta m = 0$  at  $\lambda^{-1} = 1.22 \mu^{-1}$ ,  $\Delta m = 1$  at  $\lambda^{-1} = 2.22 \mu^{-1}$ . Filled circles represent the observations of Nandy<sup>2</sup> for the Cygnus region; filled circles with error bars represent the ultra-violet observations of Stecher<sup>1</sup>.

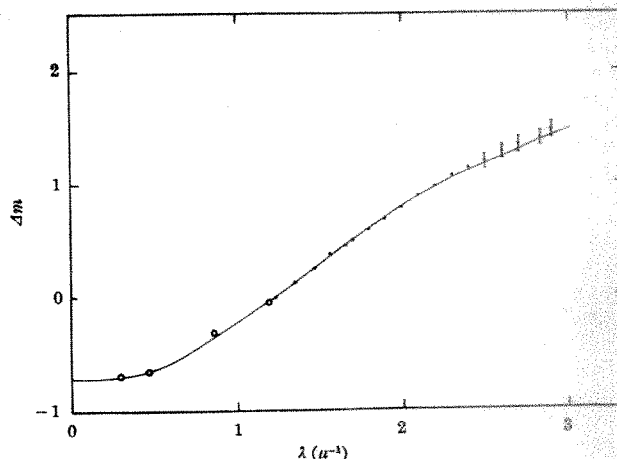


Fig. 2. Comparison of the theoretical extinction curve for a graphite core of radius  $0.054 \mu$  with a dirty ice mantle of outer radius  $0.16 \mu$  with the extinction law in the visible for the Cygnus region. Points represent Nandy's observations<sup>2</sup>; open circles represent infra-red observations of Johnson and Borgman<sup>13</sup>.

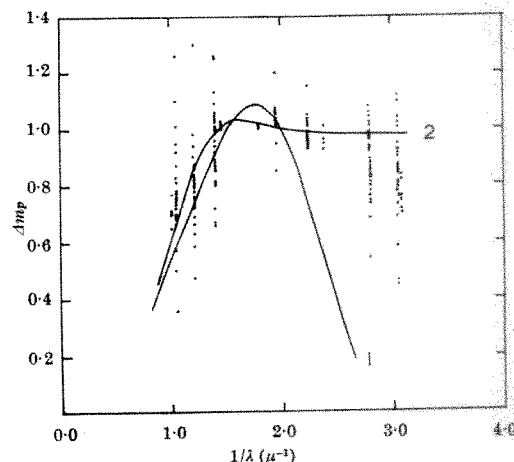


Fig. 3. Normalized polarization  $\Delta m_p$ . Normalization to  $\Delta m_p = 1$  at  $5000 \text{ \AA}$ . Curve 1 is theoretical polarization for a graphite core of radius  $0.054 \mu$  with an ice mantle of outer radius  $0.16 \mu$ . Curve 2 is theoretical polarization for a graphite flake of radius  $0.11 \mu$ . Points are observations of Coyne and Gehrels<sup>8</sup>.

In a recent article, Wickramasinghe *et al.*<sup>7</sup> have discussed the problem of matching the observed wavelength dependence of interstellar polarization with graphite grains or graphite core-ice mantle grains. It was shown that very small graphite grains of radii  $\sim 0.05 \mu$  do not give rise to the correct wavelength dependence of polarization, but that larger graphite grains or graphite core-ice mantle grains are more satisfactory in this respect. Following the procedure described in this earlier article<sup>7</sup> we have computed a normalized polarization for the present model which gives the best fit with the extinction observations. The normalized polarization curve for this case is plotted as curve 1 in Fig. 3. The points are the polarization observations of Coyne and Gehrels<sup>8</sup>. Taking account of the wide scatter of the observational points, the general fit cannot be regarded as too unsatisfactory. The lack of a closer agreement in the ultra-violet is unlikely to prove a crucial issue, however. Curve 1 is strictly appropriate for the single grain size ( $r_c = 0.054 \mu$ ,  $r_m = 0.16 \mu$ ) which fits the detailed structure of the observed extinction curve. In an actual size-distribution that would be present, it does not follow that the same grain size would produce the maximum contribution to the polarization as well. The polarization will be produced only by those grains which are effectively aligned in the magnetic field. It has been shown by Wickramasinghe<sup>9</sup> that the magnetic field required to align effectively a core-mantle grain with the dimensions in question ( $r_c = 0.054 \mu$ ,  $r_m = 0.16 \mu$ ) is about  $10^{-8} \text{ G}$ . If

the average Galactic magnetic field is about  $10^{-6}$  G, as is currently believed, the situation is that the grain with  $r_c = 0.054\mu$ ,  $r_m = 0.16\mu$  will not be effectively aligned. For a fixed value of the outer mantle radius  $r_m$ , grains with relatively larger graphite cores will be aligned preferentially from any size-distribution<sup>9</sup>. The polarization curve for such particles is, in general, flatter over the entire spectral region of interest, in a way similar to that for large graphite flakes<sup>7</sup>. The normalized polarization curve for a graphite flake of radius  $0.11\mu$ , which would be effectively aligned in a magnetic field of  $\sim 10^{-6}$  G, is given as curve 2 in Fig. 3. The general trend of this curve would be expected to be valid also for a large graphite core covered with very little ice. The agreement between curve 2 and the "mean" observations is seen to be very good.

A further requirement usually demanded of a grain model is that it has a high visual albedo. Although a precise formulation of this condition is difficult, Wolstencroft and Rose<sup>10</sup> reviewing the observational data on the diffuse galactic light have suggested the condition, at the photographic wavelength,

$$\gamma > 0.49 \pm 0.11.$$

For the present grain model with  $r_c = 0.054\mu$ ,  $r_m = 0.16\mu$  the albedo at several representative wavelengths is given in Table 2.

Table 2. ALBEDO OF GRAIN AT REPRESENTATIVE WAVELENGTHS					
$\lambda(\mu)$	0.3322	0.4016	0.5620	0.6410	0.8403
Albedo $\gamma$	0.64	0.60	0.55	0.53	0.45

The computed albedo is seen to satisfy the required condition amply.

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## PLANETARY SCIENCE

### Land's End Granites and Their Relation to the Experimental Granite System

RECENT work on the experimental granite system<sup>1,2</sup> has established a series of quaternary minima shifting in the direction of the  $\text{NaAlSi}_3\text{O}_8$  apex with increasing water vapour pressure, and becoming eutectic points at pressures in excess of 3.6 kbars. The "ternary" diagram (Fig. 1) shows the composition of six points at which components simultaneously melt under given isobaric conditions, each of these points representing minimum temperatures on the experimental granite solidus<sup>1,2</sup>. It is thought that crystallization in the natural granite system, in the presence of volatiles the vapour pressure of which

was decreasing, produced trends very similar to those of the experimental system. Field evidence and chemical data<sup>3</sup> support this view, and the widespread occurrence of tourmaline, fluorite, and hydrothermally altered granites in Cornubia<sup>3,4</sup> suggest that boron and fluorine, together with water vapour, fulfilled the role of volatiles. Because of the higher fluxing action of boron and fluorine compared with water, the shift of the quartz-feldspar boundary towards the albite-orthoclase sideline would be accentuated.

These volatiles may have existed as fluoroborates ( $M^+[\text{BF}_4]^-$ ) or trifluoroboroxole ( $\text{BOF}$ )<sub>3</sub>. The existence of fluoroborates in acid igneous magmas could be brought about by the tendency for the boron atom in boron trifluoride to complete its octet by co-ordination with molecules which have lone electrons available for valency purposes. Union with ionized fluorides of alkali metals would therefore give fluoroborates, while reaction with silica would give trifluoroboroxole and silicon tetrafluoride and thus provide a convenient mechanism for "desilicating" complex silicate minerals.

The bulk of the Land's End granite is a coarsely porphyritic rock which discordantly cuts the Mylor Beds and associated basic intrusives producing a metamorphic-metasomatic aureole. Where the coarse porphyritic granite abuts against the hornfels, thermal equilibrium was rapidly attained with the consequent absence of a chilled margin; although where equilibrium was slowly reached sodium-rich felsitic selvages developed<sup>5</sup>. Along parts of the northern contact at Land's End, medium to coarse-grained non-porphyritic granites are emplaced which post-date the main porphyritic types. (The field of marginal granites, Fig. 1, contains analyses of both the sodium-rich chilled phases and the non-porphyritic marginal types.)

Forty-two normative values of granites and aplites from Land's End are plotted in Fig. 1 in relation to the experimental granite system, and show a well defined "aplite field" extending from the silica/feldspar boundary (0.5 kbar) towards the albite corner parallel to the quaternary isobaric points; the more potassic "marginal granite field" is also parallel to this trend and merges into the "main granite field" which extends from the experimental 0.5 kbar isobaric minimum towards the orthoclase apex, thus demonstrating progressive enrichment in potassium.

The "aplite field" which has been shown to represent a "natural ternary minimum" in other south-western granites<sup>6</sup> is probably produced by the many additional

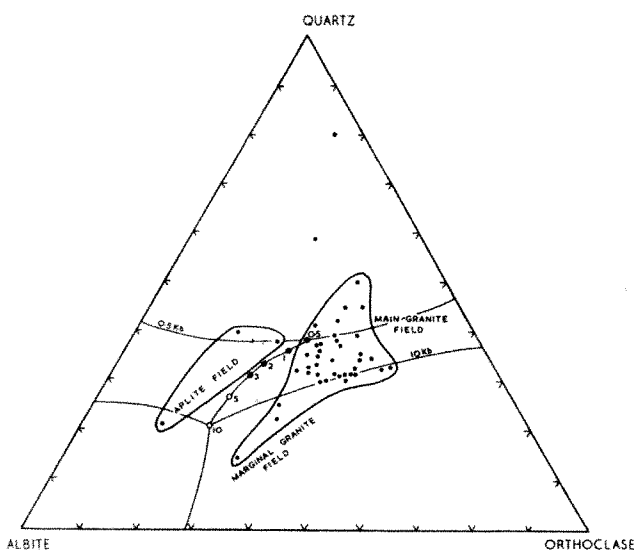


Fig. 1. Triangular variation diagram showing the relationship to the experimental granite system of normative values (catamolecular norms) of forty-two granite and aplite analyses from the Land's End granite, Cornwall. (Partly after Tuttle and Bowen<sup>1</sup> and Luth *et al.*<sup>2</sup>). O, Isobaric eutectic; ●, isobaric minimum; •, granite and aplite norms.



constituents in the natural system modifying, as it were, the less complex experimental system, and as this "natural ternary minimum" will vary areally depending on the fluxes present at any given point, the term "natural ternary minimum field" is considered more appropriate<sup>3</sup>. This field defines the composition of initial melts generated selectively during palingenesis<sup>1,6,7</sup>.

Available data from the Land's End granites (Fig. 1) illustrate the existence of two fields of relatively sodium-rich terms, one including the early granites (marginal granite field) and one including aplites and leucogranites (aplite field), thus supporting the findings of Brammall and Harwood<sup>8</sup> and Exley and Stone<sup>9</sup>. The data delineate a large "main granite field", and furthermore, demonstrate several evolutionary trends. The sodium-rich chilled marginal granites<sup>6</sup> could also represent a natural ternary minimum field and reflect the trend in composition of the invading magma, but since these and the main porphyritic granites have been subjected to potassium metasomatism<sup>3,9</sup> it seems more probable that the "marginal granite field" initially lay closer to the experimental trend, and was subsequently diverted towards the orthoclase apex on metasomatism. Loss of potassium from the centre of the granite would have left it relatively enriched in sodium, thus providing the basis for further differentiation. This, together with filter pressing, produced aplites and leucogranites which ascended through fractures in the main granite which was by now essentially solid. In several marginally "weak" areas along the north-west coastline at Land's End, the inner sodium-rich granite broke through the coarse porphyritic envelope in a similar manner to the St. Austell mass<sup>10</sup>.

It is suggested that some deep seated granite, granodiorite or granite gneiss, underwent differential anatexis<sup>7</sup>, to produce a "milieu mobilisé" the composition of which would lie within the "natural ternary minimum field" and that this quartz-feldspathic liquid ascended through the overlying sediments in much the same manner as other south-western granites<sup>6</sup> (that is, by forceful injection with overhead stoping, and assimilation).

Evidence therefore suggests that the Land's End granites progressed from a sodium-rich magma to a potassium-rich granite by endometasomatism, coevally evolving a sodium-rich core and aplitic "fluids" by differentiation. This produced a series of granites which represent most petrogenetic trends encountered in the south-western pluton, and which are consistent with its regional development.

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### Igneous Intrusions and Associated Rocks of the Mangerite-Charnockite Suite

THE demonstration that the pattern of rare earth abundance in a quartz-monzonite from Grenville Township, Quebec, is typical of the pattern shown by most sedimentary rocks<sup>1</sup> is of considerable interest in view of the controversy concerning the consanguinity of anorthosites

with mangerites and kindred rocks of the charnockite suite.

This rare earth pattern is consistent with a metamorphic or ultrametamorphic origin for the charnockitic rocks, and supports the proposals of Hargraves<sup>2</sup> and Berrangé<sup>3</sup>, who have contended that charnockites bordering major Canadian anorthosites are derived from the country rock gneisses.

The Precambrian rocks of the Kap Farvel district in South Greenland (which we are investigating on behalf of the Geological Survey of Greenland<sup>4</sup>) include charnockites with some similarities to those described from Grenville Township. The charnockites partially surround a late plutonic suite of norites, monzonites and rapakivi granites emplaced approximately  $1650 \times 10^6$  yr ago. The igneous rocks clearly intrude the charnockites and may develop an iron-rich border facies against them; however, charnockitic veins also penetrate the igneous suite. A late potash metasomatism, with the potash apparently derived from the country rock, partially obliterates primary features in the border facies.

The charnockites near to the contact with intrusive igneous rocks have extremely complex and apparently chaotic structures but microscopically show equilibrium textures indicative of annealing recrystallization in a static environment. They contain orthopyroxene with abundant clinopyroxene lamellae and feldspars with carlsbad/albite twinning thought by Philpotts *et al.*<sup>5</sup> to be typical of that found in igneous rocks. Away from the contact the charnockites grade structurally, texturally and mineralogically into the surrounding semipelitic and granitic gneisses, which have a relatively simple overall structure and amphibolite facies mineralogy. The field relationships strongly suggest that the charnockites were formed by granulite facies metamorphism and ultrametamorphism of the country rock gneisses. This suggestion is supported by the behaviour of basic intercalations in the gneisses which, as the igneous contact is approached, retain their original directional fabrics but are disrupted by the enclosing charnockitic acid gneiss. Close to the contact of the intrusive igneous rocks the basic fragments are mantled by fine grained pyroxene-rich rock with an equilibrium texture which encloses centres retaining amphibolite facies mineral assemblages and directional fabrics.

Although the charnockitic rocks of the Kap Farvel area are in part igneous, in so far as some appear to have crystallized from locally derived anatectic melts, we believe that—in common with those described by Hargraves and Berrangé—they represent contact metamorphic products better suited to classification and description in terms of mineral facies, rather than in terms of igneous nomenclature which places emphasis on compositional differences. According to this view these charnockites can be regarded neither as having been generated independently of their associated igneous suites<sup>1,5</sup>, nor as being comagmatic with them<sup>6,7</sup>.

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## PHYSICS

A Possible Restriction on CP-Noninvariance in  $K^0$ -Decay

THE generally accepted interpretation—which is the only remaining explanation within the framework of quantum mechanics—of the observed  $K_S^0 \rightarrow 2\pi$  decays<sup>1,2</sup> is that this is a manifestation of some CP-noninvariant interaction. Despite a large variety of suggestions as to the nature of the CP-noninvariant interaction, there has so far been no clear indication of CP-noninvariance in any other process and we still remain ignorant of the origin or nature of the interaction responsible for  $K_S^0 \rightarrow 2\pi$  decays. A phenomenological analysis of  $K^0 \rightarrow 2\pi$  decays has been given by Wu and Yang<sup>3</sup>, but experiments carried out so far do not permit a unique determination of the relevant parameters. In the absence of a basic theory, it may be of interest to consider a simplifying assumption, which does not conflict with any known result, which fixes the parameters for  $K_S^0 \rightarrow 2\pi$  decay.

The condition which we wish to discuss is<sup>4</sup>

$$\langle K_1^0 | K_2^0 \rangle = 0 \quad (1)$$

where  $K_1^0$  and  $K_2^0$  are the linear superpositions of  $K^0$  and  $\bar{K}^0$  states which are characterized by a purely exponential time-dependence in the Weisskopf-Wigner approximation. Assuming TCP-invariance, they are given by<sup>5</sup>

$$\begin{aligned} K_1^0 &= (1 + |r|^2)^{-1/2} (K^0 + r\bar{K}^0) \\ K_2^0 &= (1 + |r|^2)^{-1/2} (K^0 - r\bar{K}^0) \end{aligned} \quad (2)$$

where  $r$  is in general a complex constant determined by the dynamics of the  $K^0 - \bar{K}^0$  complex. From relations (2), we see that the condition (1) is equivalent to

$$|r|^2 = 1 \quad (3)$$

From the disparity of  $K_1^0$  and  $K_2^0$  lifetimes, Lee, Oehme and Yang<sup>5</sup> could conclude that  $|r|$  could not differ appreciably from unity. Taking  $|m_2 - m_1| = 0.5 \tau_1^{-1}$ , the restriction that an arbitrarily chosen neutral kaon beam can only decay with time yields the inequalities  $0.95 < |r| < 1.05$ . As is evident from (2), the phase of  $r$  depends on the choice of relative phase of  $K^0$  and  $\bar{K}^0$  states. We adopt the choice of Wu and Yang<sup>3</sup> which makes the amplitudes for  $K^0$  and  $\bar{K}^0$  to decay to the  $I = 0$   $\pi\pi$  scattering eigenstate purely real. A limit on both the magnitude and phase of  $r$  can be obtained from a knowledge of the relative  $2\pi$  decay rates of  $K_1^0$  and  $K_2^0$ . Using the formulae and notation of Wu and Yang, it can be shown quite generally that<sup>6</sup>

$$|(1-r)/(1+r)| \leq 2^{-1/2} \rho^{1/2} (2^{1/2} \rho^{1/2} - 1)^{-1} [2|\eta_{00}| + 2^{1/2} \rho^{-1/2} |\eta_{00}|] \quad (4)$$

where  $\rho$  is the branching ratio  $\rho = \Gamma(K_1^0 \rightarrow \pi^+\pi^-)/\Gamma(K_1^0 \rightarrow \pi^0\pi^0)$ . Even the rough limits which could be imposed on  $|\eta_{00}|$  from knowledge of the  $K_2^0$  lifetime and the partial rates for other decay modes beside  $\pi^0\pi^0$  sufficed to determine that  $r$  is close to unity both in modulus—consistent with condition (3)—and phase (with the Wu-Yang phase convention). Recent measurements<sup>7</sup> of the  $K_S^0 \rightarrow \pi^0\pi^0$  rate, which yield  $|\eta_{00}|$ , reinforce the conclusion<sup>7</sup>. The condition (3) then requires that the small parameter  $\varepsilon = 1 - r$  be purely imaginary,

$$Re \varepsilon = 0 \quad (5)$$

If we assume, in accordance with the  $\Delta I = \frac{1}{2}$  rule, that the  $I = 2$  amplitudes are small compared with those for  $I = 0$ , we have the approximate relations<sup>3</sup>

$$\eta_{+-} = \frac{1}{2}[\varepsilon + \varepsilon'] \quad (6a)$$

$$\eta_{00} = \frac{1}{2}[\varepsilon - 2\varepsilon'] \quad (6b)$$

where  $\varepsilon'$  is a parameter describing the CP-violation in the  $I = 2$  amplitude relative to the  $I = 0$  amplitude. From relations (6a) and (6b), we see that relation (5) requires

$$Re \eta_{00} = -2 Re \eta_{+-} = -2 |\eta_{+-}| \cos \varphi_{+-} \quad (7)$$

where  $\varphi_{+-}$  is the phase of  $\eta_{+-}$ . Equation (7) cannot be satisfied unless

$$|\eta_{00}| \geq 2 |Re \eta_{+-}| = 2 |\eta_{+-}| |\cos \varphi_{+-}| \quad (8)$$

According to Rubbia and Steinberger<sup>8</sup>, the best estimate for  $\varphi_{+-}$  is  $\varphi_{+-} = 0.60 \pm 0.23$  radians. Equation (8) then requires

$$|\eta_{00}| \geq (3.2 \pm 0.6) \cdot 10^{-3} \quad (9)$$

using the value  $|\eta_{+-}| = (1.94 \pm 0.09) \cdot 10^{-3}$  quoted by Cronin *et al.*<sup>2</sup>. The condition (9) requires the presence of appreciable  $I = 2$  amplitudes in  $K_S^0 \rightarrow 2\pi$  decay, because pure  $I = 0$  would give  $|\eta_{00}| = |\eta_{+-}|$ . The likelihood that relation (3) could only be satisfied by an appreciable departure from the  $\Delta I = \frac{1}{2}$  rule in  $K_S^0 \rightarrow 2\pi$  decay was previously noted by Bowen<sup>4</sup>.

According to equation (7), for a given  $\eta_{+-}$ , the magnitude of  $\eta_{00}$  fixes its phase (within a two-fold ambiguity),

$$\begin{aligned} \cos \varphi_{00} &= -2 (Re \eta_{+-})/|\eta_{00}| \\ \varphi_{00} &= \pi \pm \cos^{-1} [2 (Re \eta_{+-})/|\eta_{00}|] \end{aligned} \quad (10)$$

A measurement of the phase of  $\eta_{00}$  is therefore of great interest as a test of the hypothesis (1). Taking the value of  $|\eta_{00}|$  from Cronin *et al.*,  $|\eta_{00}| = (4.9 \pm 0.5) \cdot 10^{-3}$ , we obtain the estimates,

$$\begin{aligned} \cos \varphi_{00} &= -(0.65 \pm 0.20) \\ |\pi - \varphi_{00}| &= 0.86 \pm 0.30 \text{ radians} \end{aligned} \quad (11)$$

The condition (1) has several other interesting consequences. The decay curve of any neutral kaon beam becomes simply the sum of two exponentials; furthermore, there is no charge-asymmetry in leptonic decays of  $K_S^0$ , independent of the  $\Delta S = \Delta Q$  rule<sup>5</sup>. If the  $\Delta S = \Delta Q$  rule holds, the time dependent charge-asymmetry in leptonic decays from a beam which is initially pure  $K^0$  is required to be exactly the opposite to that from a  $\bar{K}^0$  beam. Also, the asymptotic decay rate into any particular channel becomes exactly the same whether we start with initial  $K^0$  or  $\bar{K}^0$  beams.

The significance of the restriction (1) is probably much deeper. Because such a condition scarcely occurs by accident, confirmation of hypothesis (1) would strongly suggest the existence of some hitherto unknown symmetry operation, of which  $K_1^0$  and  $K_2^0$  are distinct eigenstates. The possibility that there might be such a guiding principle beneath the apparent confusion created by the discovery of CP-nonconservation makes a test of the explicit prediction (10) extremely desirable.

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## Characteristics of Fibre Friction

AMONTONS'S classical law of friction, as explained by the cohesion theory, accounts satisfactorily for most cases of metallic friction. For non-metallic materials, however, and in particular the elastic solid field of polymeric mono-filaments and natural fibrous materials, many exceptions

to his law have been reported<sup>1-4</sup>. In these cases it has been observed that over an appreciable load range, the frictional coefficient ( $\mu$ ) does not remain a constant but decreases as the load increases, which suggests that local deformation occurs at the interface between the polymer and friction object. To explain the variability of  $\mu$ , it has been proposed<sup>5</sup> that the true area of contact and the shear strength vary with the load. For this to be true, Howell<sup>6,7</sup> has shown that the frictional force must be related to the load by  $F = KW^n$ , where  $K$  and  $n$  are constants. (The value of  $K$  depends on the properties of the surface materials while  $n$  is independent of these and is dependent only on the mechanism of deformation, that is,  $n$  is an indicator of the visco-elastic properties of the material under test.) For a fibre or a yarn travelling at a constant speed over a cylindrical object, the change in tension developed in the fibre or yarn is therefore found from the following equation

$$T_2^{(1-n)} = T_1^{(1-n)} + (1-n)KR^{(1-n)}\theta \quad (1)$$

where  $T_2$  and  $T_1$  are the output and input tensions, respectively,  $\theta$  is the angle of yarn wrap, and  $R$  is the radius of curvature of the test object.

In an attempt to study the frictional properties of wool yarns, an instrument was built at this laboratory which measures  $T_2$  and  $T_1$  continuously, using sensitive differential capacitor transducers accurate to  $\pm 0.1$  g. Kinetic measuring of yarn/metal, yarn/yarn frictional behaviour can be performed on most types and counts of yarn, for a variety of  $\theta$  and  $R$  values, and through a linear yarn speed range of about 0–365 m/min. The instrument and its use have been described earlier<sup>8</sup>.

A 64's quality, '92 Tex' worsted yarn (3.4, 1.8 turns/cm) was chosen as a test yarn, and its frictional characteristics were determined running over a hardened steel cylinder, 1.1 cm in diameter, at a constant linear speed of 150 m/min. Values of  $T_2$  and  $T_1$  were collected at approximate increments of  $T_1 = 3$  g, up to a maximum value of  $T_1 \sim 60$  g, and through a range of values of  $\theta$  from 0.59 to 2.97 radians. The best fit values of  $n$  and  $K$  in equation (1) were calculated for the eight values of  $\theta$  chosen (Fig. 1) using a computerized iteration technique first suggested by Rubenstein<sup>9</sup>.

Obviously in Fig. 1 there exists a definite non-linear relationship between  $n$ ,  $K$  and  $\theta$  that is not suggested by equation (1), and on the basis of the existing theory it is difficult to explain these results. It is true that over a very extensive load range  $n$  may vary somewhat<sup>10</sup> and that  $n$  and  $K$  may not be constant with changes in surface roughness and  $R$  (ref. 11), but the variability of  $n$  and  $K$  with  $\theta$  has not previously been reported. In fact, on plotting  $n$  against  $K$  it is found that  $n$  is related to  $K$  in the following curious manner

$$n = \frac{5.46 - K}{5.90} \quad (2)$$

this linear interrelationship being highly significant with a correlation coefficient of  $-0.968$ . Howell's original formula must therefore be correspondingly modified, and it may now be suggested that the frictional force is related to the normal load by the equation

$$F = pW^n - qnW^n \quad (3)$$

where, for a wool yarn, the constants  $p$  and  $q$  are equal to 5.46 and 5.90, respectively.

To explain the relationship in equation (2), E. Menefee has suggested (personal communication) that this may involve an internal fibre friction effect because the tendency of fibres to move laterally past each other will be strongly dependent on the angle involved. In other words,  $K$ , which is supposed to depend on the surface properties, may represent an overall surface effect which includes the interior interactions of the fibres; similarly  $n$ , related to the deformability of the yarn, will probably also be dependent on the ability of the yarn interior to rearrange and thereby also depend on the internal fibre surface

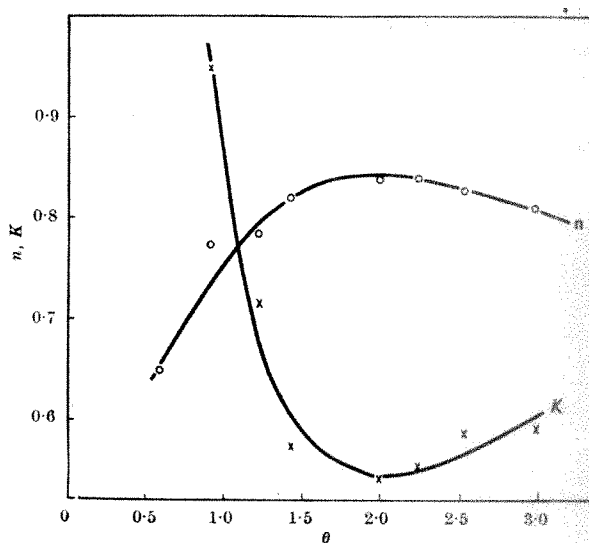


Fig. 1.  $K$ ,  $n$  versus  $\theta$  for '92 Tex' worsted yarn.

properties. Another possible explanation may be that we are observing the effects of boundary conditions of hydrodynamic lubrication, resulting from small amounts of residual natural wool oils and waxes, and not true fibre/metal friction at all. Even though the yarns tested were scoured in preparation and left unwaxed, very thin liquid films, possibly of only molecular dimensions, may still exist to influence the frictional behaviour of the yarn<sup>12</sup>. Evidence to support this possibility has recently come from similar observations made in this laboratory on a multi-filament nylon yarn which carried a known amount of surface lubrication. One other interesting observation in Fig. 1 is the maximum value of  $n$  ( $=0.85$ ) at  $\theta \sim 1.8$  radians. This value corresponds closely with the predicted value of  $n=8/9$  from the hemispherical asperity model suggested by Howell, Mieszkis and Tabor<sup>13</sup>. This result has been shown to be in very close agreement with the findings of other workers<sup>2</sup>.

It thus seems reasonable to suggest that Howell's theory is not valid over a wide range of values of  $\theta$  except at or near  $\theta=1.8$  radians, and that other theories are more likely to predict the observed frictional behaviour of fibres and yarns. In this respect, Whitney<sup>14</sup> thinks that for nylon and polypropylene monofilaments, Gralén's<sup>15</sup> equation that  $F = aW + bW^c$  (where  $a$ ,  $b$  and  $c$  are constants) more adequately agrees with experimental data. Work is continuing here to investigate the application of equation (3) and other theories to the phenomenon of wool fibre friction.

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### Increase in d.c. Dark Conductivity of Anthracene in a Magnetic Field

Frankevich and Balabanov<sup>1</sup> have reported magnetic enhancement of the photoconductivity of single crystals of anthracene. Similar effects in the dark conductivity have been observed in this laboratory.

Measurements were made of the d.c. dark conductivity at  $10^5$  V/m in an argon atmosphere, with and without a guard ring, over a period of 12 days. Fig. 1 shows the results for a crystal 1 mm thick which had been degassed in the dark for 3 days before the measurements were undertaken. Evaporated gold electrodes were used because deleterious effects had been observed with metallic pastes. It was found that the methyl-iso-butyl ketone used in "dag" silver preparation slightly reduced the resistivity of the single crystals of anthracene: on  $5\mu$  films of anthracene the resistivity was reduced by three orders of magnitude and the activation energy changed from 0.9 eV to 0.3 eV. This could have arisen from the formation of a charge-transfer complex by the ketone with anthracene or with impurities such as anthraquinone and anthrone.

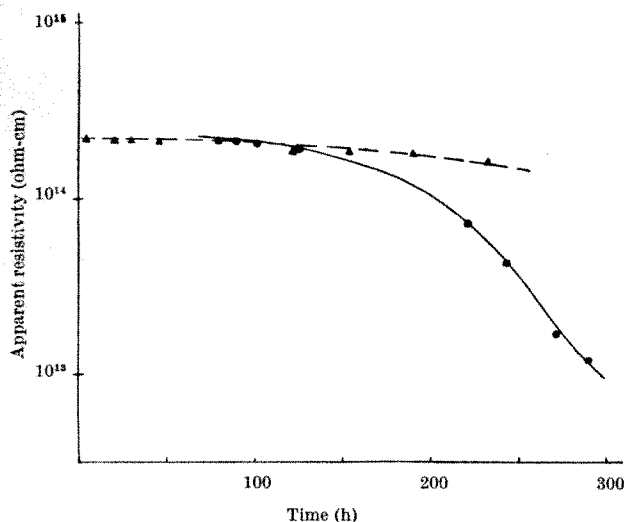


Fig. 1. Variation of the resistivity of single crystals of anthracene parallel to the "ab" plane with time at 296° K.  $\Delta$ , With guard ring;  $\bullet$ , without guard ring.

We investigated the effect of applying a magnetic field perpendicular to the current through the crystal. Care was taken in all measurements to ensure that stability of temperature and space charge had been secured: this often took up to 4 h. Fig. 2 shows the increase in dark current with magnetic field when the guard ring was used. Fig. 3 illustrates the measurements obtained without the guard ring: they suggest that the results of Fig. 2 were due to a bulk rather than a surface effect. The results were independent of current and field polarity.

The energy gaps for zero and 0.67 Tesla magnetic fields were determined between 255° K and 320° K as 0.84 eV for both conditions within the experimental error of  $\pm 0.02$  eV.

The results shown in Fig. 2 for the magneto conductivity were much greater than and of opposite sign to that allowed by conventional semiconductor theory. Measurements made on single crystals of anthracene in the dark of the Hall current (to be published) suggested a Hall mobility of the order of  $1 \times 10^{-3}$  m<sup>2</sup>/V sec, which can be compared with a similar photoconductive Hall mobility observed by Delacote and Schott<sup>2</sup>. Following Smith<sup>3</sup>, in a field of 0.7 Tesla

$$\Delta\sigma/\sigma_0 \approx -5 \times 10^{-9}$$

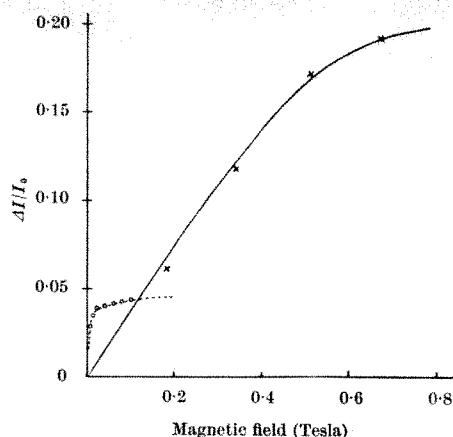


Fig. 2. Fractional increase of dark current against magnetic field, with a guard ring. Broken rule, photoconductivity result of Frankevich and Balabanov<sup>1</sup>.

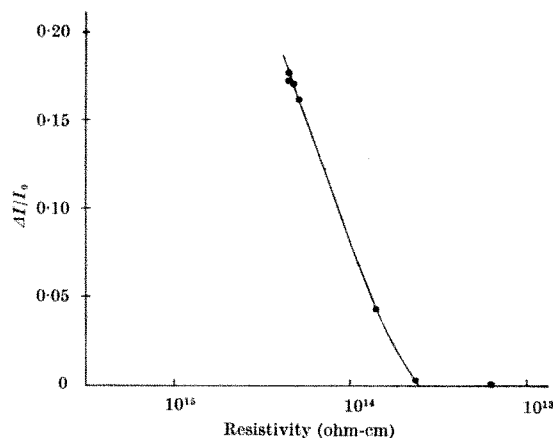


Fig. 3. Fractional increase of dark current against apparent crystal resistivity for a magnetic field of 0.51 Tesla and no guard ring.

Friedman<sup>4</sup> has shown that for narrow band semiconductors the effect would be smaller than this by a factor of  $10^4$  and the Hall effect would be anomalous. Frankevich and Balabanov explained their results for photoconductivity in terms of increased exciton lifetime.

A change in activation energy of less than  $10^{-2}$  eV would account for the magnetoconductivity increase at 300° K. This could result from small shifts and splittings of impurity and molecular energy levels within the Davydov exciton model<sup>5</sup>. Furthermore, the internal space charge reduced the initial crystal current by at least three orders of magnitude and a small decrease in this space charge by diffusion of "trapped" carriers to surface recombination centres could explain the observed magnetoconductivity effect.

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## CHEMISTRY

## Valency State of Chromium in Seawater

THE valency state in which chromium occurs in seawater not only provides important information concerning the geochemical behaviour of this element in marine environment but has also in recent years become significant in connexion with the problem of disposal of radioactive waste. For example, Osterberg *et al.*<sup>1</sup> investigated the variation of the spread of the Columbia River water along the Oregon coast of the Pacific by using chromium-51 which was originally released from the Hanford reactor as a radioactive tracer. In this case, it was reported by Cutshall *et al.*<sup>2</sup> that the major part of chromium-51 stayed in the original hexavalent state in seawater even at several hundred kilometres from the river mouth. In order to understand the cycle of radioactive chromium in the seawater, therefore, it seems indispensable to know the valency state in which chromium in seawater is stable. Unfortunately, the present day knowledge of this is very limited. In his recent compilation Riley<sup>3</sup> gave six references for the chromium content of seawater but these data are not sufficient to indicate general trends in the behaviour of chromium in seawater. In these investigations, except for the two investigations by emission spectrography, chromium was separated with hydroxides of aluminium, iron or chromium itself. These separations are only effective when chromium in seawater is present in a trivalent form.

Goldschmidt<sup>4</sup> suggested that chromium was present in seawater in the hexavalent state (chromate), and this was accepted by Krauskopf<sup>5</sup>. Arrhenius and Bonatti<sup>6</sup> supported the idea of a hexavalent state and pointed out the possibility of coprecipitation of chromate with strontium or barium sulphate *in situ*. Fukai and Huynh-Ngoc<sup>7</sup> showed by thermodynamical computations that the stable species of chromium in seawater should be hexavalent. Sillén<sup>8</sup> suggested that uncharged soluble chromic hydroxide might be present.

Because two contradictory ideas exist about the valency state of chromium in seawater, I have devised a differential method for the determination of the trivalent and hexavalent chromium which occurs in seawater. The principle of the method depends on independent measurements of two equivalent samples from the same sample of water, one treated by direct coprecipitation of chromium with iron hydroxide and the other by a similar coprecipitation after reduction with sodium sulphite in acid medium.

The results obtained by the method for samples of water collected from the Ligurian Sea are summarized in Table 1. It can be seen that hexavalent chromium was found in all the water samples. For some of the samples the trivalent chromium estimated was within the range of analytical sensitivity. These results seem to support the thermodynamical estimation of the valency state of chromium given by Fukai and Huynh-Ngoc<sup>7</sup>. The thermodynamically unstable species, trivalent chromium, however, was detected in many cases. This fact suggests that there

is some process which is working against thermodynamic equilibria.

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## Some Reactions of Triruthenium Dodecacarbonyl

WE have been investigating some reactions of triruthenium dodecacarbonyl  $\text{Ru}_3(\text{CO})_{12}$ . The structure of this compound is well established as an equilateral triangle of three ruthenium atoms with twelve terminal carbonyl groups<sup>1</sup>. The reactivity of this compound towards halogens, thiols, olefines and related compounds is, however, virtually unexplored. Some similarity in its chemical reactivity to that of tri-iron dodecacarbonyl can be expected, but whereas reactions of the iron cluster normally lead to cleavage of the trimeric unit, the ruthenium analogue appears to give stable trinuclear species. This may be correlated with an increase in the stability of metal-metal bonds on going down the transition metal triad<sup>2</sup>. This communication summarizes some of the reactions carried out on ruthenium carbonyl.

**Reaction with halogens.** Oxidation of triruthenium dodecacarbonyl with halogens ( $\text{X}_2$ ) ( $\text{X} = \text{Cl}, \text{Br}$  or  $\text{I}$ ) has led to the isolation of four classes of ruthenium carbonyl halides, namely: (i) The monomeric  $\text{Ru}(\text{CO})_5\text{X}_2$  (ref. 3). (ii) The dimeric  $\text{Ru}_2(\text{CO})_8\text{X}_4$ ;  $\text{Fe}(\text{CO})_5\text{Br}_2$  (ref. 4) and  $\text{Os}(\text{CO})_5\text{X}_2$  (ref. 5) reported previously may be of a similar type. (iii) The trimeric  $\text{Ru}_3(\text{CO})_{12}\text{X}_6$  which to our knowledge represent the first examples of a new class of trimeric carbonyl halide. This class of compound emphasizes that the metal cluster unit appears to behave in a novel way by donating six electrons to available groups. We have previously observed this behaviour in the compound  $\text{Os}_3(\text{CO})_{12}\text{OsO}_4$  (ref. 6). The fact that no analogues of iron are known may be correlated with the enhanced stability of metal-metal bonded compounds in the higher oxidation states of second and third row transition metals. The molecular weights of these compounds ((i), (ii) and (iii)) are based on the appearance of the parent molecular ions in the mass spectra. (iv) The polymeric carbonyl halides  $(\text{Ru}(\text{CO})_2\text{X})_n$  (ref. 7).

**Reaction with thiols.** Reaction of triruthenium dodecacarbonyl with thiols follows a different course from that

Table 1. TRIVALENT AND HEXAVALENT CHROMIUM IN SEAWATER SAMPLES

Sample	Location of sampling	Date of sampling	Chlorinity (per ml.)	$\text{Cr}^{3+}$ ( $\mu\text{g/l.}$ )	Soluble chromium $\text{Cr}^{6+}$ ( $\mu\text{g/l.}$ )	Total ( $\mu\text{g/l.}$ )
Surface seawater	Monaco coast	January 12, 1966	—	0.14	0.29	0.43
" "	" "	June 1, 1966	—	0.02 >	0.34 <	0.36
" "	2 km south of Monaco	June 8, 1966	—	0.02 >	0.36 <	0.38
" "	Cap d'Ail Coast	July 19, 1966	21.15	0.02 >	0.28 <	0.30
" "	Roquebrune Bay	September 13, 1966	20.95	0.18	0.05	0.23
" "	Cap d'Ail Coast	September 30, 1966	—	0.19	0.19	0.38
Seawater from 5 m depth*	42° 47' N. 7° 29' E.	October 25, 1966	20.92	0.23	0.16	0.39
Seawater from 500 m depth*	" "	" "	21.08	0.19	0.16	0.35
Seawater from 1,000 m depth*	" "	" "	21.03	0.20	0.21	0.41

\* The seawater was not filtered.



Table 1. INFRA-RED SPECTRA OF COMPLEXES IN CO STRETCHING REGION

Compound	$\nu(\text{C-O})$			
$\text{Ru}(\text{CO})_5\text{Br}_2^*$	2180 (m)	2138 (s)	2110 (m)	2078 (s)
$\text{Ru}(\text{CO})_5\text{I}_2^*$	2160 (m)	2106 (s)	2097 (s)	2069 (s)
$\text{Ru}_2(\text{CO})_9\text{Cl}_4^\dagger$	2143 (s)	2075 (s)	2015 (m)	
$\text{Ru}_2(\text{CO})_9\text{Br}_4^\dagger$	2138 (s)	2073 (s)	2010 (w, br)	
$\text{Ru}_2(\text{CO})_9\text{I}_4^\dagger$	2128 (s)	2069 (s)	2012 (w, br)	
$\text{Ru}_2(\text{CO})_{12}\text{Cl}_6^*$	2138 (m)	2068 (s)	2007 (m)	
$\text{Ru}_2(\text{CO})_{12}\text{Br}_6^*$	2133 (m)	2065 (s, br)	2009 (w)	
$(\text{Ru}(\text{CO})_5\text{Cl})_n^*$		2066 (m)	1988 (m)	
$(\text{Ru}(\text{CO})_5\text{Br})_n^*$		2059 (s)	1990 (m)	
$(\text{Ru}(\text{CO})_5\text{I})_n^*$		2053 (s)	1995 (s)	
$(\text{Ru}(\text{CO})_5\text{SPh})_2^\dagger$	2105 (w)	2080 (m, sh)	2060 (s, br)	2018 (m, br)
$(\text{Ru}(\text{CO})_5\text{SBU})_2^\dagger$	2110 (w)	2080 (m)	2056 (s)	2010 (s)
$(\text{Ru}(\text{CO})_5\text{SEt})_2^\dagger$	2110 (w)	2080 (m)	2058 (s)	2010 (s)
$(\text{Ru}(\text{CO})_5(\text{SPh})_2)_2^\ddagger$	2105 (w)	2042 (s)	1985 (s)	1945 (sh)
$(\text{Ru}(\text{CO})_5(\text{S-nBu})_2)_2^\ddagger$	2100 (w)	2022 (s)	1962 (s)	1930 (w)
$(\text{Ru}(\text{CO})_5(\text{SEt})_2)_2^\ddagger$	2096 (w)	2023 (s)	1963 (s)	1932 (w)
$(\text{Ru}(\text{CO})_5(\text{SMe})_2)_2^\ddagger$	2102 (w)	2022 (s)	1962 (s)	1930 (sh)
$(\text{Ph}_3\text{P})_3\text{Ru}_2(\text{CO})_8^\S$	2046 (vw)	1978 (sh)	1970 (s)	1933 (s)
			1929 (s)	1920 (sh)
$\text{C}_6\text{H}_5\text{Ru}(\text{CO})_5^\S$		2061 (s)	1990 (s, br)	
$\text{C}_6\text{H}_5\text{OMeRu}(\text{CO})_5^\S$	2060 (s)	1985 (s)	1955 (w)	
$(\text{C}_6\text{H}_5(\text{OMe})\text{Ru}(\text{CO})_5)^\ddagger$		2120 (m)	2055 (m)	

\* Spectra in chloroform.

† Spectra in carbon tetrachloride.

‡ Spectra in nujol.

§ Spectra in cyclohexane.

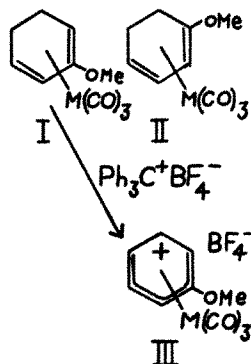
observed with halogens, and in these reactions the compounds  $\text{Ru}_2(\text{CO})_9\text{S}_2\text{R}_2$ ,  $(\text{Ru}(\text{CO})_5\text{S}_2\text{R}_2)_n$  ( $\text{R} = \text{Me}, \text{Et}, n\text{-Bu}, \text{Ph}$ ) and  $\text{Ru}(\text{SR})_3$  ( $\text{R} = \text{Ph}$ ) are the principal products. The structure of  $\text{Ru}_2(\text{CO})_9\text{S}_2\text{R}_2$  is expected to be similar to that of  $\text{Fe}_2(\text{CO})_9\text{S}_2\text{Et}_2$  which is well established<sup>8</sup> as dimeric with sulphur bridges. The polymeric  $(\text{Ru}(\text{CO})_5\text{S}_2\text{R}_2)_n$  compounds are similar to the polymeric halides  $(\text{Ru}(\text{CO})_5\text{X}_2)_n$  which are believed to have a halogen-bridged kinked chain structure with *cis* carbonyl groups<sup>9</sup>.

**Reaction with phosphines.** Reaction of triruthenium dodecacarbonyl with  $\text{PPh}_3$  gives  $(\text{Ph}_3\text{P})_3\text{Ru}_3(\text{CO})_9$ . A complex of this analytical description has already been reported<sup>10</sup>. The infra-red spectrum in the carbonyl stretching region (Table 1) of the complex produced in this work is, however, substantially different from that reported earlier. We believe that this discrepancy may be accounted for by the formation of isomers.

**Reaction with dienes.** Triruthenium dodecacarbonyl reacts with 2,5-dihydroanisole in benzene at reflux temperature to give dihydroanisole ruthenium tricarbonyl. Unlike the reaction with iron carbonyls, which produces two isomers (I) and (II) in yields<sup>11</sup> of about 30 per cent and 70 per cent, respectively, isomer (I) is the principal product. Previous work has shown that the free diene corresponding to isomer (I) is thermodynamically unstable. The production of this isomer as the stable species on co-ordination to the  $\text{Ru}(\text{CO})_3$  group is therefore of particular significance. As with the iron derivative, reaction with trityl tetrafluoroborate leads to hydride abstraction from the organic ligand (see reaction scheme).

A similar reaction with dihydrobenzene yields the dihydrobenzene ruthenium tricarbonyl derivative.

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### Chemiluminescence of Humic Acids

THE concept that humic acids (HA) are heteropolycondensates of phenolic substances is now widely accepted<sup>1,2</sup>. All the humic acids have hydroxyl, methoxy, or carbonyl groups connected with the aromatic parts of the nucleus. Our earlier investigations suggested that oxidation of hydroxyl groups attached to the aromatic ring may be accompanied by chemiluminescence<sup>3-5</sup>. It seems probable that chemiluminescence by analogy with fluorescence<sup>6</sup> may be able to provide information about the physical and chemical properties and the mechanism of oxidation of the humic acid. Furthermore, the emission of radiation in the course of the oxidation of humic acids may have physiological effects, especially if ultra-violet radiation is present. We have undertaken an investigation in order to gain more detailed information on this problem.

Table 1. CHARACTERISTICS OF HUMIC ACIDS

Sample No.	Origin	Extraction	Purifying procedure	Elementary analysis (per cent)			
				C	H	N	ash
1	Podzol soil	0.5 normal sodium hydroxide after decalcification with 2 per cent hydrochloric acid	Precipitation, centrifugation, dialysis	40.32	4.12	3.43	7.9
2	Black soil			41.59	4.26	2.15	10.1
3a	High peat	0.1 normal sodium hydroxide	Precipitation, centrifugation, dialysis, extraction with ethanol	Ethanol insoluble fraction			
3b				ethanol soluble fraction			
				46.2	3.79	3.94	-
				40.6	5.12	4.02	-

Table 1 contains the characteristics of humic acid samples. The equipment used for the luminescence measurements consists of an externally silvered, hemispherical cuvette mounted on a multiplier phototube, sensitive in the spectral region 390–500 nm<sup>4,7</sup>. Aqueous solutions of sodium humates (1, 2, 3a, Table 1) and a 0.05 per cent ethanolic solution of hymatomelanic acid (3b, Table 1) were oxidized with 0.185 molar hydrogen peroxide in the presence of 0.125 molar sodium carbonate. The luminescence intensity (*I*) was then recorded as a function of time (*t*) under varying concentrations of humic acids, hydrogen peroxide, and hydroxyl ions, at various temperatures and in the presence of the same catalysts and inhibitors (Fig. 1).

Weak visible chemiluminescence was found in the spectral region between 400–600 nm. The curves of light intensity against time gave  $I_{\text{max}}$  and  $E_{h\nu/3} = \int_{t=0}^{t=3} I dt$ , where *t* is the time in min (Table 2). We found that the maximum light intensity and the maximum value of  $E_{h\nu/3}$  are

Table 2. EFFECTS OF CATALYSTS AND INHIBITORS ON THE RELATIVE CHANGES OF MAXIMUM INTENSITY  $I'$  AND LIGHT SUM  $E'$  OF HUMIC ACIDS

Humic acid	Catalysts (moles/l.)*				Inhibitors (moles/l.)†			
	Fe(CN) <sub>6</sub> <sup>3-</sup> 10 <sup>-3</sup>	Iron, copper and cobalt 10 <sup>-3</sup>	Horseradish peroxidase ‡ 10 <sup>-4</sup>	Horseradish peroxidase + ascorbic acid ‡ + 1.5 × 10 <sup>-4</sup>	Ascorbic acid ‡	Boracic acid saturated	10 <sup>-2</sup>	$\alpha$ -Naphthol 5 × 10 <sup>-4</sup>
Humic acid $I'$	581.8	254.5	90.9	138.9	125.0	27.1	76.5	47.1
3a $E'$	231.3	187.5	113.8	56.2	88.3	20.4	47.4	30.2
Humic acid $I'$	309.1	136.4	340.0	—	35.4	20.7	—	—
3b $E'$	223.7	288.1	184.3	—	49.4	23.1	—	—

\* Acetic buffer, pH 5.0.

† Sodium carbonate, 10<sup>-5</sup>.

‡ Phosphate buffer, pH 7.4.

$$I' = \left( \frac{I - I_k}{I_k} 100 \right)_{\max}$$

$$E' = \left( \frac{E - E_k}{E_k} 100 \right)_{h/3 \text{ min}}$$

$I$ ,  $E$ , Maximum intensity and light sum, respectively, in the presence of catalysts or inhibitors;  $I_k$ ,  $E_k$ , the same without catalysts or inhibitors.

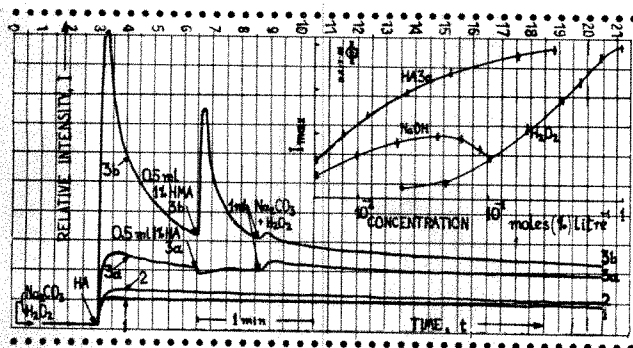


Fig. 1. Time course of chemiluminescence during oxidation of different humic acids (0.05 per cent) with 0.180 molar hydrogen peroxide and 0.125 molar sodium carbonate. Temperature, 30° C; supply voltage, 1,040 V. Humic acids or hydrogen peroxide and sodium carbonate were injected at the points indicated by the arrows. The inset in the upper right portion of the figure is a graph of maximum intensity  $I_{\max}$  against concentration for particular reagents.

reached where there is a large excess of hydrogen peroxide and hydroxyl ions:

$$\frac{[\text{H}_2\text{O}_2] \%}{[\text{HA}] \%} = 140, \quad \frac{[\text{NaOH}] \%}{[\text{HA}] \%} = 2.4$$

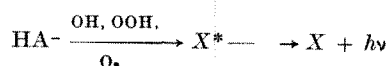
The temperature coefficient  $k_t = \frac{(I_{\max})_t + 10}{(I_{\max})_t}$  is  $1.89 \pm 0.18$

for the humic acid (row 3a of Table 1) and  $1.58 \pm 0.33$  for hymatomelanin (row 3b) in the range 15–30° C. A plot of  $\ln I_{\max}$  against  $1/T \times 10^3$  gives a straight line both for the HA and HMA. Activation energies calculated from the slope of these lines are  $12.78 \pm 2.11$  and  $8.03 \pm 1.01$  kcal/mole, respectively. The values of activation energies, quenching effects of  $\alpha$ -naphthol, phenol and an activation chemiluminescence by ions of heavy metals ( $M^{+n}$ ) suggest that the light emitting species are generated in radical reactions.

The first step in the consecutive reaction involves the decomposition of hydrogen peroxide



In the next stages, the anions and/or radicals OH, OOH, O<sub>2</sub> react with the humate anion, producing a smaller and simpler molecule in an excited state



The results shown in Table 2, concerning the effects of boracic acid, peroxidase and ascorbic acid, indicate that this step involves an oxidation of phenolic groups and breakdown of the aromatic rings of humic acid molecules.

In order to confirm this suggestion, absorption spectra of the humic acids from 320–600 nm were investigated.

$\Delta \log k$ , which is logarithm of the ratio of absorption coefficient at 400 nm to that at 600 nm, was 0.83 and 0.63 before oxidation for the humic acid and hymatomelanin acid samples shown in Table 1 (3a and b) respectively and 0.87 and 0.59 after oxidation. Decreases of absorption at 400 nm ( $\Delta E_{1\text{cm}}^{400}$ ) after oxidation for 3 min were 12.0 and 6.3 for samples 3a and 3b. This fact shows that the oxidation products of humic acids are less condensed than HA due to the breakdown of aromatic rings.

Further work is in progress to determine the spectrum and quantum yield, and, if possible, to establish the physiological role of the excited states of oxidation products of humic acids and that of emitting radiation.

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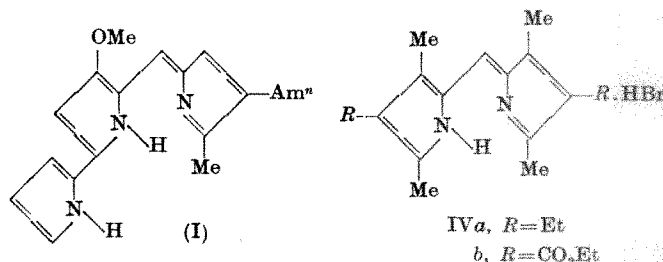
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### Antimalarial Activity of Prodigiosin

SINCE Babès in 1885 claimed the complete inhibition of cholera vibrio by an old culture of *Micrococcus prodigiosus* there have been numerous descriptions<sup>1-3</sup> of antibiotic properties associated with the bacterium *Chromobacterium prodigiosum* (*Serratia marcescens*) and the bipyrrylpyrrolylmethene prodigiosin (I), which occurs in the red bacterial pigment. The purity of the metabolite<sup>4,5</sup> used in the earlier investigations is suspect and the earlier claims like those for the bacterium lack definition of the true causative factor. In this regard the prodigiosin used has been considered too toxic for therapeutic use, although it has evidently been used in the clinical treatment of some cases of disseminated coccidioidomycosis (San Joaquin Valley



fever)<sup>6</sup>. Goble and Boyd<sup>7</sup> examined a number of porphyrins, bilirubinoids, pyrroles and congeners against *Trypanosoma congolense* in mice and found chlorophyllins, containing either magnesium or copper, and haematoporphyrin to be effective in delaying death and curative with prolonged treatment. We have investigated the antimalarial properties of purified prodigiosin (I), the cyclotetradepsipeptide serratamolide (II)<sup>4,8,9</sup>, an ethyl alcoholic extract of *Serratia marcescens* (III) from which I, II and other metabolites can be derived<sup>4</sup>, and two dipyrrolylmethenes (IV a and b) against *Plasmodium berghei* in mice. Prodigiosin showed definite activity (Table 1) against the parasite, which took about twice as long to kill the mice as compared with untreated infections, whereas the other materials exhibited only weak activity at the same or comparable concentrations.

Table 1. ANTIBIOTIC ACTIVITY OF PRODIGIOSIN AGAINST *Plasmodium berghei* IN MICE

Prodigiosin (mg/kg body wt.)	Deaths on indicated day (post infection)				Mean survival time (days)		Toxic deaths
					Treated	Control	
40	1/9	1/10	1/13	1/14	—	—	0
40	1/9	1/10	1/13	1/14	1/17	12.6	6.3
40	2/9	1/10	1/13	1/14	—	11.0	6.2
40	2/9	1/10	1/13	1/14	—	11.0	6.2
40	2/9	1/10	1/13	1/14	—	11.0	6.2
160	1/5	1/13	2/16	—	—	—	1
160	1/5	1/13	2/16	1/17	15.5	6.3	1
160	2/5	1/14	1/15	—	—	6.2	2
160	2/5	1/14	1/15	1/19	16.0	6.2	2

For each test, five 30 day old female mice were infected with a lethal dose of parasites 3 days before subcutaneous administrations of prodigiosin in peanut oil.

At 640 mg/kg all treated mice were dead by the fifth day, with the mean survival time for controls at 6.2–6.3 days.

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## BIOCHEMISTRY

### Phylogenetic Relationships of Echinoderms : Biochemical Evidence

THE conclusion that present day asteroids and ophiuroids have descended from the crinoids by way of somasteroids<sup>1</sup> has been challenged by G. M. Philip<sup>2</sup>, who argued that palaeontological evidence would not bear out the apparent homologies of structure of living sea-stars and crinoids. Fell, the proponent of the theory, has given a seemingly adequate reply<sup>3</sup>. Both authors, however, appear to be unaware of, or have chosen to ignore, evidence from comparative biochemistry that clearly has a bearing on the problem of the relationships of the echinoderm phyla:

it suggests that crinoids may lie on a different line of descent from any of the other four classes. In fact, in so far as biochemistry constitutes acceptable evidence, it makes Fell's proposal that Somasteroidea, Asteroidea and Ophiuroidea are sub-classes of class Crinoidea<sup>1</sup> appear to be untenable.

In a review published posthumously<sup>4</sup>, Bergmann discussed the comparative biochemistry of the sterols. He pointed out that comprehensive studies of the sterols of echinoderms<sup>5</sup> had confirmed his previous observation that the predominant sterols of ophiuroids and echinoids were the strongly laevorotatory  $\Delta^5$ -sterols. Cholesterol was usually the only component, although it was sometimes accompanied by elionasterol, poriferasterol and stigmasterol. On the other hand, the sterols of asteroids and holothurians were invariably a mixture of weakly dextrorotatory compounds,  $\Delta^7$ -stellasteruol, and its 22,23-dehydro derivative, stellasterol\*. The latter two classes also contain batyl alcohol<sup>6,7</sup>, the 1-O-octadecyl ether of glycerol. Extracted with the lipids, and non-saponifiable, batyl alcohol had once been thought to be a sterol and had been variously named astrol<sup>8</sup> and asteriasterol<sup>9</sup>. It is not generally found in the fats of ophiuroids and echinoids<sup>5</sup>.

The distribution of energy-rich amidine phosphates (phosphagens) should also be considered here. Although Huennekens and Whitely have concluded that the type of N-phosphate compound present in a given phylum does not appear to constitute a valid criterion of phyletic relationship<sup>10</sup>, there is a certain regularity of occurrence among the echinoderms. The exceptions have been found only in sperm tissue and not among adult animals, the distribution for which<sup>11</sup> is shown in Table 1, which also summarizes the distribution of sterols and batyl alcohol.

If we consider, as Florkin does<sup>12</sup>, that compounds with a common chemical lineage are homologous (while compounds exercising the same function in different organisms are analogous), then it is clear from the boxed-in portion of Table 1 that the Asteroidea and Holothuroidea contain homologous constituents. Echinoidea and Ophiuroidea contain different constituents which are themselves homologous. From these considerations it appears that the Asteroidea and Holothuroidea are more closely related to each other phylogenetically than to the Echinoidea, or the Ophiuroidea, while the latter two classes are also related to each other.

Table 1. DISTRIBUTION OF STEROLS, BATYL ALCOHOL AND PHOSPHAGENS AMONG THE CLASSES OF ECHINODERMS

Class	Arginine phosphoric acid	Creatine phosphoric acid	$\Delta^5$ sterols	$\Delta^7$ sterols	Batyl alcohol†
Crinoidea	+	—	+	—	+
Asteroidea	+	—	—	+	±
Holothuroidea	+	—	—	+	+
Echinoidea	+	+	+	—	—
Ophiuroidea	—	+	+	—	—

† Determined by titration with periodic acid<sup>4</sup>. The sign ± for the Asteroidea is intended to indicate that three of the nine species examined contained a small enough amount of batyl alcohol to make its presence questionable. One ophiuroid may have contained a small quantity of batyl alcohol.

This conclusion lends support to well known embryological evidence<sup>12</sup> that the first larval form (Dipleurula) in the development of the echinoderm is common to four classes, while the later larvae take the forms outlined in Fig. 1. From the recapitulation theory this evidence leads to the same conclusions as comparative biochemistry.

Fell, having previously classed this application of the recapitulation theory as a "reductio ad absurdum" in the case of the echinoderms<sup>13</sup>, more recently described the inferred relationships as "too preposterous to warrant further serious consideration"<sup>1</sup>. Nevertheless, he acknowledged that most authors of zoological texts have accepted the views of embryologists.

\* Up to now, the only other groups of animals found to contain  $\Delta^7$ -sterols are sponges of the genus *Chondrilla*.

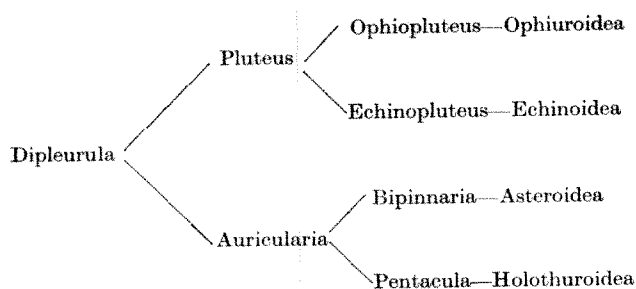


Fig. 1. Embryological development of echinoderms.

When a mass of evidence from anatomy and palaeontology is contradicted only by embryological evidence, it may indeed be permissible to dismiss the latter as "clandestine evolution, in response to temporarily planktonic food-gathering phases in the life history". When the reasoning from embryology receives independent support from a second, entirely different, type of scientific evidence, however, they cannot both be so easily turned aside. It is clear that biologists must reconsider their views of the phylogeny of the echinoderms. It should be remembered that the comparative biochemistry of sterols has already rendered useful service in revision of the taxonomy of some sponges<sup>4</sup>.

Returning now to Table 1, it is clear that the Crinoidea fall into a class by themselves, because they contain batyl alcohol and arginine phosphate, and  $\Delta^5$  rather than  $\Delta^7$  sterols. In fact, the one crinoid sterol, crinosterol, for which a reasonable structure has been proposed<sup>14</sup> is completely different from any other sterol of the animal or plant kingdoms. This suggests that the crinoids may have followed an entirely different line of evolution from the other echinoderms, just as they are believed to exhibit independent development beyond the Dipleurula stage.

The place of somasteroids in this scheme may well be clarified by a study of their chemistry.

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### Crinosterol: a Unique Sterol from a Comatulid Crinoid

THE discussion of the ancestry of the sea-stars<sup>1,2</sup> has prompted me to review work I did some 15 years ago<sup>3</sup> on a biochemical aspect of the problem<sup>4,5</sup>. In that work I isolated from a species of *Comatula* (a crinoid) a sterol of unique properties, and believed it to be unknown at that time; I assigned it the name crinosterol<sup>5</sup>. These results have not, however, been published until now because the structure deduced from physical properties was the same

as that accepted for a known sterol, chalinasterol, and the conflict could not be resolved. Since then, the structure of chalinasterol has been reformulated, and established as 24-methylenecholesterol<sup>6</sup>. Thus it is now possible to suggest that crinosterol has the structure originally proposed.

The crinoid from which the sterol was extracted was obtained by Dr. W. Bergmann in 1950 from Dr. René Catala of Noumea, New Caledonia. The species was not definitely identified, but there is little doubt that it was a member of the genus *Comatula*.

From 800 g of specimen dried in air, extraction with acetone yielded 11.6 g of lipid, and hydrolysis of the fat in potassium hydroxide solution gave 3.39 g of unsaponifiable material, of which 1.18 g crystallized from methanol as crude sterol. Without further purification, the crude sterol was acetylated directly with acetic anhydride, and the acetate was recrystallized six times from absolute ethanol and twice from acetone to yield 128 mg, which melted at 144°–145°. One recrystallization from ether raised the melting point to 146°–147°,  $[\alpha]_D^{24} - 47.2^\circ$  (26.2 mg,  $\alpha = 0.40^\circ$ ), and the melting point did not change on further recrystallization. The elementary analysis gave: carbon, 81.40, and hydrogen, 11.14 per cent; the calculated results for the compound  $C_{30}H_{48}O_2$  are carbon, 81.76; hydrogen, 10.98 per cent. Hydrolysis of the acetate in 20 per cent potassium hydroxide in methanol (2 h on steam bath), and recrystallization of the product, twice from methanol and once from acetone, yielded the free sterol, which melted at 137°–138° (unaltered by further recrystallization),  $[\alpha]_D^{20} - 47.2^\circ$  (24.9 mg,  $\alpha = 0.38^\circ$ ). The free sterol was converted to its benzoate using benzoyl chloride in pyridine at room temperature. The benzoate, recrystallized first from chloroform-methanol, then five times from absolute ethanol, had a double melting point: it melted first at 135°, resolidified (with fluorescence), then melted again at 141°–143°. The elementary analysis gave carbon, 83.01; hydrogen, 10.12; calculated for  $C_{33}H_{50}O_2$ : carbon, 83.61; hydrogen, 10.03.

A sample of crude acetate (263 mg), melting at 116°–124°, was dissolved in ether, and brominated by adding a 5 per cent solution of bromine in glacial acetic acid. The product required extensive purification, and the final yield of tetrabromide, melting point 185°–186°, was only 10.8 mg; elementary analysis showed 43.25 per cent bromine; the calculated percentage of bromine for  $C_{30}H_{48}Br_4O_2$  is 42.04. The authenticity of this tetrabromide as a derivative of crinosterol acetate and not of some impurity was established by debromination with zinc and acetic acid. The debrominated compound, after one recrystallization from methanol, melted at 144°–146°, and an admixture with the crinosterol acetate described here melted at 145°–146°.

The quantities of pure materials isolated precluded the application of rigorous methods of determination of structure. The results of bromination, however, clearly established the presence of two double-bonds. That one of them was at C-5 could readily be deduced from the molecular rotations of the sterol, acetate, and benzoate<sup>7-9</sup>:  $[M]_{\text{acetate}} - [M]_{\text{sterol}} = -20$ , and  $[M]_{\text{benzoate}} - [M]_{\text{sterol}} = +98$ . Further consideration of molecular rotation differences showed that the only alterations of the structure of cholesterol which could logically account for them were a double bond at C-22 and a 24 $\beta$ -methyl group. While the possibility of a 24-ethyl group could not be eliminated on these grounds alone, its presence would require that the sterol be identical with stigmasterol (melting point 170°, ref. 5) or poriferasterol (melting point 156°, ref. 5). It is therefore proposed that crinosterol is the C-24 epimer of brassicasterol, and is 24 $\beta$ -methyl-22-dehydrocholesterol.

The crinoids *Antedon rosacea* and *Cenocrinus asteria* also yielded laevorotatory sterols, with acetates melting at 120°–122°,  $[\alpha]_D - 41.1^\circ$ . The sterol acetate from *C. asteria* gave a crystalline tetrabromide, melting point

180°. These sterols were probably not crinosterol, but insufficient material was available for further study.

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### Formation and Destruction of Pyrimidine Dimers in Polynucleotides by Ultra-violet Irradiation in the Presence of Proflavine

THE ultra-violet irradiation of polynucleotides in solution results in the reversible formation of dimers of *cyclobutane* type between adjacent pyrimidine residues<sup>1</sup>. In DNA, such dimers produce important effects in many biological systems<sup>2</sup>. Beukers has also found that the presence of proflavine during irradiation at 2537 Å inhibits the formation of thymine dimers in DNA and that dimers produced in the absence of proflavine are destroyed by further irradiation in its presence<sup>3</sup>. Although Beukers was unable to show that the destruction of the dimers in DNA consists of a simple reversion to monomers, he suggested that this might be demonstrated by means of experiments with organized polymer dI-dC consisting of strands of polydeoxyinosinic acid and polydeoxycytidylic acid. Such a polymer has already been used to show that the enzymatic destruction of uracil dimers by photo-reactivating enzyme in the presence of visible light consists of monomerization<sup>4</sup>.

We have followed Beukers's suggestion, and have obtained data which indicate that his hypothetical scheme is correct. Our data indicate that proflavine affects only the rate of formation of dimers and not their monomerization. It follows that the steady state concentration of dimers, which depends on the balance of the forward and backward reactions, is altered by the presence of proflavine. By using proflavine to inhibit the formation of dimers, we have been able to measure the monomerization of dimers in DNA at several different wavelengths and have shown that at long wavelengths dimers containing cytosine may be selectively split by radiation in the presence of proflavine. The biological analogue of this finding is reported in an accompanying note<sup>5</sup>. Because the quantum yield for dimer formation in DNA is small<sup>6</sup> (approximately 0.01), any slight change in the configuration of DNA would be expected to alter this value drastically. Changes in DNA structure on binding of the dye molecules<sup>6,7</sup> are therefore sufficient to explain the results, and energy transfer mechanisms do not have to be invoked.

Monochromatic radiation was obtained by the use of a mercury arc and a large quartz prism monochromator. The radiant fluxes are computed as averages from the incident flux and the measured absorbance<sup>8</sup>. We investigated the polymers <sup>3</sup>H-cytosine-labelled dI-dC (40,000 c.p.m./μg) and tritium thymine labelled *E. coli* DNA (300,000 c.p.m./μg). Irradiation was carried out as described previously<sup>9</sup> in the presence of proflavine and under conditions in which polynucleotide and dye bind strongly together<sup>10</sup>. After irradiation, the solutions containing buffer, dye and polymers were heated and dried

Table 1. THE PERCENTAGES OF TOTAL RADIOACTIVITY IN URACIL DIMERS (UU) AND URACIL (U) AFTER ACID HYDROLYSIS OF <sup>3</sup>H-CYTOSINE-LABELLED dI-dC IRRADIATED IN THE ABSENCE OR PRESENCE OF PROFLAVINE (PRO)

Experiment	Wave-length Å	Flux erg mm <sup>-2</sup> × 10 <sup>-4</sup>	PRO absent		PRO present	
			UU	U	UU	U
(per cent activity)						
A	2800	4	10.4	1.2	3.6	1.0
B	2800	4				
(with PRO absent and followed by)						
	2800	1	—	—	3.8	1.4
	2390	1	3.0	1.0	1.6	1.4
C	2800	4				
(with PRO absent and followed by 60° C, 60 min and then by)						
	2800	4	13.2	2.0	11.2	1.7
	2390	1	7.0	4.3	6.9	4.7

Samples (0.2 ml.) had the following concentrations: polymer (in terms of nucleotides)— $1.6 \times 10^{-4}$  molar; proflavine— $1.8 \times 10^{-5}$ ; buffer—0.05 molar phosphate pH 7.0.

and the residues hydrolysed with 98 per cent formic acid. Radioactivity in dimers and thymine was assayed by paper chromatography followed by scintillation counting<sup>9,11</sup>. The dimers detected with dI-dC were uracil dimers—the deamination products of cytosine dimers. With DNA we detected thymine-thymine and uracil-thymine dimers—the deamination products of cytosine-thymine dimers. The presence of proflavine did not affect the hydrolysis, chromatography or counting efficiency.

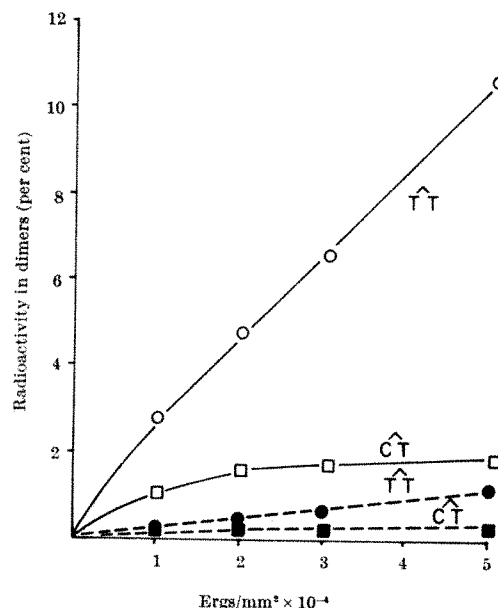


Fig. 1. The effect of proflavine on the formation of dimers in DNA. *E. coli* DNA labelled with <sup>3</sup>H-thymine was irradiated with 2800 Å radiation in the absence (○, □) and the presence (●, ■) of proflavine and was then analysed for the percentage of radioactivity in thymine-thymine (TT) and cytosine-thymine (CT) dimers. Samples of 0.2 ml. of 0.01 M phosphate buffer pH 7.0 contained 1.2 μg/ml. of DNA and  $1.5 \times 10^{-5}$  M proflavine. The radiation flux is corrected for absorption by the dye.

Table 1 presents data from three types of experiments on the effects of proflavine on dimer formation and destruction in dI-dC. Experiment A indicates that the presence of proflavine inhibits dimer formation in this polymer. (The uracil that does appear is largely the result of deamination of cytosine during acid hydrolysis and not of irradiation<sup>9</sup>.) Experiment B shows that short wavelength (2390 Å) radiation can reverse the effects of long wavelengths and that dimers once formed may be destroyed in the presence of proflavine by further irradiation at 2800 Å. The destruction of dimers by short wavelengths is the more complete in the presence of proflavine.



When irradiated dI-dC is heated, the cytosine dimers deaminate to form uracil dimers, which have small absorption coefficients at long wavelengths and which are therefore not monomerized by such radiation. Experiment C illustrates the effects of further irradiation on dI-dC already containing uracil dimers. A large dose at a long wavelength has practically no effect, whereas at short wavelengths there is a significant decrease in uracil dimers and an equal increase in the amount of uracil produced both with and without proflavine. Thus the destruction of uracil dimers consists of monomerization, and this process is not affected by the presence of proflavine. These data support Beukers's description<sup>3</sup>.

The results of the experiments with DNA are given in Fig. 1, which shows that the presence of proflavine during irradiation at 2800 Å inhibits the formation of both thymine-thymine and cytosine-thymine dimers, and that there is a ten-fold decrease of dimer formation. The data in Fig. 2 indicate that for DNA as for dI-dC (Table 1) there is a large net destruction of cytosine dimers with continued irradiation at long wavelengths in the presence of proflavine. Thus irradiation at 2800 Å selectively destroys dimers containing cytosine, but thymine-thymine dimers are relatively unaffected because of their extremely low absorption coefficient at this wavelength. At shorter wavelengths (2650 Å and 2390 Å), both thymine-thymine and cytosine dimers are destroyed.

At 2390 Å the initial rate of monomerization of dimers in DNA is independent of the presence of proflavine. Moreover, in the presence of proflavine, the destruction of dimers as a function of wavelength is not qualitatively similar to the absorption spectrum of the dye, which is a maximum at 2600 Å. Thus, energy transfer mechanisms do not play an important part in dimer destruction in the presence of proflavine, and the best description of the effects of proflavine on dimer formation and monomerization in DNA is that the dye inhibits only the formation of dimers and does not affect their monomerization.

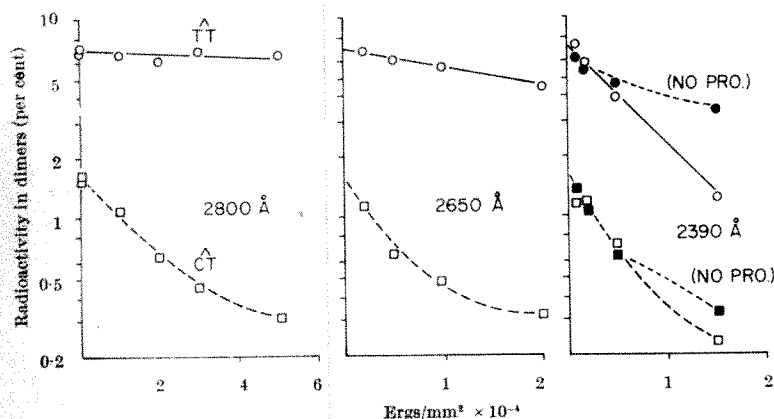


Fig. 2. The destruction of thymine-thymine and cytosine-thymine dimers by three different wavelengths of ultra-violet light in the presence of proflavine (pro) and at 2390 Å in its absence (no pro). *E. coli* DNA at 1.2 µg/ml. in 0.01 molar phosphate buffer pH 7.0 and labelled with <sup>3</sup>H-thymine was first irradiated at 2800 Å with  $3 \times 10^4$  erg/mm<sup>2</sup>. Proflavine was then added to a concentration of  $1.5 \times 10^{-5}$  molar, and irradiation was continued at the indicated wavelengths.

The finding that long wavelength irradiation in the presence of proflavine selectively monomerizes dimers containing cytosine has led to an estimate of the relative importance of cytosine dimers in the inactivation of transforming DNA (ref. 4). Because the net monomerization by short wavelengths is more extensive in the presence of proflavine, it may be possible to carry out reversal experiments on biologically active systems at much smaller initial doses than have been used so far.

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### Contribution of Dimers containing Cytosine to Ultra-violet Inactivation of Transforming DNA

BEUKERS<sup>1</sup> has shown that dimers containing thymine in DNA irradiated at 2537 Å can be eliminated by further irradiation at 2537 Å in the presence of proflavine, and later work<sup>2</sup> has confirmed his hypothesis that the elimination represents splitting of the dimers. We have shown that the biological inactivation of ultra-violet-irradiated transforming DNA from *Haemophilus influenzae* may similarly be reversed by irradiation in solutions containing proflavine. An investigation of the wavelength dependence of this reactivation in the presence of proflavine, together with information on the reversal of different types of pyrimidine dimers<sup>3</sup>, has made it possible to evaluate the role of dimers containing cytosine in inactivation.

Ultra-violet irradiation and the transformation assay were done as previously described<sup>4</sup>. The incident doses were corrected for absorption by the solutions. For irradiation, transforming DNA at room temperature was in 0.01 M phosphate buffer, pH 7, at a concentration of 1 µg/ml. Proflavine was used at a final concentration of 5–6 µg/ml. After a 30-fold dilution, the DNA was assayed for ability to transform *H. influenzae* cells to resistance to 2.5 µg/ml. cathomycin. Under these conditions proflavine did not itself affect the number of transformants.

Fig. 1 shows that transforming DNA inactivated to 1 per cent survival by 2520 Å radiation is reactivated by further irradiation at the same wavelength after proflavine has been added to the solution. Proflavine also protects transforming DNA from inactivation when it is present during irradiation, as shown in Fig. 2, and as previously shown by Beukers<sup>1</sup> and by Lerman<sup>4</sup> for acridine orange. The data are presented on a square root plot<sup>5</sup>, which gives a straight line for inactivation in the presence and in the absence of proflavine. It is seen in Fig. 2 that the ratio of the sensitivities for the inactivation of *H. influenzae* transforming DNA by 2800 Å radiation with and without proflavine is 10.8, close to the value of 10 observed under similar experimental conditions for the ratio of the numbers of dimers containing thymine in *E. coli* DNA irradiated with and without proflavine. These data provide additional evidence for the importance of dimers in inactivation.

We have previously shown that transforming DNA exposed to a large dose at long wavelength may be reactivated by subsequent irradiation at short wavelength<sup>6</sup>. Fig. 3 shows that the amount of reactivation is greater when proflavine is present during the short-wavelength irradiation. Similar results have been found for elimination of dimers by short-wavelength irradiation with and without proflavine<sup>2</sup>. Proflavine appears not to alter the initial rate of the reaction but rather the final level. Thus proflavine does not affect the probability of dimer splitting but apparently decreases the probability of the dimerization reaction.

The amount of reactivation by 2650 Å radiation in the presence of proflavine following 2800 Å inactivation is intermediate between the large reactivation produced by 2390 Å radiation and the small reactivation at 2800 Å. This wavelength dependence is not similar to the absorption spectrum of proflavine, which peaks at 2600 Å and drops markedly on either side of the peak, but is similar to that of pyrimidine dimers<sup>7</sup>, suggesting that energy for reactivation is absorbed in pyrimidine dimers rather than in the dye itself.

Fig. 4 shows the reactivation of transforming DNA in the presence of proflavine by 2800 Å radiation following

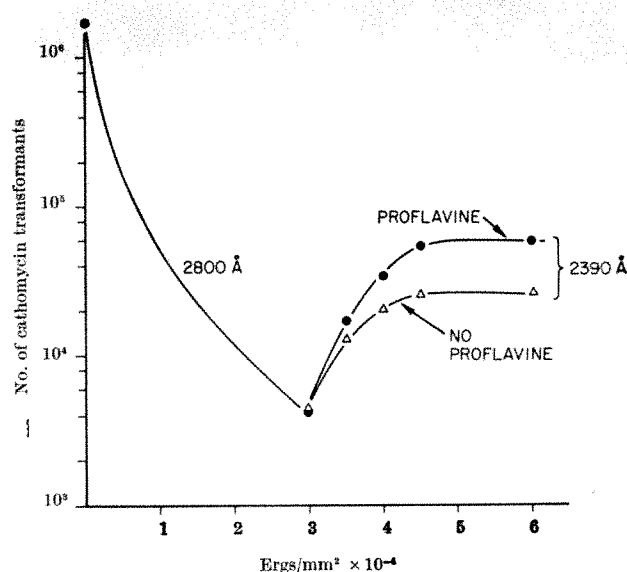


Fig. 3. Short-wavelength reactivation of *H. influenzae* transforming DNA with and without proflavine present. The DNA was first irradiated at 2800 Å without proflavine, and then at 2390 Å either with or without proflavine.

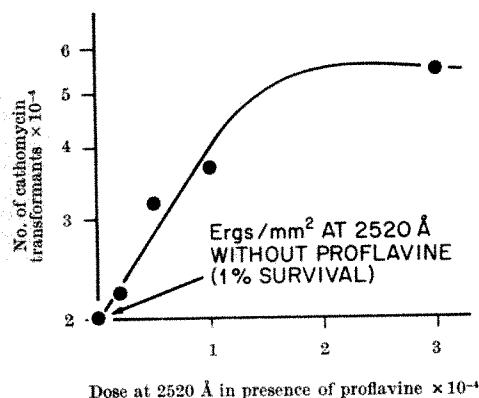


Fig. 1. Reactivation of *H. influenzae* transforming DNA inactivated by 2520 Å radiation by further irradiation in the presence of proflavine.

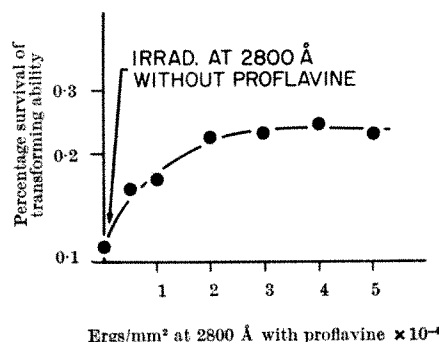


Fig. 4. Reactivation at 2800 Å of *H. influenzae* transforming DNA inactivated at the same wavelength by a dose of  $8 \times 10^4$  ergs/mm<sup>2</sup>. After maximum reactivation, the survival of transforming DNA was the same as the survival after an initial dose of  $2 \times 10^4$  ergs/mm<sup>2</sup>. Thirty-three per cent of the biological damage has therefore been eliminated.

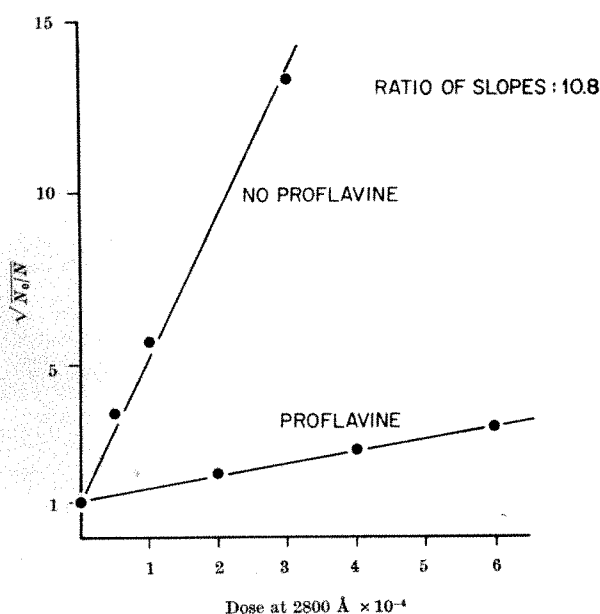


Fig. 2. Inactivation of *H. influenzae* transforming DNA with and without proflavine in the solution of DNA. The numbers of transformants produced by unirradiated and irradiated DNA are  $N_0$  and  $N$ , respectively.

inactivation by the same wavelength without proflavine. The amount of reactivation is small but significant. At this wavelength there is a clear differential effect of irradiation in the presence of proflavine on thymine-thymine and cytosine-thymine dimers, in that there is negligible splitting of thymine-thymine dimers<sup>2</sup>. From the data of several experiments similar to that shown in Fig. 4, we have determined that between 14 and 33 per cent of the biological damage produced by 2800 Å radiation can be removed by irradiation at 2800 Å in the presence of proflavine. We estimate from data for *E. coli* DNA that in *H. influenzae* DNA under the same experimental conditions, 20 per cent of the total dimers are split, of which no more than 4 per cent are thymine-thymine dimers<sup>2</sup>. Because there are very few cytosine-cytosine dimers in *H. influenzae* DNA<sup>7</sup>, cytosine-thymine dimer splitting must therefore account for almost all the biological effect shown in Fig. 4. Furthermore, the correspondence between the fraction of biological damage removed and the percentage of total dimers eliminated—as measured by ultra-violet absorbance<sup>6</sup>—suggests that cytosine-thymine and thymine-thymine dimers are similar in their contribution to inactivation.

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### *cis-syn* Thymine Homodimer from Ultra-violet Irradiated Calf Thymus DNA

THE present interest in the ultra-violet irradiation of nucleic acids<sup>1-3</sup> seems to warrant the tedious effort required to isolate in small quantities and to identify the product(s) formed in biological material, at doses compatible with biological assays of the radiation-induced damage.

The appearance of two areas of radioactivity on paper chromatograms of the acid hydrolysates of DNA labelled with thymine and irradiated with ultra-violet light was recently reported by Boyce and Howard-Flanders<sup>4</sup> and by Riklis<sup>5</sup>. The eluent used was *n*-butanol-acetic acid-water (80:12:30; v/v). Other eluents were also shown to give good separations<sup>6</sup> in a study of the chemical nature of these products from irradiated DNA. On the basis of these findings, a reliable procedure was established for obtaining these products not only in high purity but also in quantities large enough to allow the elucidation of structure using ultra-violet, nuclear magnetic resonance, and infra-red spectra. In this communication, we wish to report the identification of the *cis-syn* thymine homodimer (T=T)<sup>7</sup> as one of these products.

Native calf thymus DNA (5 g for each run) was dissolved at a concentration of about 60 µg/ml. (O.D. 1.2 at the maximum) in 0.15 molar sodium chloride solution.

The DNA solution was irradiated in enamel pans (40 × 25 cm), and 750-ml. portions of the dilute solution

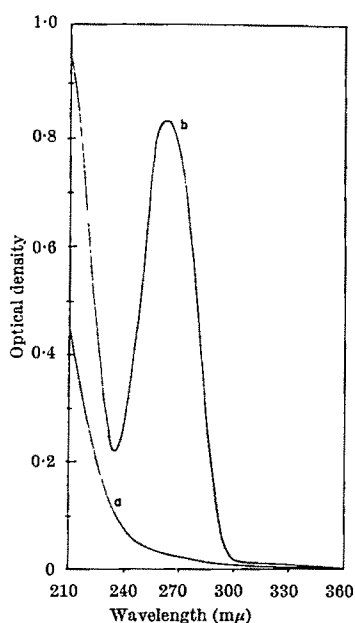


Fig. 1. Ultra-violet absorption spectra of *a*, *cis-syn* thymine homodimer from acid hydrolysates of ultra-violet irradiated DNA, and *b* after 10 min irradiation in aqueous solution.

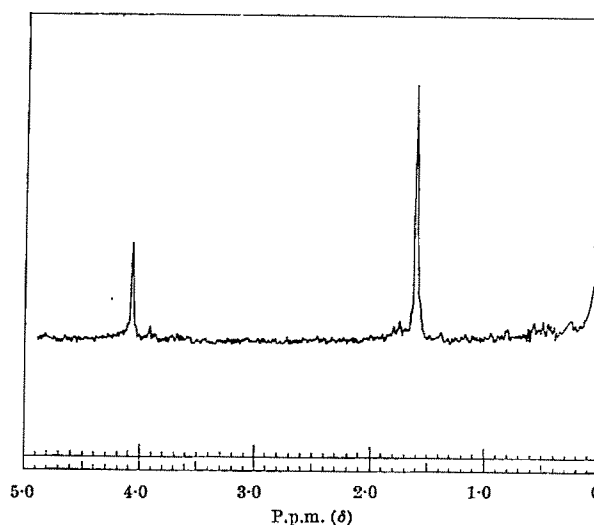


Fig. 2. Nuclear magnetic resonance spectrum of *cis-syn* thymine homodimer isolated from ultra-violet irradiated DNA. Solvent is CF<sub>3</sub>COOD. Values of δ are shown in p.p.m. from tetramethylsilane.

were irradiated for 25 min with a total dose of  $5 \times 10^4$  ergs/mm<sup>2</sup>. The choice of this irradiation condition was based on a time study which indicated that under these conditions irradiated DNA gives optimal competitive inhibition of the photoenzymatic reactivation of irradiated transforming DNA<sup>8</sup>.

The irradiated solution (72 l. per run) was concentrated to 10 l. and dialysed overnight against a continuous flow of water. The dialysed solution was concentrated to dryness.

The completely dried sample was taken up in about 200 ml. of trifluoroacetic acid (TFA), and then transferred to and sealed in twenty 'Pyrex' glass tubes with heavy walls. These were heated at 170°C for 90 min. The hydrolysate was filtered to remove some insoluble material. Both the tubes and the residue were washed twice with 0.1 normal hydrochloric acid. After the filtrates had been evaporated to dryness, the residue was dissolved in about 75 ml. of TFA.

The hydrolysates in TFA were then spotted on twenty-five sheets of Whatman No. 3 paper (57 × 13.5 cm), and developed with *n*-butanol-acetic acid-water for 20 h by the descending technique. The areas between *R<sub>F</sub>* 0.15 and 0.35 of the dried chromatograms were cut out and extracted three times with 500 ml. of 0.1 normal hydrochloric acid. In order to determine whether the extraction was complete, spectra were taken for each sample before and after irradiation. The extract of hydrochloric acid was evaporated to dryness and subjected to rechromatography several times using different solvent systems. The chromatography procedure used was similar to that already described; the conditions and data are summarized in Table 1.

Table 1

Chromatography	Residue dissolved in 0.1 normal hydrochloric acid (ml.)	Sheets of paper	Eluent* (time, h)	Area cut-out ( <i>R<sub>F</sub></i> )	Extract in 0.1 normal hydrochloric acid (ml.)
Second	15	8	A (20)	0.29	3 × 200
Third	8	4	B (23)	0.54	3 × 150
Fourth	5	2	C (20)	0.15	3 × 100

\* Solvent systems: A, *n*-butanol-acetic acid-water (80:12:30); B, isopropanol-concentrated hydrochloric acid-water (68:15.5:16.5); and C, *n*-butanol-water (86:14). (All proportions given by volume.)

In order to locate the desired areas on the chromatograms, DNA labelled with thymine-2-<sup>14</sup>C was used in parallel runs under identical conditions.

Four milligrams of a slightly yellow material, obtained by concentrating the final hydrochloric acid extract, was washed twice with 5 ml. of absolute ethanol to remove the colour.

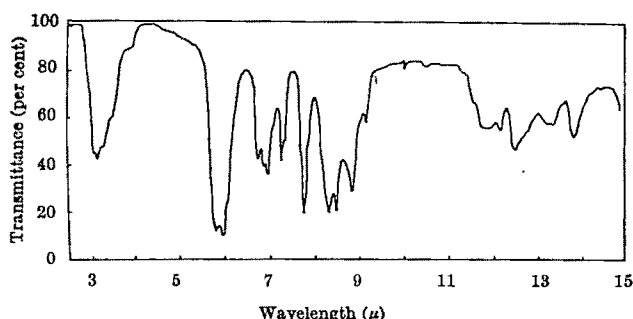


Fig. 3. Infra-red spectrum of *cis-syn* thymine homodimer isolated from ultra-violet irradiated DNA.

The residue was dissolved in 25 ml. of hot water. The filtrate, concentrated to 3 ml., was allowed to stand in a refrigerator overnight. The colourless crystalline product which separated from water after two crystallizations gave ultra-violet, infra-red, and nuclear magnetic resonance spectra identical to those of the thymine homodimer obtained from frozen solutions.

Our experiments provide evidence that T=T is a product of DNA irradiated with ultra-violet light. According to a recent report<sup>9</sup> on the naturally abundant <sup>13</sup>CH satellite proton spectra of T=T, this product should have the *cis-syn* configuration. Because the isolation procedure involved acid hydrolysis, it has yet to be shown that T=T is a photo-product formed directly on irradiation. This is especially true because it has recently been shown that irradiation of cells in which the DNA contained thymine labelled at both the 2-carbon and 5-methyl hydrogen yielded T=T with a 30 per cent reduction in the ratio of <sup>3</sup>H/<sup>14</sup>C when compared with thymine<sup>6</sup>. Because direct formation of T=T does not involve the 5-methyl group<sup>8</sup>, it would appear that the formation of T=T on cellular DNA may involve at least one intermediate step.

On the basis of chromatographic mobility and photo-reversal to thymine in solution, it has been suggested<sup>10,11</sup> that T=T is the product formed when DNA is irradiated with ultra-violet light. In recent reports<sup>4,5</sup>, however, two chromatographically separable products were observed, and T=T was again assumed to be the principal product. Blackburn and Davies<sup>12</sup> have attempted to establish the structure of the product of irradiated DNA by using T=T from ice. Although this approach is interesting, we must emphasize that the so-called principal product consists of at least one other compound which is chromatographically indistinguishable and co-crystallizable with T=T although it is not T=T (unpublished results).

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### Influence of Protracted Infusion of Glucose and Insulin on the Composition and Regeneration Activity of Liver after Partial Hepatectomy in Rats

In the liver tissue which remains after partial hepatectomy (resection of 65–70 per cent of the liver) marked changes in the composition and the energy metabolism<sup>1,2</sup> develop even before the occurrence of increased mitotic activity. The energy metabolism in the liver tissue is shifted from a predominant utilization of glycides to an increased utilization of lipids<sup>3</sup> with a corresponding decrease in the content of glycogen and an increase in the content of lipids. We wished to find out whether the regenerative activity of the liver would be affected if the development of these changes was arrested.

The experiments were carried out on male rats weighing 200 g, which were fed on a standard laboratory diet until the operation<sup>4</sup>. In the experimental groups of rats (groups 2–4) partial hepatectomy was performed by the method of Higgins and Anderson<sup>5</sup>. Group 1 included normal rats in which the rest of the liver remaining after resection was analysed. After partial hepatectomy the rats of the second group were given an infusion of Ringer's solution, rats of the third group 20 per cent glucose (in Ringer's solution), rats of the fourth group 20 per cent glucose solution containing 0.02  $\mu$  of crystalline insulin (*SPOFA*) per 1 ml. The infusion apparatus administered the solutions into the left jugular vein of the rats each hour for 5 min, the dose being 0.3 ml./100 g body weight. Thirty-four hours after the operation the rats were killed by decapitation. Two hours before death

Table 1. WEIGHT OF LIVER REMAINING AFTER PARTIAL HEPATECTOMY, DRY TISSUE AND COMPOSITION, BEFORE AND AFTER INFUSION OF RINGER'S SOLUTION, 20 PER CENT GLUCOSE OR 20 PER CENT GLUCOSE WITH INSULIN

	Before infusion (control values)	After infusion—36 h after partial hepatectomy		
		Ringer's solution	20 per cent glucose	Insulin and 20 per cent glucose
Weight of the liver g/100 g body weight	1.40 $\pm$ 0.36	2.10 $\pm$ 0.28 <0.001	2.18 $\pm$ 0.38 <0.01	1.70 $\pm$ 0.24 <0.05
Dry liver tissue (per cent)	31.6 $\pm$ 1.18	32.5 $\pm$ 2.03	33.1 $\pm$ 2.71	29.3 $\pm$ 1.66
Lipids	56.0 $\pm$ 18.2	222.0 $\pm$ 40.6 <0.001	205.0 $\pm$ 18.6 <0.001	104.0 $\pm$ 20.4 <0.001
Protein nitrogen	15.6 $\pm$ 3.64	24.3 $\pm$ 4.69 <0.01	26.8 $\pm$ 5.47 <0.01	17.8 $\pm$ 2.00
Ribonucleic acids	11.2 $\pm$ 1.36	17.6 $\pm$ 2.80 <0.001	20.4 $\pm$ 1.56 <0.001	15.7 $\pm$ 2.50 <0.01
Deoxyribonucleic acids	4.1 $\pm$ 0.37	6.3 $\pm$ 1.15 <0.001	6.8 $\pm$ 2.15 <0.01	4.3 $\pm$ 0.86

Infusions were started immediately after partial hepatectomy.

tritiated thymidine (200 mc./0.4 ml./200 g) with a specific activity of 6.0 c./mmole was injected into the femoral vein of each rat. Incorporation of thymidine-<sup>3</sup>H into the nuclei of the liver cells was monitored by the autoradiographic method<sup>6</sup>. In liver sections stained with iron haematoxylin, the mitotic index (number of mitoses found in 1,000 nuclei of parenchymal liver cells) was ascertained. The content of lipids<sup>7</sup>, nucleic acids<sup>8</sup>, and protein nitrogen<sup>9</sup> was determined in the samples of liver tissue. The results were evaluated statistically using Student's *t* test.

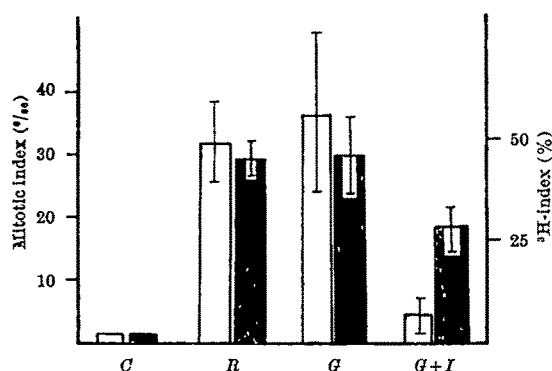


Fig. 1. Mitotic index of parenchymal liver cells (per cent; white columns) and number of nuclei in these cells labelled by thymidine-<sup>3</sup>H (per cent; <sup>3</sup>H-index; black columns) in rats with intact livers (C), and in rats with partial hepatectomy following infusion of Ringer's solution (R), 20 per cent glucose (G) or 20 per cent glucose with insulin (G+I). Standard deviations are mentioned with the average values.

Thirty-four hours after partial hepatectomy there was a smaller increase in the weight of the liver which remained after resection and also a smaller total amount of lipids, protein nitrogen, RNA and DNA in the liver tissue of rats which were injected with the solution of insulin and glucose (Table 1). The values of the mitotic index were considerably lower ( $p < 0.001$ ) in the rats into which solutions of insulin and glucose were infused than in the rats of the other experimental groups (Fig. 1). The number of nuclei of parenchymal liver cells labelled with thymidine-<sup>3</sup>H also decreased in the group of rats which had been infused with insulin and glucose as compared with the rats which were infused with Ringer's solution ( $p < 0.001$ ) or glucose solution alone ( $p < 0.01$ ) (Fig. 1). Insulin administered in the infusion with glucose increases lipogenesis, especially in the adipose tissue, and at the same time inhibits liberation of free fatty acids from this tissue<sup>10,11</sup>. The slackening of the growth of the lipid content in the liver is probably connected with the limited mobilization of free fatty acids from the fat deposits which usually occur after partial hepatectomy. This shift in liver lipid content has also been confirmed by our recent, still unpublished experiments, in which the mobilization of free fatty acid after partial hepatectomy was partially blocked by hexamethonium or nicotinic acid. It is well known that the liver takes up free fatty acid from the blood, in a similar way as the heart or the skeletal muscles, for example in dependence on their concentration in the blood. After partial hepatectomy the increased supply of free fatty acid to the liver leads to an increased lipid content in the liver simultaneously, however, with an increased utilization of this substance<sup>2,3</sup>. This shift in the energy metabolism secures the necessary energy for the liver tissue being under the condition of an increased functional strain in which a regenerative process characterized by a predominance of endergonic reactions is just going on. We believe that as a consequence of the partial inhibition of the growth of lipids in the liver a shift of the metabolism of the liver takes place. We suppose that the decrease in the regenerating activity of

the liver observed by us is probably also connected with the inhibited development of these changes.

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### Catabolism of Tryptophan by the Isolated, Perfused Liver and Intestine

THE metabolism of tryptophan has received considerable attention from biochemists<sup>1,2</sup> and has been of particular interest because a number of physiologically active agents, such as serotonin, are among the many metabolites of tryptophan. Information about tryptophan metabolites is extensive in a qualitative sense, but quantitative data on tryptophan catabolism in various organs are scarce. The present communication deals with certain quantitative aspects of tryptophan metabolism in the perfused liver and intestine of the rat.

The perfusion of the isolated liver or intestine of the rat was performed by the Miller technique<sup>3</sup> as described in an earlier communication<sup>4</sup>. Forty millilitres of fresh, heparinized rat blood diluted with one third of its volume of Ringer's solution (Fixanal Ringer-Riedel de Haën) and containing 200 mg of glucose was used as perfusion fluid. In the tryptophan loading experiment 50  $\mu$ moles of unlabelled L-tryptophan was added to the perfusion fluid. The animals were maintained on a standard diet (A.M. and R.M.H. 1/1 from Kray—Oost-Knollendam—Holland) which contained all important vitamins including 6.5 mg/kg of pyridoxine. The animals were starved from 18 h before perfusion.

Tryptophan labelled with tritium primarily in the aromatic ring (more than 75 per cent of total activity of tritium) and with carbon-14 in carbon atom 2 of the indole ring was used in all these experiments. Tritiated L-tryptophan had a specific activity of 136 mc./mmole whereas DL-tryptophan (indolyl-2-<sup>14</sup>C) had a specific activity of 5.2 mc./mmole. The purity of the compounds was ascertained by paper chromatography in two solvent systems. Only one peak coinciding with authentic tryptophan was seen.

The labelled material was added to the perfusate at the beginning of the experiments and samples of perfusate and organ tissue, bile in the case of the liver, and carbon dioxide were obtained at various times during the perfusion. Carbon dioxide was collected in ethanolamine and methyl cellosolve (1:2). Samples of perfusate, and homogenates of liver and intestine prepared by homogenization with a pestle after addition of four volumes of water, were treated with one volume of acetone. The resulting precipitate was removed by centrifugation and the supernatant used for the determination of carbon-14 and tritium activity as well as of the concentration of tryptophan. The radioactivity of carbon-14 and tritium was determined by liquid scintillation spectrometry



using a Packard Tri-carb apparatus. Tryptophan concentration was determined by the fluorometric method of Duggan and Udenfriend<sup>5</sup>. Labelled carbon dioxide and the radioactivity in bile were determined in aliquots of the collected material without further treatment.

Data on radioactivity in liver, perfusate and bile expressed in percentage of the total radioactivity initially added to the perfusate are presented in Fig. 1. Perfusions were carried out either at physiological concentrations of tryptophan (4  $\mu$ moles) or in conditions of tryptophan loading after addition of 50  $\mu$ moles of L-tryptophan to the perfusion fluid. Pertinent data on the specific activities of tryptophan and the concentration of tryptophan during perfusion are also shown. The use of the DL-form of tryptophan labelled with carbon-14 introduces certain difficulties in interpreting the changes with time, particularly in the perfusion experiment involving physiological concentrations of tryptophan in the perfusion fluid. Evidence reported in the literature<sup>2</sup> indicates that D-tryptophan is metabolized at one half of the rate of L-tryptophan in liver slices. This finding may explain the slower rate of decrease in activity of carbon-14 as compared with that of activity of tritium in the perfusion carried out at physiological concentrations of tryptophan. This effect is not noticeable in the perfusion in conditions of loading, presumably because of the presence of massive amounts of L-tryptophan.

When tryptophan (indolyl-2-<sup>14</sup>C) is used, it is clear that all of the activity of carbon-14 is lost in the metabolic step in which *N*-formyl kynurenine is converted to kynurenine. Metabolic labilization of tritium in the tritium-labelled tryptophan used in these experiments can,

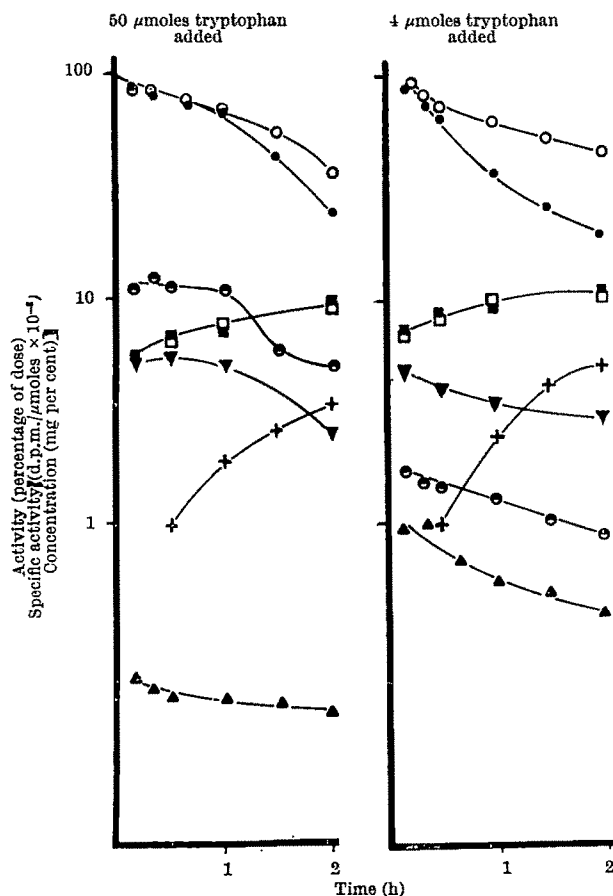


Fig. 1. Catabolism of 4 or 50  $\mu$ moles of tryptophan (mixture of tritiated benzene and <sup>14</sup>C-2-indolyltryptophan) in the isolated perfused liver. The curves for radioactivity of tryptophan decrease only slightly more rapidly than those for non-volatile tritium activity. ○, Carbon-14 in perfusate; ●, non-volatile tritium in perfusate; □, carbon-14 in liver; ■, non-volatile tritium in liver; +, tryptophan concentration in perfusate; ×, tryptophan concentration in liver; +, tritium in bile; ▲, tryptophan specific activity.

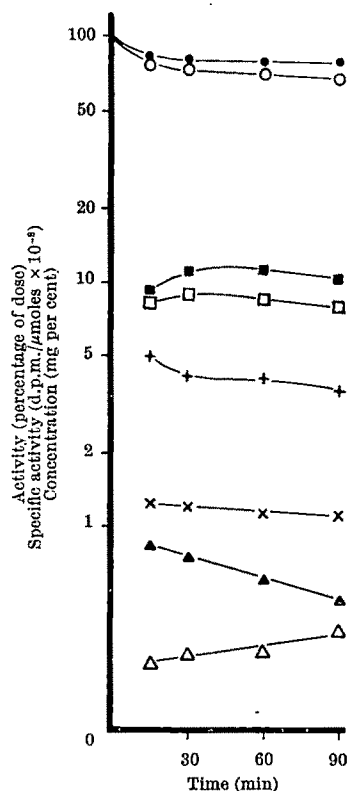


Fig. 2. Catabolism of 4  $\mu$ moles of tryptophan (mixture of tritiated benzene and <sup>14</sup>C-2-indolyltryptophan) in the isolated perfused intestine. There is no significant difference between values for carbon-14 and tritium and no volatile activity is found. ●, Total activity, and ○, tryptophan activity in perfusate; ■, total activity, and □, tryptophan activity in intestine; +, tryptophan concentration in intestine; ×, tryptophan concentration in perfusate; ▲, tryptophan specific activity in perfusate; △, tryptophan specific activity in intestine.

however, be expected during the formation and further degradation of 3-hydroxyanthranilic acid and should result in the formation of tritium oxide, thus enriching the body water with tritium. Catabolism of tryptophan occurs rapidly (Fig. 1), primarily by way of the kynurenine pathway, and results in the formation of compounds devoid of carbon-14. Only a small fraction (about 10 per cent), however, of the total activity of carbon-14 added to the perfusate is found in labelled carbon dioxide at the end of a 2 h perfusion. On the assumption that some of the activity of carbon-14 can enter the pool of the "one-carbon fragment", certain amino-acid residues in liver-protein isolated from perfused livers have been examined. It was found that about 20 per cent to 30 per cent of the total activity of carbon-14 was present in protein bound serine and alanine after 2 h of perfusion. On the other hand, chromatography of the acetone soluble fraction of blood and liver revealed the presence of only small quantities of radioactive substances other than tryptophan, even in conditions of tryptophan loading.

Fig. 1 shows that substantial quantities of tryptophan metabolites are excreted into the bile, but some of these metabolites are devoid of carbon-14 and contain only tritium. One of the main products of excretions in the bile has been tentatively identified as 3-hydroxyanthranilic acid. Initially, tryptophan disappears more slowly in conditions of tryptophan loading, but its catabolism increases as the perfusion progresses, probably as a result of the induction or activation of tryptophan pyrrolase<sup>6</sup> after the addition of the 50  $\mu$ moles of L-tryptophan.

The data presented in Fig. 2 demonstrate that the kynurenine pathway of tryptophan catabolism plays only a very small part in the intestine if this pathway exists at all in this organ. This conclusion is based on the finding that, in contrast to the finding in liver, there is

(a) no loss of carbon-14 and (b) no detectable formation of tritium oxide. The formation of at least one metabolite of tryptophan, however, was noted and it was tentatively identified as indole acetic acid.

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### Connective Tissue and the Pentose Phosphate Pathway in Normal and Denervated Muscle

DIRECT oxidation of glucose-6-phosphate through the pentose phosphate pathway is known to occur in various animal tissues. The enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH), which are involved in the first two steps of this pathway, have been found in many organs. The activity of these enzymes in skeletal muscle is exceptionally low compared with that of most other mammalian tissues studied<sup>1</sup>. Their activity does, however, increase in muscles undergoing atrophy. This increase appears to be relatively unspecific, as it has been observed in denervation atrophy, hereditary muscular dystrophy, muscle necrosis due to trauma or viral infection, and in myopathy induced by drugs<sup>2-4</sup>. We have studied the G6PDH and 6PGDH activity in normal and denervated soleus and gastrocnemius muscles of the rabbit. Our findings suggest that the increase in activity of these enzymes may be the result, not of specific changes in the muscle cell itself, but rather a reflexion of the increase in connective tissue.

Adult male albino rabbits underwent transection of one sciatic nerve. Their soleus and gastrocnemius muscles were removed 2, 4 and 8 weeks after denervation. The preparation of the specimens and the reference base used were the same as those indicated in a previous report<sup>5</sup>. G6PDH and 6PGDH were assayed according to Lowry *et al.*<sup>6</sup> except that the formation of NADPH was followed directly in a fluorometer. Collagen was estimated from the hydroxyproline content, measured by the method of Neuman and Logan<sup>7</sup> as modified by Martin and Axelrod<sup>8</sup>.

Fig. 1 shows the results obtained. Non-collagenous protein is used as reference base because it appears to be a good index of the functioning muscle mass<sup>5</sup>. Each point represents the mean value from six rabbits. All the differences between the controls and the denervated groups were significant at the level  $P=0.01$ . It can be seen that there are differences between both the enzyme and collagen content of the normal soleus and gastrocnemius. The enzyme activities and the amount of collagen per each unit of non-collagen protein were about three times higher for the soleus than for the gastrocnemius of unoperated animals. After denervation a gradual increase in these values occurred. After 8 weeks, all three values had risen about 2.5 times in the denervated soleus and 8.5 times in the gastrocnemius. Using the total fresh weight of each muscle as reference, an

increase in collagen could still be seen (Table 1). Thus, the elevation noted using protein as a reference base partly reflects an absolute increase in collagen. Similar results were obtained from an estimation of total enzyme activity. To rule out the loss of an inhibitor or the appearance of an activator, homogenates from control and denervated muscles were mixed in different proportions. The enzyme activity of the mixtures was that expected from simple addition.

Table 1. TOTAL COLLAGEN CONTENT OF THE MUSCLES STUDIED

Type of muscle	Control*	Denervated*		
		2 Weeks after denervation	4	8
Soleus	13.6 ± 0.8	13.3 ± 0.7	14.1 ± 1.5	17.2 ± 1.2
Gastrocnemius	51.2 ± 4.7	54.7 ± 5.1	59.8 ± 8.9	79.3 ± 9.1

\* Values ± standard errors of the mean expressed as mg collagen/entire muscle.  
†  $P < 0.05$ .

The differences found between normal soleus and gastrocnemius are not very surprising. These muscles are known to differ from one another not only functionally but also in their chemical composition<sup>9</sup>. The soleus is a tonic or slow muscle, mostly involved in postural regulation; the gastrocnemius is a phasic or fast one, mainly used in voluntary movements.

This parallelism between the activities of G6PDH and 6PGDH and the content of collagen is remarkable. It suggests that connective tissue, the source of collagen, is responsible for the presence of these enzymes in muscle homogenates. Thus the elevation of their activity observed in a variety of muscle disorders would reflect the increase of connective tissue in the affected muscles. Direct evidence for this would only be possible by histochemical methods. Localization of these enzymes using histochemical techniques is, however, difficult as they are soluble<sup>1</sup> and tend to diffuse rapidly. Supportive evidence of an indirect nature can be found in a study by Woessner and Boucek<sup>9</sup>. They measured the enzyme concentrations of rat connective tissues on implanted polyvinyl sponge. The activities of G6PDH and 6PGDH that they reported were twenty to forty times greater for this tissue than those found in rat skeletal muscle by numerous authors using a variety of assay methods<sup>1,10</sup>. A similar relationship may exist between connective tissue increase and the elevation of some hydrolytic enzymes in diseased muscle. Acid phosphatase and 5-nucleotidase, both high in sponge connective tissue<sup>9</sup>, are increased in homogenates of denervated and dystrophic muscles<sup>2,3,11</sup>. Both enzymes

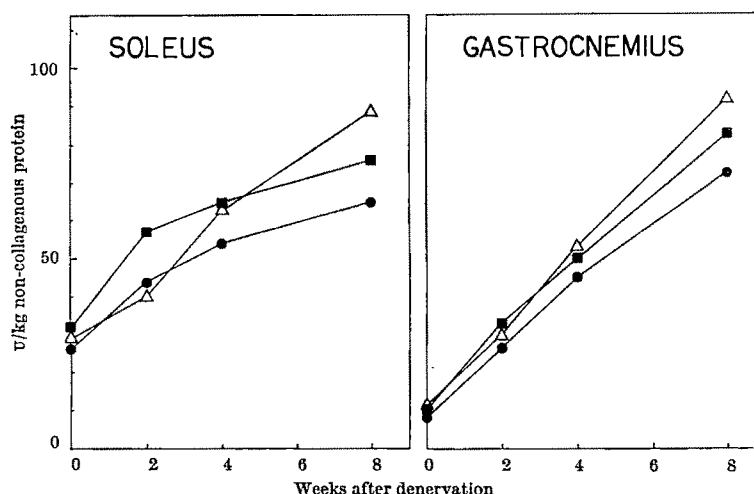


Fig. 1. Changes in enzyme activity and collagen content of soleus and gastrocnemius after denervation. G6PDH (■) and 6PGDH (●) activities are expressed in  $\mu\text{moles/h}$ , and collagen ( $\Delta$ ) in  $\text{grams} \times 2/3$  (the fraction is introduced as a factor to make the curves more readily comparable).

have been found mainly in the proliferating connective tissue of dystrophic muscle<sup>12,13</sup>.

If, as our results suggest, the activity of the pentose phosphate pathway in muscle homogenates arises mostly from the connective tissue present in these, the possibility exists that connective tissue might have a hitherto unreported role in regeneration by furnishing some metabolites to the muscle cell. Pentose sugars are produced in this pathway. There is a parallel between the activity of the pentose shunt and the synthesis of RNA in normal tissues<sup>14</sup>. Although the activity of the pathway is low in muscle homogenates, it has been estimated by Hiatt and Lareau to be sufficient to furnish the ribose required for RNA synthesis in normal muscle<sup>15</sup>. This synthesis is probably increased in the regenerative processes that follow necrosis of muscle tissue. In such conditions the proliferation of connective tissue might fulfil the increased need of muscle fibres for some metabolites. If this were so, the question would arise as to how such metabolites would reach these fibres from the connective tissue cells. Fibroblasts apparently can release portions of their cytoplasm into the intercellular spaces by a mechanism comparable with that of apocrine secretion<sup>16</sup>. It is conceivable that ribose-5-phosphate might reach the intercellular space in this way and then enter the muscle cell after having been split by the phosphatases which are abundant in connective tissue.

At present, there is active research into the quantitative biochemical changes occurring in diseased organs. An increase in the activity of G6PDH and of some lysosomal enzymes is a relatively common occurrence in pathological tissues. It is unfortunate that there are not better means of determining whether some of these changes represent intracellular alterations or simply reflect a variation in the proportion of several cell populations. In this respect, studies of tissue using implanted sponges in different species, combined with an evaluation of the degree of proliferation of connective tissue in the organs under observation, might be helpful in cases where histochemical techniques do not provide an answer.

The part played by connective tissue in removing cellular debris and as a supporting matrix is well recognized. In addition, connective tissues may play an active part in the repair and regenerative processes of organs by furnishing some of the needed materials.

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## Phenylketonuria and Vitamin B<sub>6</sub> Function

THE essential role of vitamin B<sub>6</sub> (used as a group name to include pyridoxine, pyridoxal and pyridoxamine) in maintaining the proper function of the central nervous system has been established<sup>1</sup>. Mental retardation, hyperirritability, and convulsive seizures are manifestations of deficient vitamin B<sub>6</sub> metabolism. The similarity of the clinical syndrome of phenylketonuria suggests some malfunction of vitamin B<sub>6</sub>. Results of our studies on experimental phenylketonuria in rats indicate that inhibition of B<sub>6</sub> function contributes to the pathogenesis of phenylketonuria.

Recently we reported that exogenous phenylalanine and pyridoxine are precursors of a new metabolite (designated A) found in phenylketonuric urine but not in normal urine<sup>2</sup>. Metabolite A has been characterized as pyridoxylidene phenylethylamine. The question arises whether this compound interferes in some basic way with vitamin B<sub>6</sub> metabolism or whether synthesis of excessive amounts of it creates a deficiency of vitamin B<sub>6</sub> in the brain, thus causing mental derangement. Attempts to distinguish between these two possible mechanisms are described in this communication.

Experiments were conducted to determine whether the retarding effect of phenylalanine on learning in the rat<sup>3-5</sup> is influenced by pyridoxine in the diet, whether a deficiency of B<sub>6</sub> impairs learning, and whether phenylalanine affects recovery from B<sub>6</sub> deficiency symptoms.

Weanling male rats of the Holtzman strain were freely fed one of two diets: A, which contained an excess of pyridoxine, or B, from which pyridoxine was omitted. Three weeks later the animals on diet A were separated into four groups; 1 and 2 served as controls while 3 and 4 received phenylalanine. Rats fed diet B were also divided into four sets. Group 5 served as a control, while group 6 received phenylalanine. Groups 7 and C were fed the B<sub>6</sub> antagonist deoxypyridoxine<sup>6</sup> (DOP). When deficiency symptoms appeared in group C, the inhibitor was removed from the diet and recovery was observed under four dietary regimens (8-11) (see Table 1).

Table 1  
Composition of diet

Diet A group	Pyridoxine (mcg/g)	L-Phenylalanine (mg/g)	Time fed (days)
1	30	0	42
2	30	0	28
	100	0	14
3	30	0	28
	100	70	14
4	30	50	28
	100	70	14
Diet B group			
5	0	0	42
6	0	50	28
		70	14
7	0	0	21
	0 + DOP (5 mcg/g)	0	11
	0 + DOP (2.5 mcg/g)	0	10
C	0 + DOP (5 mcg/g)	0	11
	0 + DOP (2.5 mcg/g)	0	10

At the end of 3 weeks, B<sub>6</sub> deficiency symptoms had appeared in group C. Treatment with DOP was ended and the animals were maintained on diets 8-11 (Table 2).

Table 2

Diet B group	Pyridoxine (mcg/g)	L-Phenylalanine (mg/g)	Time fed (days)
8	100	0	18
9	100	70	18
10	0	0	18
11	0	70	18

Learning capacity was measured by counting errors made in the six channel maze (Fig. 1) during the log-phase of the learning curve (Fig. 2). Results shown in Table 3 established the validity of the water maze test to measure rate of learning.

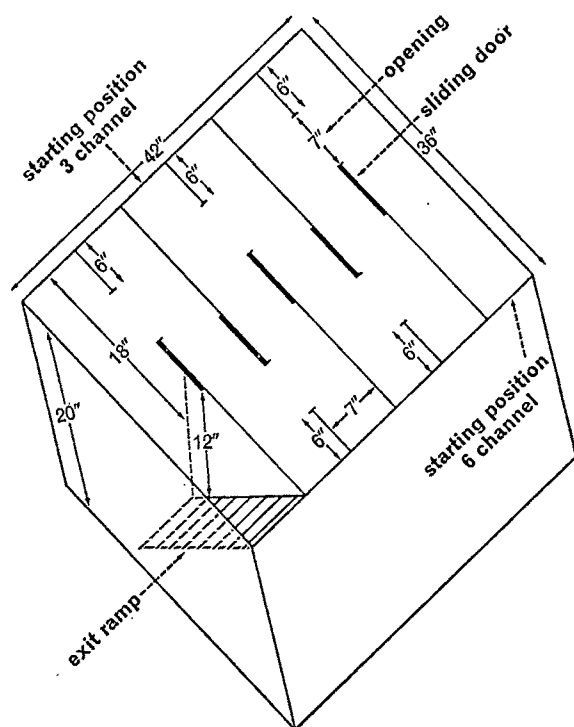


Fig. 1. Swimming maze of stainless steel is similar to that described by Pilgrim *et al.*<sup>14</sup>. The tank is filled with water up to the level of the ramp, and the temperature is maintained at 15° C. To accustom the animals to water and to acquaint them with the openings and the exit ramp in the maze and to enable the investigator to weed out the inherently poor learners, a training period preceded the testing period. Throughout the training and testing periods the animals were maintained on their respective diets. Rats, 9–10 weeks old, were trained in the maze with three open channels. Two weeks later they were tested in the maze with six open channels. Three trials per rat per day were executed on four consecutive days. A rest period of at least 20 min was allowed between trials. The animals were dried with a towel after each swim. Errors were counted during trials 4–12 and consisted of the number of times the rat missed an opening, entered a blind alley, or backtracked. To prevent overfatigue, animals that failed to reach the ramp within 3 min were assisted through the openings and the errors cumulated during this period were recorded. Animals that failed to master the three-channel maze in 10–12 trials during the training period were eliminated from the experiment as inherently poor learners.

Data were analysed statistically by the method of variance<sup>7</sup>. There was no significant difference between replicated experiments conducted with different lots of animals.

Table 3. ANALYSIS OF WATER MAZE TEST

Group	No. rats	Average body weight (g)	Mean errors ± standard error			Mean swimming times ± standard error Trials 10, 11, 12 (sec/trial)
			Trials 4, 5, 6	Trials 7, 8, 9	Trials 10, 11, 12	
1	10	275	8.9 ± 1.2	3.6 ± 1.0	2.1 ± 0.8	14.9 ± 1.9
4	9	265	15.2 ± 3.9	8.1 ± 1.8	3.0 ± 1.0	12.7 ± 1.1
5	10	193	14.8 ± 2.8	7.9 ± 2.2	4.3 ± 0.8	12.0 ± 0.7
6	9	199	13.1 ± 2.6	3.3 ± 1.1	2.0 ± 0.6	13.6 ± 0.4
7	14	189	24.5 ± 4.0	10.7 ± 1.6	4.2 ± 0.7	14.4 ± 1.1

There is no significant difference between any two groups of animals in either the number of errors made or in the time required to swim through the maze of six channels during trials 10–12. The differences observed in the rate of maze learning, as measured by the number of errors made during trials 7–9, is attributed to imposed dietary regimens.

Table 5. EFFECT OF L-PHENYLALANINE ON RECOVERY FROM VITAMIN B<sub>6</sub> DEFICIENCY

Group	Diet Py (mcg/g) PAS (mg/g)	No. rats	Learning test		Growth		Acrodynia, loss of fur		
			Mean errors ± standard error	P (groups compared)	Mean weight gain ± standard error g/day/rat	P (groups compared)	Recovered	No change (No. of rats)	More severe
8	100	13	3.3 ± 0.6	0.05–0.01 (8, 9)	4.6 ± 0.3	0.001 (8, 9)	13	0	0
9	100	20	6.8 ± 1.0	0.2 (9, 11)	1.9 ± 0.3		1	17	2
10	70	14	3.8 ± 0.8	0.05 (10, 11)	2.7 ± 0.2	0.001 (10, 11)	4	17	0
11	0	20	8.6 ± 1.6		1.2 ± 0.2		1	17	2

Py = pyridoxine HCl, PAS = L-phenylalanine supplement.

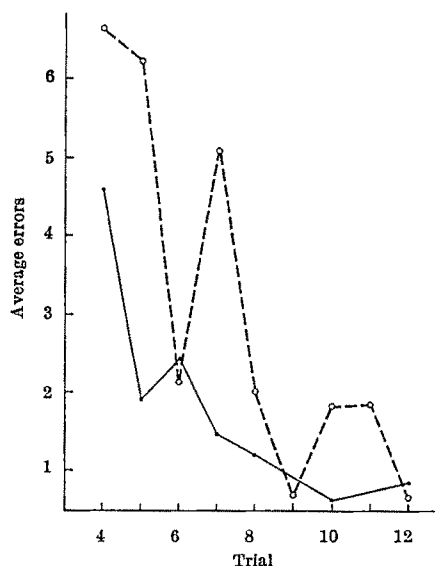


Fig. 2. Learning curves. Animals behaved very erratically during the first 5–6 trials. The total number of errors made during trials 7–9 served as a criterion of learning. ●—●, Group 1 (10♂) control diet; ○---○, group 2 (9♂) control + L-phenylalanine.

It is apparent from the results, summarized in Tables 4 and 5, that diets containing large amounts of phenylalanine and pyridoxine inhibit learning in rats. The data further suggested that deficient mental performance is caused not by lack of pyridoxine but rather by interference of pyridoxine metabolism.

A sub-optimal intake of pyridoxine (group 5) did not produce any decided effect on maze learning; neither did a supplement of phenylalanine added to a pyridoxine free diet (group 6). In contrast, a diet high in phenylalanine as well as pyridoxine (groups 3 and 4) not only caused a marked reduction in maze learning but also produced retarded growth, loss of fur and loss of balance.

Table 4. EFFECT OF DEOXYPYRIDOXINE AND OF L-PHENYLALANINE SUPPLEMENT ± PYRIDOXINE ON MAZE LEARNING

Group	No. rats	Composition of diet			Mean errors ± standard errors	Groups compared	P
		Py mcg/g	PAS mg/g	Weeks fed			
1	38	30	0	6	4.3 ± 0.7	1 and 4	0.01–0.001
4	19	30	50	4	8.6 ± 1.3	4 and 3	0.2
		100	70	2			
2	17	30	0	4	2.4 ± 1.0	2 and 3	<0.001
		100	0	2			
3	18	30	0	4	11.8 ± 2.0	3 and 6	0.01–0.001
		100	70	2			
5	20	0	0	6	6.2 ± 1.3	5 and 6	0.2
6	18	0	50	4	4.1 ± 1.0	4 and 6	0.01–0.001
		0	70	2			
7	17	0	0	3	10.7 ± 1.6	7 and 5	0.05
		+DOP				7 and 1	<0.001
						7 and 2	<0.001

All of the diets contained 32 per cent potato starch, 28 per cent sucrose, 5 per cent corn oil, 20 per cent vitamin-free casein (Borden), 4.4 per cent salt mixture (Hegsted), 9 per cent cellulose and adequate amounts of all of the vitamins<sup>15</sup> with the exception of pyridoxine, the amount of which was varied. The content of cellulose was correspondingly decreased in the diets that were supplemented with L-phenylalanine. Py = pyridoxine hydrochloride, PAS = L-phenylalanine supplement, DOP = deoxypyridoxine.

A few animals of groups 4 and 7 could not maintain balance in the water maze; these were eliminated from the experiment. Loss of fur was observed in a few animals of Group 4.

These effects were also observed in animals treated with the B<sub>6</sub> inhibitor deoxypyridoxine (group 7).

Retarded learning, poor growth, acrodynia and loss of fur were observed in animals fed deoxypyridoxine. Addition of pyridoxine to the diet (group 8) brought about full recovery in all the animals. When pyridoxine, however, was fed with phenylalanine (group 9) these B<sub>6</sub> deficiency symptoms<sup>8,9</sup> persisted in 95 per cent of the animals and became more severe in a few. Apparently the utilization of pyridoxine for recovery was prevented.

The experimental evidence suggests that in phenylketonuria vitamin B<sub>6</sub> function is impaired by an inhibitory substance formed from dietary phenylalanine and pyridoxine. The enzyme for the decarboxylation of phenylalanine to phenylethylamine<sup>10</sup> is present in brain tissue. Pyridoxal is found in normal brain<sup>11</sup>. Thus the reaction of phenylethylamine with pyridoxal to yield the Schiff base pyridoxylidene phenylethylamine may readily take place in the brain. This Schiff base has been detected in phenylketonuric urine but not in normal urine. Phenylketonuric patients are known to form abnormally large amounts of phenylethylamine<sup>12</sup>. Among the known products of phenylalanine metabolism formed in excessive amounts by phenylketonurics, phenylethylamine has the most potent effect on the central nervous system. This supports the suggestion that pyridoxylidene phenylethylamine may interfere with vitamin B<sub>6</sub> function and thus cause the mental derangement associated with phenylketonuria.

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### Phenylketonuria in Rats: a Model for Biochemical Studies

Polidora, Cunningham and Waisman<sup>1</sup> reported that induced phenylketonuria (PKU) in rats does not adequately simulate the irreversible intellectual impairments found in the phenylketonuric child, although they point out that there is somewhat general agreement in the literature that this technique provides an adequate model of PKU for biochemical studies. The results of our experiments on the metabolism of phenylalanine in normal and PKU rats suggest, however, that even from the biochemical point of view there is no adequate simulation of impairments in the phenylketonuric child.

Weanling male rats of the Wistar strain were made phenylketonuric by feeding a normal protein test diet containing 5 per cent L-phenylalanine for 1 week. This was followed by a diet containing 7 per cent L-phenylalanine<sup>2</sup>.

Our contention that these PKU rats do not provide an adequate biochemical model for the study of PKU is based on the following facts: (1) All the rats had very high concentrations of free tyrosine in the plasma. In most cases, soon after the initiation of the high phenylalanine diet the concentration of tyrosine in the plasma was much higher than that of phenylalanine. One week later all the rats had higher free tyrosine concentrations. (2) The rats all gave positive results when tested with ferric chloride and dinitrophenylhydrazine early in the experiment. These tests are indicative of high phenylpyruvic acid excretion. On the other hand, when the rats were maintained on the special diet for 3-4 weeks they no longer gave these positive tests although the plasma concentrations of phenylalanine and tyrosine were still high. Instead of the blue-green colour indicative of a positive ferric chloride test, a dark brown spot was obtained. The dinitrophenylhydrazine test showed small traces of a yellow precipitate but only after the mixture had stood for several hours at room temperature. (3) When the rats were taken off the high phenylalanine diet for only a few hours there were drastic changes in the plasma levels of phenylalanine and tyrosine. There were also alterations in the concentrations of other amino-acids in the plasma. After 2 weeks on the diet rich in phenylalanine, one rat had 0.90 mmoles phenylalanine/ml. and 1.50 mmoles tyrosine/ml. in the plasma. Fasting for 24 h lowered these values to 0.033 and 0.067 mmoles/ml. The rat was then fed the diet rich in phenylalanine for 5 days and the phenylalanine and tyrosine plasma concentrations were then 0.35 and 0.94 mmoles/ml. A 6-h fast lowered these values to 0.090 and 0.490 mmoles/ml.

Because of these changes in the amino-acid levels it was impossible to obtain reproducible results in the study of the metabolism of D,L-phenylalanine-3-<sup>14</sup>C unless the high phenylalanine diet was kept in the metabolic cage during the 24-h experimental period.

(4) In human phenylketonurics only 10 per cent of the ingested phenylalanine is converted to tyrosine while 85 per cent is excreted in the urine as phenylalanine, O-hydroxyphenylacetic acid, phenylpyruvic acid, phenyllactic acid and phenylacetic acid<sup>3,4</sup>. Our results show that 24 h after the injection of D,L-phenylalanine-3-<sup>14</sup>C, 26.0 per cent of the carbon-14 was found in the expired carbon dioxide, while only 7.4 per cent was excreted in the urine. The remaining carbon-14 was incorporated into tissue proteins.

(5) After the injection of radioactive phenylalanine, the proteins obtained from the plasma were hydrolysed and analysed. 2 h after injection 88 per cent of the carbon-14 incorporated into the protein could be accounted for by radioactive phenylalanine while 12 per cent was in the form of radioactive tyrosine. After 24 h these values were 80 and 20 per cent respectively. In normal rats 24 h after the same injection, 78 per cent of the radioactivity in the proteins was in the form of phenylalanine and 22 per cent in the form of tyrosine. In PKU patients Udenfriend and Bessman<sup>5</sup> have found that only about 1.5 per cent of the radioactivity of the plasma proteins comes from labelled tyrosine derived from injected radioactive phenylalanine.

(6) There is a considerable distortion of the pattern of distribution of the plasma amino-acids in phenylketonuria. Linneweh and Ehrlich<sup>6</sup> reported that plasma concentrations of lysine, threonine, serine, aspartic acid, alanine and tyrosine were significantly lower than normal. Another study<sup>6</sup>, reviewed in Table 1, showed that the concentrations of most of the plasma amino-acids of the PKU patient are lower than those of the normal individual. We have determined the free amino-acid concentration in the plasma of four rats which were maintained for 10 days on a diet rich in phenylalanine. The distribution of the amino-acids in the plasma of PKU rats is quite different from that in PKU patients (Table 1). There is a greater variation from normal in the amino-acid concentrations in the PKU rats; the levels of glutamic acid, glycine and



arginine are lower, while those of certain sulphur and basic amino-acids, as well as the aromatic amino-acids, were greatly increased. The values for isoleucine and leucine varied little, while glutamine is not present at all, although it is found in the plasma of normal rats.

It appears that the modification of amino-acid and protein metabolism in PKU rats is different from that found in human beings with PKU.

Table 1. PERCENTAGE VARIATION OF PLASMA AMINO-ACIDS IN PKU AND NORMAL HUMANS (A) AND PKU AND NORMAL RATS (B)

	A PKU patient (ref. 7) (per cent)	B PKU rats (per cent)
thr	-14.6	-28.2
ser	-6.6	-27.6
glu	-48.0	-71.6
pro	-41.0	-56.9
gly	-10.5	-72.1
ala	-20.0	-26.9
val	-38.4	-25.6
cys	-42.9	+40.0
met	-24.0	+34.6
ileu	-33.9	-9.4
leu	-32.6	-3.0
tyr	-68.8 (6)	+1,740.0
orn	-16.7	-48.0
lys	-13.5	+9.0
his	-31.5	+8.3
arg	-51.8	-77.7
phe	+180.0	+590.0
asp	—	-64.3

These findings indicate that induced PKU is a very temporary state in which the high plasma phenylalanine concentration can change rapidly with variations in the diet. The very high plasma tyrosine concentration suggests that the rats are in a state of tyrosyluria<sup>7</sup> rather than phenylketonuria. Further, most of the injected radioactive phenylalanine is still metabolized by way of the normal tyrosine pathway. All these observations differ from those reported in human beings with PKU.

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### Effects of Steroids on Chrysanthemum in relation to Growth and Flowering

THE physiology of flower initiation has been much investigated for more than half a century. It is now established that in conditions of inductive day length the leaves produce a hormone which induces differentiation in the apical meristem. The chemical nature of the hormone has not yet been definitely established. There have been many investigations of the effect of different organic compounds on plants in relation to flowering. The primary motive has been to look for compounds that could possess "florigenic" activity. Some workers have used various antimetabolites in an attempt to determine whether any of them block the synthesis of the floral stimulus, which could provide some indirect indications as to the nature of the hormone. Antimetabolite investigation has suggested that the flowering hormone may be a steroid or an isoprenoid-like compound<sup>1</sup>. Work on a strawberry variety has shown that unsaponifiable lipid fractions from flowering plants promote flowering in

the vegetative ones<sup>2</sup>. The active substance may include vitamin E and certain unidentified sterols. This communication reports the effects of steroids on flowering of chrysanthemum plants in non-inductive conditions.

Eleven common steroids of both plant and animal origin were selected at random (ranging from C<sub>19</sub> to C<sub>30</sub>). These were as follows: cholesterol (C<sub>27</sub>);  $\beta$ -sitosterol (C<sub>29</sub>); cholesteryl acetate (C<sub>29</sub>); lanosterol (C<sub>30</sub>);  $\Delta$ -5-3-acetoxy-bisnorcholeonic acid (C<sub>24</sub>); digitalin (C<sub>23</sub>); glycocholic acid (C<sub>26</sub>); androsterone (C<sub>19</sub>); androstane (C<sub>19</sub>); 21-acetoxypregnenolone acetate (C<sub>25</sub>); stigmasterol.

Concentrations of 100 and 500 p.p.m. of each steroid were applied as sprays to chrysanthemum plants. The steroids were dissolved in 5 ml. of peanut oil, mixed with water along with 5 ml. of 'Plyac', an emulsifying and surface active agent, and made up to volume with water.

Rooted cuttings of chrysanthemum, variety 'Princess Anne', were obtained from Yoder Brothers, Ohio. On receipt, the cuttings were planted in 4 in. earthen pots. Garden soil rich in organic matter was used as the growth medium. Two days after transplanting, the plants were transferred to a greenhouse bench and were exposed to a 22 h photoperiod. Hundred watt bulbs were spaced 4 ft. apart and 3 ft. above the plant surface, and provided a minimum light intensity of 150 ft.-candles<sup>3</sup>. The lights were automatically turned on at 6 p.m. every day and were turned off at 3 a.m. the following day. The greenhouse was kept moderately warm with a steam-heat unit. Plants were watered daily with tap water and once a week 50 ml. of a complete nutrient solution was applied to each plant. Plant heights were recorded once every month.

A randomized block design with two replications of five plants each was employed. Plants were selected for their uniformity and vigour. On November 9, 1964, when the average height of the plants was approximately 9 in., they were treated with the steroid solutions. Applications were made with hand spray guns, and the leaves were drenched with the solution. A second application of the steroid sprays was made after 1 week.

Fourteen weeks after the initial treatment, the plant tips were collected, and immediately immersed in a killing and fixing solution ('Craft III'). Slides were prepared from the shoot apex, then stained with safranin and fast green before floral initiation was evaluated<sup>4,5</sup>. Evaluations were made by comparing them with the slides made from chrysanthemum buds, collected at different stages of initiation. The results are presented in Table 1.

The results of steroid sprays in stem elongation are illustrated in Table 1. The differences between certain treatments may have appeared to be significant, but when analysed statistically the observation did not hold true, because of the wide variability present in the individuals within a treatment. The two concentrations of the steroids used also did not show any marked difference as far as stem elongation was concerned.

The effects of steroids on intact plants or on the growth of isolated plant parts have been investigated by many

Table 1. EFFECTS OF STEROID SPRAYS AT TWO CONCENTRATIONS ON STEM ELONGATION AND FLOWER BUD INITIATION IN CHRYSANTHEMUM PLANTS

Treatments	Plant heights in in.		Per cent of plants initiated <sup>a</sup>	
	100 p.p.m.	500 p.p.m.	100 p.p.m.	500 p.p.m.
Cholesterol	22.18	21.45	10	10
$\beta$ -Sitosterol	22.05	22.70	50	10
Cholesteryl acetate	23.80	23.15	20	None
Lanosterol	21.05	23.20	40	20
Stigmasterol	22.55	22.75	10	20
Digitalin	21.55	21.75	10	None
$\Delta$ -5-3-Acetoxybisnor- choleonic acid	22.00	22.18	10	10
Glycocholic acid	22.45	21.25	30	50
Androsterone	21.75	22.10	10	None
Androstane	22.20	23.70	10	10
21-Acetoxypregnenolone	22.75	22.15	30	None
Control	23.05	22.08	20	10

Results are average of ten test plants.

Heights were measured 6 weeks after the initial treatment.

Terminal buds were collected 14 weeks after the initial treatment and then dissected to evaluate floral response.

workers. Their work suggests that oestrone and saponins are active in the promotion of plant growth; and all other steroids of either plant or animal origin tested so far have been found to be either ineffective or slightly inhibitory in their action<sup>6</sup>. Results of this investigation are in agreement with previous findings.

From the data in Table 1 in relation to flower bud initiation, it can be seen that two steroids,  $\beta$ -sitosterol and lanosterol, at a concentration of 100 p.p.m., have resulted in flower initiation in 50 per cent and 40 per cent plants respectively. Only 10 per cent of plants were found to be initiated in each of the following treatments: cholesterol, stigmaterol, digitalin,  $\Delta$ -5-3-acetoxybisnorcholenic acid, androstosterone, and androstane. With the exception of glycocholic acid and 21-acetoxypregnenolone acetate, 30 per cent of plants were initiated; 20 per cent of plants were found to be initiated in the other two treatments, including the control.

When plants were treated with 500 p.p.m. of glycocholic acid, 50 per cent of plants set flower buds. Four other steroids at this concentration were effective, while lanosterol and stigmaterol have initiated buds in 20 per cent of the treated plants. Ten per cent of plants were found to have initiated flower buds in all other treatments, including the control.

Induction of flowering with sterols has not been reported before. Only Chouard<sup>7</sup> has demonstrated that application of oestradiol in short day conditions greatly enhances the onset of flowering in *Callistephus sinensis*. The present finding that  $\beta$ -sitosterol, lanosterol and glycocholic acid may in some way promote floral initiation needs to be investigated in some detail.

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### Inhibitory Effect of Salicylates and Cinchophen Derivatives on Amino-acid Incorporation

CINCHOPHEN is an ulcerogenic drug which depresses gastroduodenal mucus formation<sup>1</sup>. Salicylic acid also causes alimentary bleeding. It is possible that failure to secrete mucus for protection against peptic digestion of the gastroduodenal wall, in addition to chemical changes in the wall structure, is the ultimate cause of bleeding. We have therefore undertaken to investigate the effects of cinchophen, salicylic acid and their derivatives on protein synthesis.

There are discrepancies in published reports on the effect of salicylate on the cell-free incorporation of amino-acid into protein. According to Weiss *et al.*<sup>2</sup> salicylic acid stimulates the incorporation of labelled leucine into protein, whereas Dawkins *et al.*<sup>3</sup> reported an inhibitory effect.

Female rats aged 4-5 months fed *ad libitum* were killed by a blow on the head, bled and the liver excised and

homogenized immediately in three volumes of ice cold 0.25 molar sucrose buffered to pH 7.4 with 50 mmolar *tris* hydrochloric acid buffer containing 10 mmolar magnesium chloride and 50 mmolar potassium chloride. The homogenate was centrifuged at 15,000g for 10 min, and the precipitate discarded. The supernatant was used as such or treated either to isolate the ribosomes or to prepare the pH 5 fraction. The ribosomes were isolated as follows: 0.5 per cent of deoxycholate was added to a sample of supernatant which was stirred carefully for 2 min. It was then centrifuged at 130,000g for 1 h through layers of 15 and 20 per cent sucrose containing magnesium and potassium chlorides as the homogenizing mixture. This produced a precipitate of the ribosome preparation. The fraction at pH 5 was prepared as follows: the pH of another sample of the 15,000g supernatant was adjusted to 5.2 with an acetic acid-potassium acetate buffer, pH 5.0. The sample was centrifuged at 3,000g for 15 min and the precipitate washed and dissolved in sucrose-*tris* solution as before and used as the pH 5 fraction. All operations were carried out in either an ice bath, a cold room or a refrigerated centrifuge. In experiments with synthetic messengers (Tables 2 and 3) the mixture was preincubated for 15 min before the addition of polyuridylic acid on polyadenylic acid, amino-acid and inhibitor. In all experiments the final incorporation mixture was incubated in a water bath at 37° C for 30 min. The reaction was stopped by the addition of 1 ml. of 10 per cent TCA, the precipitate separated by centrifugation at 1,500g for 10 min, washed twice with 2 ml. of 5 per cent TCA, twice with ethanol containing 10 per cent sodium acetate and once with ethanol-ether 1:1. The washed precipitate was dissolved in 0.2 ml. of 1 molar hyamine hydroxide in methanol and its radioactivity determined in a liquid scintillation counter after mixing with 4 ml. of toluene-based POP-POPOP liquid scintillation mixture. The results were corrected for quenching by internal standardization. The inhibition was calculated as the percentage decrease of the standards without an inhibitor.

The strongest inhibition is shown by salicylaldehyde, decreasing somewhat in the case of salicylic acid, cinchophen and its 3-hydroxy derivative, and decreasing still more in the case of acetylsalicylic acid; whereas salicylamide and the other hydroxy derivatives of cin-

Table 1. INHIBITION OF LEUCINE INCORPORATION INTO THE 15,000g SUPERNATANT FRACTION OF RAT LIVER. THE RESULTS ARE EXPRESSED AS PER CENT DECREASE OF INCORPORATION COMPARED WITH UPTAKE IN AN INHIBITOR-FREE SYSTEM

Inhibitor concentration:	10 <sup>-3</sup> molar	
Cinchophen	20%	33%
3-Hydroxy cinchophen	37	36
6- " "	4	16
7- " "	4	6
8- " "	2	0
Salicylic acid	34	36
Acetylsalicylic acid	19	22
Salicylamide	0	5
Salicylaldehyde	71	65

Incubation mixture contained: 50 mmolar *tris*-hydrochloric acid buffer pH 7.4, 1 mmolar ATP, 10 mmolar creatine phosphate, 0.4 mmolar GTP, 50 mmolar potassium chloride, 10 mmolar magnesium chloride, 10 mmolar GSH, 0.2 ml. 15,000g supernatant of liver homogenate 1:3, 250 m $\mu$ C-leucine, 100  $\mu$ g other amino-acids and inhibitor in a total volume of 1 ml. Columns represent two independent experiments.

Table 2. PER CENT INHIBITION OF POLYURIDINE DIRECTED PHENYLALANINE INCORPORATION

Inhibitor concentration:	10 <sup>-3</sup> M	3 $\times$ 10 <sup>-3</sup>	
	per cent inhibition	per cent inhibition	
Cinchophen	37	41	55
3-Hydroxy cinchophen	45	42	52
6- " "	10	10	
7- " "	1	0	
8- " "	-2	6	
Salicylic acid	39	35	62
Acetylsalicylic acid	23	23	43
Salicylamide	0	2	28
Salicylaldehyde	71	77	96
Salicylic acid + DNA (5 mg)	47	37	
Salicylic acid + albumin (5 mg)	10	15	

The incubation mixture was the same as in Table 1 except that instead of the homogenate supernatant it contained pH 5 fraction (0.3 mg protein), ribosomes (1.4 mg protein) and polyuridylic acid 100  $\mu$ g. The only amino-acid added was phenylalanine labelled with carbon-14, 250 m $\mu$ C.

cinchophen investigated had almost no effect. The addition of extra protein to the incorporation mixture resulted in protection against inhibition (Table 2), whereas addition of DNA was without effect. Equilibrium dialysis showed that these drugs were bound to albumin but not to DNA or RNA. Thus the decreased inhibition indicated in Table 1 when compared with Tables 2 and 3 can be assumed to be the result of a lower free drug concentration caused by a higher protein concentration.

Our earlier experiments have shown that hydroxylation of cinchophen does not abolish its inhibitory action on aspartate: 2-oxoglutarate and alanine: 2-oxoglutarate transaminases<sup>4</sup> and uridine diphosphate glucose dehydrogenase *in vitro*. On the other hand, the hydroxy derivatives of cinchophen with the exception of 3-hydroxy cinchophen are not ulcerogenic in dogs *in vivo*, and this may be explained by their failure to inhibit protein synthesis.

Table 3. PER CENT INHIBITION OF POLYADENINE DIRECTED LYSINE INCORPORATION

Inhibitor concentration:	10 <sup>-3</sup> M		3 × 10 <sup>-3</sup> M	
	per cent inhibition		per cent inhibition	
Cinchophen	32	35	55	44
3-Hydroxy cinchophen	42	33	50	53
6- " "	12	11		
7- " "	7	8		
8- " "	16	4		
Salicylic acid	44	32	61	60
Acetylsalicylic acid	17	26	40	43
Salicylamide	5	2	25	20
Salicylaldehyde	70	71	98	97

Incubation mixture was the same as in Table 2 except that polyuridylic acid was substituted by polyadenylic acid and phenylalanine by lysine labelled with carbon-14, 250 mμ.

The results obtained are in good agreement with those reported by Dawkins *et al.*<sup>3</sup> in investigations of salicylic acid action.

A brief report of this investigation was presented earlier<sup>5</sup>.

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### Photochemical Degradation of Diquat

DIQUAT as the dibromide (I) is the active constituent of 'Reglone'\* herbicide, which is being used in a wide variety of weed control problems<sup>1</sup>. It has been shown<sup>2,3</sup> that the related bipyridylum herbicide 'Gramoxone\*', based on the paraquat cation, is subject to significant photochemical degradation in sunlight at certain times of the year; the present communication is concerned with the breakdown of diquat dibromide when exposed to sunlight, which has been studied in the course of a detailed examination of the fate of the chemical after application.

'Reglone' is used to control water weeds at concentrations of about 1 p.p.m. of ion in the water. Consequently,

\* Registered trade mark of Plant Protection, Ltd.

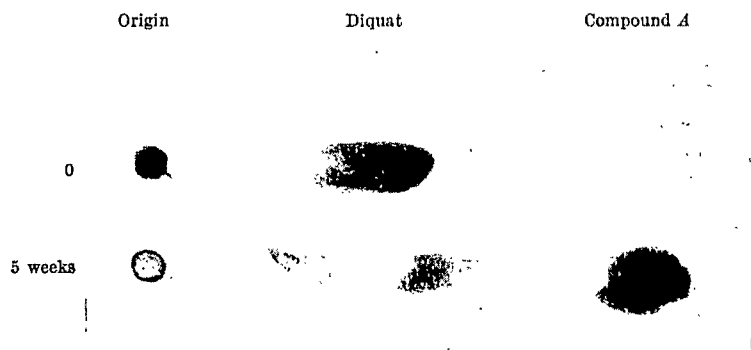


Fig. 1. Degradation of dilute aqueous <sup>14</sup>C-bridge-labelled diquat dibromide in sunlight. (Autoradiograph of paper chromatogram.)

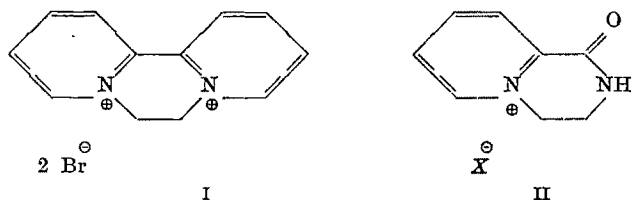
the first experiments on the photochemical degradation of diquat were undertaken using very dilute solutions of the chemical. A solution of 0.4 mg of <sup>14</sup>C-ethylene bridge-labelled diquat dibromide in 100 ml. of water contained in a crystallizing dish covered by a sheet of polythene 0.1 mm thick was exposed to sunlight during the summer for several weeks. (There is very little radiation in sunlight with a wavelength below 300 mμ and none below 290 mμ (ref. 4); it has been shown that polythene sheet 0.1 mm thick does not absorb any radiation above 290 mμ.) 0.5-Ml. portions of the irradiated solution were removed at intervals, concentrated and applied to the origin of a strip of Whatman No. 1 chromatography paper. The chromatogram was developed using a mixture of *n*-butanol, acetic acid and water (4 : 1 : 2) and placed in contact with X-ray film. The resulting autoradiograph showed that one principal radioactive decomposition product was formed (compound A in Fig. 1). Diquat, when present on the chromatogram, was revealed by means of potassium iodoplatinate solution<sup>6</sup>; the decomposition product quenched the induced fluorescence of the paper when the chromatogram was observed under ultra-violet light.

In order to study the photochemical degradation more conveniently, trial experiments were carried out by stirring 10–20 ml. of <sup>14</sup>C-diquat dibromide in 0.1 per cent (w/v) aqueous solution in a beaker and exposing them to ultra-violet light. When a 'Hanovia' Model 16 lamp, without a filter, was used as the light source, very rapid and complete degradation of the diquat occurred. Using either <sup>14</sup>C-ethylene bridge or <sup>14</sup>C-ring-labelled material, almost no radioactivity remained in solution within 24 h of the start of irradiation; apparently the diquat was degraded to volatile fragments. When the light from this lamp was filtered through a sheet of borosilicate glass 2 mm thick, however, it caused the same type of degradation of the chemical as occurred in sunlight. (Borosilicate glass effectively absorbs all radiation below about 300 mμ.) Compound A was the chief radioactive degradation product whether <sup>14</sup>C-bridge or <sup>14</sup>C-ring-labelled diquat was irradiated.

The reaction was then conducted on a larger scale using a 'Hanovia 10L' photochemical reactor with a borosilicate glass down-tube which contained a mercury vapour arc tube at medium pressure. Oxygen was passed at a rate of approximately 2 ml./sec through an irradiated solution of 15 mg <sup>14</sup>C-ring-labelled diquat dibromide in 1 l. of water, and 10-ml. portions were removed at intervals and their ultra-violet spectra examined. As the reaction proceeded, the light absorption of diquat (λ<sub>max</sub> = 310 mμ) decreased, and a new absorption band at 270 mμ appeared, due to the formation of compound A. The samples were evaporated to small bulk and applied to a paper chromatogram which showed on development that the degradation of diquat was similar to that which occurred in sunlight. The reaction was repeated using 10 l. of solution containing 200 mg of diquat dibromide to enable enough of the degradation product to be obtained to allow it to be

identified: this larger scale reaction took 14 days to complete. The resulting solution was percolated through a column of 'Dowex 50W-X8' cation-exchange resin in the hydrogen form, elution being effected with increasing specified strengths of aqueous formic acid and followed by measuring the ultra-violet absorption of the eluate at 270 m $\mu$ . Compound A was eluted with 40 per cent formic acid and removal of the solvent gave a yellow amorphous solid. Paper chromatography of this solid showed that several minor components were present, but compound A predominated. The crude solid was converted to the hydrochloride by treatment with saturated methanolic hydrogen chloride, and after removal of the excess reagent the material was recrystallized from methanol and ethyl acetate to give the chromatographically pure compound as a white amorphous solid whose melting point was greater than 300°C.

Compound A was radioactive whether it was prepared from  $^{14}\text{C}$ -ring-labelled or  $^{14}\text{C}$ -bridge-labelled diquat and therefore contained at least one intact ring and at least one of the bridge carbon atoms. The reaction with potassium iodoplatinate to give a black precipitate indicated the presence of a quaternary nitrogen atom. Elemental analysis, together with infra-red, ultra-violet, nuclear magnetic resonance, and mass spectral data of the compound, were consistent with the tetrahydro-oxo-pyridopyrazinium structure (II,  $X = \text{Cl}$ ).

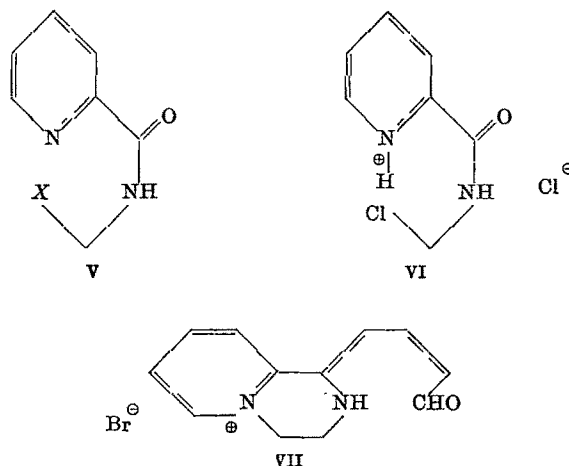


The nuclear magnetic resonance spectrum showed a complex multiplet between 0.8 and 1.8  $\tau$  assignable to the signals from the four aromatic protons. Two triplets at 4.92 and 5.96  $\tau$  ( $J = 7$  c/s in both instances) are attributed to the  $\geq \text{N}-\text{CH}_2-$  and the  $-\text{CH}_2-\text{NH}-$  proton resonances respectively, this type of splitting being typical of an  $A_2B_2$  proton system. The mass spectrum of the pure solid supported the structural assignment. A peak at  $m/e$  149 corresponds to the mass number of the ion derived from II ( $\text{C}_8\text{H}_8\text{N}_2\text{O}$ ), while a peak observed at  $m/e$  120 suggests loss of a  $-\text{CH}_2-\text{NH}-$  fragment. Further peaks at  $m/e$  106 and  $m/e$  78 can be attributed to the ions (III) and (IV) respectively.



The structure was finally confirmed by synthesis. The condensation product (V;  $X = \text{OH}$ ) prepared by heating ethyl picolinate with ethanolamine was treated with thionyl chloride to give the chlorethylamine hydrochloride (VI). The free base (V;  $X = \text{Cl}$ ) obtained by exact neutralization of the amide hydrochloride (VI) with aqueous sodium hydroxide was heated, in the absence of solvent, when ring closure took place smoothly to give 1,2,3,4-tetrahydro-1-oxo-pyrido [1,2,  $\alpha$ ]-5-pyrazinium chloride (II;  $X = \text{Cl}$ ) in excellent yield.

The synthetic compound and compound A had superimposable infra-red spectra, and their chromatographic properties were identical.



Compound II is interesting in that it has a unique ring structure and its formation from diquat presumably involves an intermediate such as (VII) which then undergoes oxidation.

Compound II is also formed when diquat adsorbed on filter paper and silica gel is exposed to sunlight. There is an interesting contrast between the photochemical behaviour of diquat and paraquat. The latter, with maximum ultra-violet light absorption at 257 m $\mu$ , is not degraded in solution by sunlight. When it is adsorbed on a surface, the maximum light absorption moves to a higher wavelength (Talbot, P. J., personal communication) so that sufficient radiation from sunlight above 290 m $\mu$  is absorbed to lead to some degradation of paraquat. Diquat, with its light absorption maximum at 310 m $\mu$ , is able to absorb sufficient radiation from sunlight to be degraded even in solution.

In addition, diquat is degraded photochemically after it has been applied to plants (metabolic degradation of the chemical in plants does not occur<sup>1</sup>). Unlike the photochemical degradation of paraquat on plants, that of diquat is very extensive, and very little of the latter remains on plants after exposure to sunlight for a week or so in summer. Compound II seems to be formed, but it is unlikely that this material, or any others formed in the photochemical degradation, will be translocated into harvested parts of the plants, because the degradation takes place on plants largely after they are dead. (It has been shown<sup>2</sup> that no translocation of the photochemical degradation products of paraquat occurs from dead leaves.) Furthermore, compound II ( $X = \text{Cl}$ ) has a very low oral toxicity to mammals. Details of the work on the degradation of diquat on plants will be published elsewhere: work is continuing on the structural elucidation of the minor photochemical breakdown products.

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### Reversibility of a Penicillamine Induced Defect in Collagen Aggregation

WE have recently reported that the administration of penicillamine ( $\beta,\beta$ -dimethylcysteine) results in a marked accumulation of soluble collagen in the skin of rats<sup>1</sup>. Ultra-centrifugal analysis of this thermally denatured material indicated that it consists almost exclusively of strands of  $\alpha$ -collagen<sup>2</sup>. The pattern obtained appeared to be identical to that observed after the administration of lathyrogenic compounds such as  $\beta$ -aminopropionitrile<sup>3</sup>. In addition to the changes in solubility, animals treated with penicillamine also manifested a decrease in the tensile strength in the intact skin and in healing wounds<sup>1</sup>.

These observations prompted us to investigate the relationship between the duration of penicillamine administration to skin changes in: *a*, soluble collagen; *b*, insoluble collagen; and *c*, tensile strength, as well as in the rate and degree of reversibility of these changes on withdrawal of the drug.

DL-Penicillamine was fed to young male rats (60–70 g) of the Holtzman strain. The drug was incorporated into the diet at 0.25 per cent concentration. The dose was calculated on the average of food intake of the rats and is stated in Figs. 1 and 2. Pyridoxine was also added to the diet at a concentration of 100 mg/kg of diet, inasmuch as Wilson and du Vigneaud found that DL-penicillamine produced a pyridoxine deficiency, presumably by the formation of a thiazolidine complex<sup>4</sup>. This degree of supplementation has been shown to overcome the inhibitory effects of DL-penicillamine on growth. D-Penicillamine was used in recent experiments in which it showed the same properties as the DL isomer. The D form is more satisfactory because of its decreased toxicity and lesser interaction with pyridoxine. Initial values were obtained by killing eight rats at the start of the experiment. The animals were then placed on penicillamine and groups of four or five rats were killed after 2, 3, 5, 6, 10 and 14 days. At this time the remaining animals were kept on the same basal diet with penicillamine removed. Skin specimens were analysed for collagen content and its solubility properties as previously described<sup>5</sup>. Only those fractions soluble in 0.5 molar sodium chloride and insoluble are reported in this communication, as they reflect the most significant and pertinent changes. In addition, a strip of skin (5 mm  $\times$  50 mm) cut from the dorsal area of the animal and parallel to the longitudinal axis was cut and used to measure the tensile strength in an 'Instron' machine. The force in grams required to rupture the skin is used as an index of tensile strength. The changes in the concentration of the 0.5 molar sodium chloride collagen fraction and insoluble collagen of skin during and after administration of penicillamine are shown in Fig. 1. After the first day of treatment a significant accumulation of soluble collagen occurred without

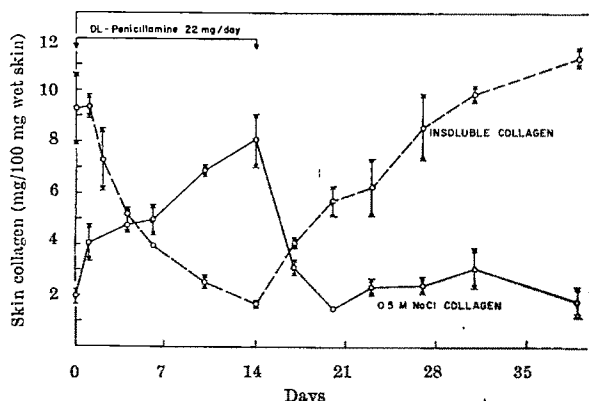


Fig. 1. Changes in collagen soluble in 0.5 molar sodium chloride and insoluble collagen of young rats treated with DL-penicillamine during 14 days and their reversal on discontinuing treatment.

any appreciable change in the amount of insoluble material present. After the first day, however, the amount of 0.5 molar sodium chloride collagen increased rapidly while the amount of insoluble material began to fall rather precipitously. Fig. 1 also demonstrates the rapid reversibility of the changes induced by penicillamine after withdrawal of the drug. The concentration of soluble collagen dropped quite rapidly with a concomitant rise in the insoluble collagen indicating that the process of aggregation was being re-established. There is a clear correlation between insoluble collagen concentration and tensile strength (Fig. 2). This was found to be the case in relating variations of insoluble collagen with age and tensile strength of rabbit skin<sup>6</sup>. Nevertheless, the slopes of the curves are different, probably because the insoluble collagen material is not a homogeneous substance but is composed of materials of different degrees of aggregation and inertness. It is reasonable to suppose that penicillamine causes a depletion of the more recently synthesized and consequently less cross-linked collagen. Inasmuch as the tensile strength of skin is primarily a manifestation of a more inert and cross-linked fraction of collagen, this parameter would not be expected to change as rapidly as the total insoluble collagen which includes both old and newly synthesized fibres. A similar assumption can be made to explain the reversal of these changes which occur when treatment is discontinued. The rate of increase of chemically determined insoluble collagen is greater than the simultaneous gain in tensile strength. It is interesting to note that a very marked and rapid increase in tensile strength occurred at the end of the second week of recovery. These changes show an overall similarity to the pattern we have observed in investigations of the changes of tensile strength of collagen in normal rabbit skin during the early stages of ageing<sup>6</sup>.

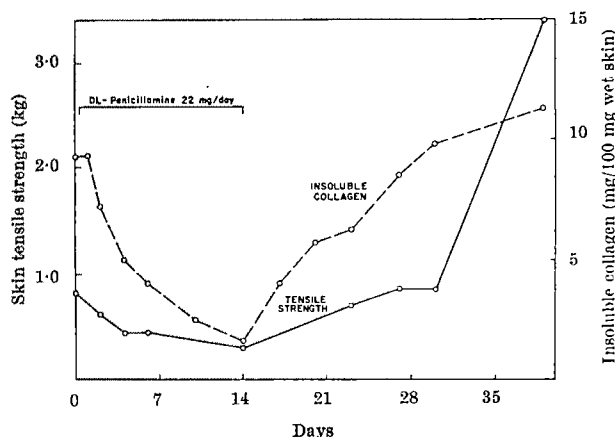


Fig. 2. Comparison of the insoluble collagen present in skin with its tensile strength during and following penicillamine treatment.

Our findings indicate that penicillamine definitely inhibits the aggregation of the soluble tropocollagen molecules to form insoluble fibrous material. The depletion of insoluble collagen may be explained by its rapid turnover in animals of this age. Consequently, the collagen which is broken down in this process is not replaced because of the blockage of aggregation brought about by penicillamine. Preliminary evaluation of our results supports this assumption, but a more detailed and quantitative analysis based on rates of turnover, syntheses and changes in pool size is necessary in order to ascertain the mechanism involved.

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### Double-disc Electrophoresis of Proteins

GEL electrophoresis in discontinuous buffer systems<sup>1</sup> is a valuable method of examining dilute protein solutions. By selecting pH conditions it is possible to concentrate and then separate either cationic or anionic proteins, but only in separate analyses. Thus, the *tris*-glycine system can be used, in which the running pH is 9.5 and most proteins migrate as anions<sup>1</sup>. The usual cationic proteins will either not migrate or will move backwards as a large zone equal in size to the origin. Conversely, an acidic system, such as  $\beta$ -alanine-acetate, may be chosen in which cationic proteins migrate at pH 4.0 (ref. 2), but in this case the anionic proteins are lost to further analysis. Apart from the loss of time and research material necessitated by separate analyses, there is an unavoidable ambiguity about the direction of migration of a particular protein, because it is quite possible for the same protein to migrate as an anion at pH 9.5 and as a cation at pH 4.0. Clearly, a single system allowing the simultaneous separation of oppositely charged proteins would be desirable. To this end the method described couples two discontinuous buffer systems which provide concentrating and running conditions for both cationic and anionic proteins in the same sample.

The apparatus consisted of two separate gel tubes joined by a 3 cm section of clear plastic hose pierced with a small opening for the introduction of liquid sample. One gel tube contained a weak acid (taurine,  $pK_a$  8.7) and its potassium salt, while the other contained a weak base (imidazole,  $pK_a$  7.0) and its chloride. The sample was photo-polymerized within the plastic hose and completed the ionic connexion between the gel tubes. In principle, the doubly discontinuous system should behave in the manner described by Ornstein<sup>3</sup>. That is, there would be an initial stacking of ions in the order of fast inorganic ions followed by slower charged proteins followed by imidazole<sup>+</sup> or taurine<sup>-</sup>. The stacking of ions towards opposite ends of the sample gel would produce sharp zones of cationic and anionic protein. Finally, these would be over-run by imidazole<sup>+</sup> or taurine<sup>-</sup> when the ion stack reached the running gel and the proteins would begin to separate on the basis of size and charge.

The gel and buffer formulations were as follows. (a) Cation running gel buffer: 1.2 ml. 1 molar potassium hydroxide, 0.75 g taurine, 25  $\mu$ l. tetramethylethylenediamine, made up with water to 10 ml. This system had an original pH of 8.1 and a running pH of 7.5. (b) Anion running gel buffer: 1.2 ml. 1 normal hydrochloric acid, 0.50 g imidazole, 50  $\mu$ l. tetramethylethylenediamine, made up with water to 10 ml., giving an original pH of 7.7 and a running pH of 8.3.

Running gels were formed by mixing two volumes of one of these buffer systems with two volumes of a solution of 20 g acrylamide, 0.8 g *N,N'*-methylbisacrylamide, 7.5 mg potassium ferricyanide, and water to 100 ml. with four volumes of 0.14 per cent ammonium persulphate

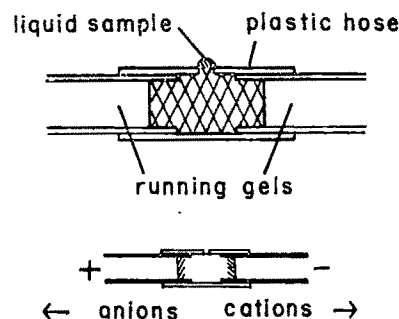


Fig. 1. Double-disc electrophoresis apparatus. Top, side view in cross-section showing the sample before polymerization. Bottom, same view during electrophoretic stacking of the sample anions and cations (shaded lines).

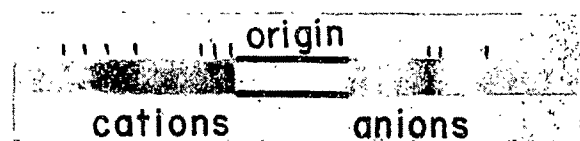


Fig. 2. Bean leaf peroxidases separated by double-disc electrophoresis. The photograph is a composite to show the original running position of the gel tubes. Peroxidase zones, some of which photograph poorly, are marked by short, vertical lines.

in water. The mixture was pipetted rapidly into glass tubes 55 mm long and 8 mm in internal diameter to within 1/8 in. of the top and layered carefully with water. Polymerization occurred in about 10 min as indicated by the formation of a refractile zone below the initial layer.

The sample gel (c) contained 200  $\mu$ l. of water containing enough safranin O and bromphenol blue to produce visible zones during electrophoresis; it also contained 150  $\mu$ l. of 1 per cent tetramethylethylenediamine in water, 150  $\mu$ l. of a solution containing 4 mg of riboflavin in 100 ml. of water, 150  $\mu$ l. of a solution of 40 per cent acrylamide and 10 per cent *N,N'*-methylenebisacrylamide in water, and 1.75 ml. of the sample in 0.025 molar phosphate at pH 7.0.

The components were quickly mixed and injected into the sample chambers, care being taken not to entrain air bubbles. In the present instance, 1 ml. of the mixture provided sufficient sample for a single analysis which was usually run in duplicate. The running gel tubes were pushed into the sample chamber so as to exclude air and to force a drop of sample up through the chamber opening (Fig. 1). A piece of plastic film was slid over the drop to seal off air and the chambers were exposed to bright fluorescent light until polymerization produced an opalescent gel. Alternatively, the sample chamber was closed by sliding in one of the gel tubes so as to cover the opening.

The units were connected vertically between two electrode boxes which were then filled with the following solutions: negative electrode box, 0.038 molar taurine and 0.0038 molar potassium hydroxide; positive electrode box, 0.038 molar imidazole and 0.0038 molar hydrochloric acid.

A typical run required about 2 h at 100 V, at which time the cationic (safranin O) and anionic (bromphenol blue) marker dyes had migrated 4 to 5 cm into their respective tubes. The gels were removed by the usual rimming technique<sup>1</sup> and stained for enzyme activity or protein. The analysis of bean leaf protein revealed three anionic peroxidases and seven cationic peroxidases using guaiacol and hydrogen peroxide as chromogenic substrates (Fig. 2).

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## IMMUNOLOGY

**Serological Differentiation of *Pythium aphanidermatum* from *Phytophthora parasitica* var. *nicotianae* and *Ph. parasitica***

THIS report describes the use of serological techniques to distinguish two morphologically similar pathogenic fungi in soil. *Pythium aphanidermatum* (Edson) Fitzp. causes damping-off in many plants, including the tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.) plants<sup>1</sup>. Recently Dukes *et al.* reported that this fungus also causes dark brown to black stem cankers on tomato seedlings grown in fields in South Georgia<sup>2</sup>. *Phytophthora parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker [= *Phytophthora nicotianae* var. *nicotianae*<sup>3</sup>] causes black shank of tobacco in this same area of South Georgia<sup>4</sup>, and has been reported to cause damping-off of tobacco seedlings, as well as a disease of eggplant, tomato and other plants<sup>5</sup>. It is difficult to differentiate quickly between these two indigenous soil-borne fungi in the vegetative state, whether in culture or in host tissues. To identify these pathogens in host tissue when reproductive structures are lacking, it is necessary to isolate and grow them in culture on media suitable for production of asexual bodies. Some isolates of both species may produce reproductive structures very slowly or not at all<sup>6,7</sup>. Proper identification of these species thus may require considerable time and effort, especially by workers not familiar with this group of fungi.

It was therefore decided to apply serological techniques towards developing a rapid method of distinguishing these species. In addition, it was decided to compare serological properties of *Ph. parasitica* [= *Ph. nicotianae* var. *parasitica*<sup>3</sup>] with those of *Ph. parasitica* var. *nicotianae*, because the only apparent difference between these two fungi is the ability of the latter to cause black shank of tobacco<sup>8</sup>. Recently Haasis reported that isolates of *Phytophthora* from several hosts other than tobacco caused a disease on a susceptible tobacco variety<sup>9</sup>. Whether these isolates caused typical black shank symptoms, however, is not known.

Previous serological work with pythiaceae fungi includes that of Burrell *et al.*<sup>10</sup>, who found that species-specific sera were efficient for the identification of *Ph. cactorum* (Leb. & Cohn) Schroet., *Ph. cinnamomi* Rands and *Ph. erythroseptica* Pethyb. Antiserum of *Ph. cactorum* cross-absorbed with *Ph. erythroseptica* did not react with antigens of several other fungi, including *Ph. parasitica* and three *Pythium* species.

To prepare the antisera, the three fungi were grown for 1 week at 28° C on a rotary shaker in potato glucose broth which consisted of the filtered juice from 200 g of fresh boiled potatoes, 20 g glucose, and distilled water to make up to 1,000 ml. Mycelia were then separated on a fine mesh nylon screen, washed twice with physiological saline, homogenized for 2 min in a blender and separated by centrifugation. They were resuspended in physiological saline, disintegrated in an ice bath for 10 min with a 'Branson S-75 Sonifier' at 6 amp, preserved with 1:10,000 'Thimersol', and kept at 4° C during the inoculation series. At first the preparations were injected intravenously into rabbits; however, 100 per cent mortality resulted with *Ph. parasitica* var. *nicotianae* inoculations. A modified injection schedule was therefore used as follows for each preparation: intramuscular injections of 1 ml. fungus suspension, plus 1 ml. 4 per cent sodium alginate adjuvant on the first and tenth day; intraperitoneal injections of 1, 2, 3, 4 and 5 ml. on the first, fourth, seventh, tenth and eighteenth days, respectively; and exsanguination on the twenty-eighth day. The antisera were frozen without preservatives.

Fungal materials for absorption studies were similarly prepared as used for the injection series except that no preservative was included. Antisera were absorbed by

mixing, intermittently, 2 ml. of absorbent with 2 ml. of antisera for 2 h at 37° C, and then clarifying antisera by centrifugation. Control antisera were treated similarly except that physiological saline was used instead of absorbent. Resulting preparations of antisera were tested in immuno agar diffusion plates against suspensions of disintegrated mycelia; reactant wells were 10 mm apart. Also, attempts were made to detect *Py. aphanidermatum* mycelium serologically in tomato stem lesions by homogenizing diseased tissue from several plants in physiological saline, filtering out coarse debris with a cheese cloth, concentrating the material by centrifugation, disintegrating the material by ultrasonification and testing the material against *Py. aphanidermatum* antiserum in immuno agar diffusion plates.

The results showed that *Py. aphanidermatum* antiserum was serologically specific; it was therefore unnecessary to absorb it. Undiluted *Ph. parasitica* var. *nicotianae* antiserum produced a faint band against *Py. aphanidermatum*, which was not evident after the antiserum was diluted 1:1 with physiological saline and incubated as an absorption control. The antiserum caused two sharp bands against each *Phytophthora* variety, but absorption with either variety removed all detectable antibodies. *Ph. parasitica* antiserum was serologically distinct from *Py. aphanidermatum* but was otherwise similar to *Ph. parasitica* var. *nicotianae* antiserum.

No precipitin lines formed when *Pythium*-infected tomato stems were tested against *Pythium* antiserum.

It is concluded that *Py. aphanidermatum* can be readily distinguished serologically from either *Ph. parasitica* var. *nicotianae* or *Ph. parasitica*, and that the two varieties of *Ph. parasitica* are serologically very similar. It was not possible to detect *Py. aphanidermatum* in infected tomato stems using immuno agar diffusion methods; however, suspected fungi could be isolated and then identified serologically. Also, the specificity of *Py. aphanidermatum* antiserum suggests that immunofluorescent techniques could effectively detect the species in mounts of infected tissue.

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### Development of Immunological Memory during the Primary Immune Response

THE existence of immunological memory has been classically recognized by the appearance of a "secondary" type of antibody response to antigenic challenge of a previously immunized animal. Whereas the kinetics of the early phases of antibody production have been intensively investigated<sup>1</sup>, little is known about the development of immunological memory during the early stages of

the immune response. Lymphoid cells from animals previously stimulated with an antigen can respond to the same antigen in tissue culture with antibody production<sup>2</sup>, cellular proliferation<sup>3,4</sup> and increased synthesis of DNA<sup>5</sup>. Available evidence indicates that this *in vitro* response can be taken as evidence for the presence of specific immunological memory<sup>6</sup>. We have investigated the development of this capacity by measuring the incorporation of tritiated thymidine into the DNA of lymphoid cells from immunized rabbits on re-exposure to the antigen *in vitro*.

Albino rabbits were immunized with a single injection of 3-6 mg of keyhole limpet haemocyanin (KLH), either intravenously or in divided doses, into the hind foot pads. Cell suspensions of spleen and popliteal lymph nodes obtained at different intervals after immunization were cultured in minimum essential medium and 15 per cent pooled normal rabbit serum. Tritiated thymidine (<sup>3</sup>H-thymidine) was added after 24 h and cultures were terminated at the end of 3 days. Buffy coat cells of the peripheral blood, obtained after sedimentation in 6 per cent dextran (molecular weight 250,000), were cultured for 4 days, and tritiated thymidine was added on the second day of culture. Cultures without antigen and with varying amounts of KLH (0.0008-0.2 mg/ml.) were set up in triplicate. After culture, cells were collected, frozen and thawed, and extracted successively with 0.3 molar cold trichloroacetic acid, 44 per cent dimethylsulphoxide and twice with cold absolute ethanol. Samples were then dissolved in 0.1 normal sodium hydroxide and radioactivity was determined by liquid-gel scintillation. Absolute counts incorporated in cultures without antigen varied markedly from rabbit to rabbit, and so results have been expressed as the ratio of counts with antigen to counts without antigen. An increase of twofold or more was considered significant. Variation between duplicate cultures was usually less than 20 per cent.

Three days after intravenous administration of KLH, no detectable response was elicited in any of the cultures (Fig. 1). After the fourth day, however, a significant

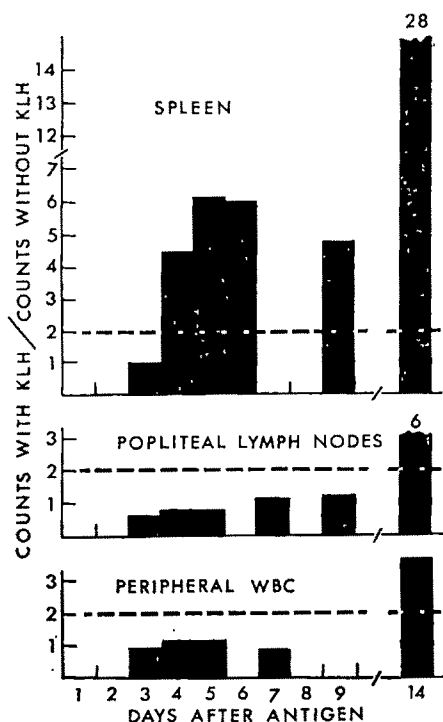


Fig. 1. Proliferative response of cell cultures from spleen, popliteal lymph nodes and buffy coat after primary immunization with keyhole limpet haemocyanin (KLH) intravenously. Response expressed as: counts of tritiated thymidine incorporated into DNA with antigen/tritiated thymidine counts incorporated into DNA without antigen.

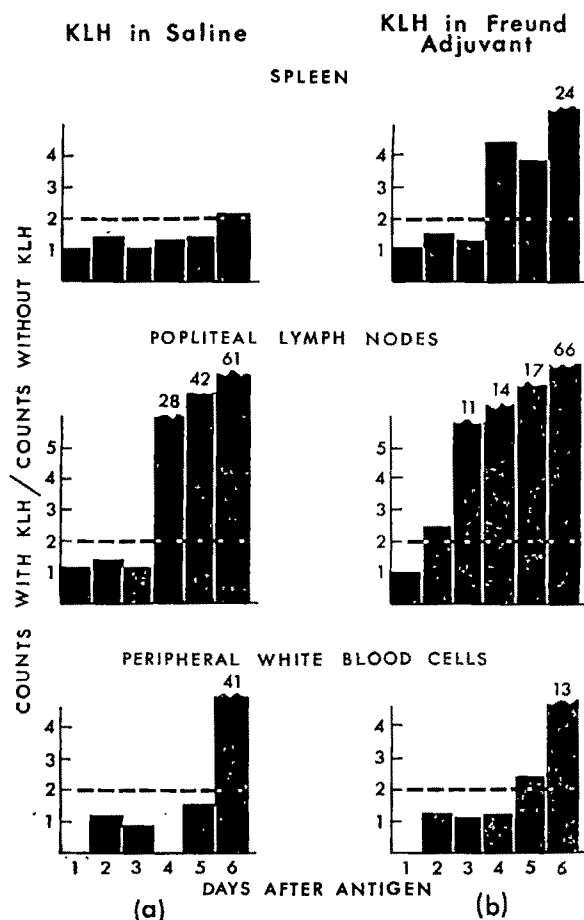


Fig. 2. Proliferative response of cell cultures from spleen, popliteal lymph nodes and buffy coat after primary immunization with KLH into the hind foot pads. Response expressed as: counts of tritiated thymidine incorporated into DNA with antigen/tritiated thymidine counts incorporated into DNA without antigen. (a) KLH in saline, and (b) KLH in complete Freund adjuvant.

response was regularly obtained in the spleen cell suspensions. As can be seen in Fig. 1, the capacity for this reaction persisted in the spleen, but appeared in the lymph node and peripheral white blood cells only after 2 weeks. Results of some typical experiments are presented in Table 1. On the twelfth day (Table 1), lymph node and blood cells had still not responded. On the fourteenth day there was a response. Control cultures consisting of either non-immunized rabbits or rabbits immunized with other antigens such as bovine serum albumin, brucella melitensis, or egg albumin did not show a response.

When KLH in saline was given into the hind foot pads, the initial response appeared in the regional popliteal lymph nodes (Fig. 2a). The onset of responsiveness occurred on the fourth day after administration of antigen. Two days later, a similar response was detected in the spleen and in the blood. When KLH was given into the foot pads in complete Freund adjuvant, a response (Fig. 2b) was noted as early as the second day in cells from the local lymph nodes. In this case, a response in the spleen cell cultures was observed on the fourth day and in the peripheral blood cells on the fifth day.

Proliferation of lymphoid cells has been shown to be an early feature of the immune response, which begins almost immediately after either the primary or secondary administration of antigen<sup>6</sup>. Proliferation of lymphocytes *in vitro* has also been produced by phytohaemagglutinin and other non-specific agents<sup>3,4,7</sup>, but it appears from our experience and that of previous workers<sup>5</sup> that the response of lymphocytes to antigen is specific and occurs only after previous exposure to the same antigen *in vivo*. Cells from

Table 1. INCORPORATION OF TRITIATED THYMIDINE INTO LYMPHOID AND PERIPHERAL WHITE BLOOD CELLS IN TISSUE CULTURE AT VARIOUS TIMES AFTER PRIMARY INTRAVENOUS IMMUNIZATION

Days after antigen	c.p.m. tritiated thymidine* Without antigen	With antigen	Counts with antigen Counts without antigen
	Spleen		
3	669	627	0.94
4	1,043	2,611	2.50
5	698	3,450	4.94
9	1,025	4,734	4.62
12	667	10,664	15.98
14	177	4,964	28.05
	Lymph node		
3	1,555	1,585	1.02
4	429	539	1.26
5	235	259	1.10
9	153	190	1.24
12	194	146	0.75
14	757	4,188	5.53
	Peripheral blood		
3	124	118	0.95
4	1,124	1,331	1.18
5	178	155	0.88
9	237	215	0.91
12	90	115	1.28
14	81	251	3.54

\* Values given represent the means of total counts of triplicate cultures from individual rabbits.

non-immunized animals did not respond to antigen, and so it seems reasonable to assume that the detection of proliferation in the experiments here reported can be taken as evidence of a secondary immune response and therefore of immunological memory.

Responsive cells were detected a few days after the administration of antigen in the present experiments. The nature of the events during the latent period which lead to the relatively abrupt emergence of cells capable of a specific immune response on re-exposure to antigen is not known. It is likely that after initial exposure to antigen, primarily stimulated cells undergo a number of mitoses to establish a population of immunologically competent cells which are committed to this antigen. Within this population, presumably, some cells differentiate fully to become antibody producing cells while others early acquire the capacity to respond only after re-exposure to the specific antigen. After removal from the animal and placement in tissue culture, the latter cells apparently continue in a resting stage in the absence of added antigen. It is possible that *in vivo* restimulation of such early appearing memory bearing cells by available antigen is involved in the normal development of the primary response. If this were so, a response of essentially a secondary type would form an integral part of the primary immune response. It is possible, therefore, that the cellular events described are related to the development from the early 19S or IgM antibody to the later 7S IgG variety<sup>1,8,9</sup>, especially because predominantly 7S antibodies are produced in the secondary response following most antigens<sup>1</sup>.

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## Effect of the Number of Antigens on the Quantity of Cells producing Several Kinds of Antibody

DATA concerning the quantity of cells which synthesize two kinds of antibody simultaneously are contradictory<sup>1-3</sup>. The kinetics of such cells has not been extensively investigated. Accordingly, we have attempted to investigate the effect of the number of antigens used for immunization on the quantity of cells synthesizing more than one kind of antibody. For this purpose, several groups of Wistar rats were given single subcutaneous injections, in both hind foot pads, of endotoxins from *Salmonella cerro*, *S. aberdeen* and *Shigella flexneri* in different combinations. The dose of each of the antigens was 5 µg/injection. The animals were killed 5 days after the injection of antigen and cell suspensions prepared from the tissues of their popliteal lymph nodes<sup>4</sup> were assayed for antibody producing cells. The assays were carried out by techniques of motile bacteria adherence<sup>5</sup> and indirect haemadsorption<sup>6</sup>. Test antigens utilized for the first procedure were live cultures of active motile *S. cerro* or *S. aberdeen* bacteria and those used for the second procedure were small round sheep erythrocytes or large dog erythrocytes sensitized by specific antigens. In control experiments, designed to verify the specificity of the chosen tests, the cells of the animals immunized with only one antigen were checked by all three test antigens. To rule out the possibility of passive "induction" of adherence reactions, cells of intact animals treated with serum or saline extracts of lymph nodes of immunized rats have also been tested. The results of the control experiments have shown that the techniques used are of adequate specificity.

Four groups of rats, eighteen or nineteen animals each, were used in most of the experiments (Table 1).

Table 1. ANTIBODY PRODUCTION BY SINGLE CELLS OF ANIMALS IMMUNIZED WITH *S. cerro*, *S. aberdeen* AND *Sh. flexneri* ANTIGENS

Complex antigens used for immunization	No. of cells tested	No. of cells synthesizing antibody to the antigens:			
		<i>Sh. flexneri</i>	<i>S. cerro</i>	<i>S. aberdeen</i>	Two antigens
<i>S. cerro</i>	49,023	—	287	576	6
<i>S. aberdeen</i>			0.6 per cent 0.03	1.2 per cent 0.05	0.7 per cent† 0.29
<i>S. cerro</i>	82,270	300	414	—	5
<i>Sh. flexneri</i>		0.4 per cent* 0.02	0.5 per cent 0.02	—	0.7 per cent 0.31
<i>S. aberdeen</i>	56,084	258	—	522	6
<i>Sh. flexneri</i>		0.5 per cent 0.03	—	0.9 0.04	0.7 per cent 0.29
<i>S. cerro</i>	45,168	180	233	362	18
<i>S. aberdeen</i>		0.4 per cent 0.03	0.5 per cent 0.03	0.8 per cent 0.04	2.2 per cent 0.52
<i>Sh. flexneri</i>					

\* Percentage of active cells out of cells tested.

† Percentage of double producers out of active cells.

On the basis of the available data it seems logical to draw the following conclusions. Only a small fraction of the population participates in the production of antibodies; this agrees well with the evidence of different investigators<sup>7-10</sup>. When animals are immunized with two antigens, most of the cells (99.3 per cent), in accordance with Nossal's experiments<sup>1</sup>, are found to synthesize only one kind of antibody and not more than 0.7 per cent of cells is synthesizing both kinds of antibody simultaneously. Animals immunized with three antigens, however, show a marked increase in the quantity of cells with "double activity" (2.2 per cent). These differences are statistically significant ( $P < 0.01$ ).

To eliminate the possibility that double producers are the result of methodological errors or artefacts, the cells isolated from animals immunized with different antigens were mixed. All three test antigens were added to the test mixture. Examination of 22,239 cells of this "mixed control" did not show any cell to which red blood cells and bacteria or red blood cells of two different types would attach simultaneously. Cells synthesizing three kinds of antibody have not been demonstrated. It is noteworthy that the quantity of cells producing antibody

to any of the antigens was roughly identical for all the groups tested. Thus, for example, 0.4 per cent of antibody producers to *Sh. flexneri* was found among the cells isolated from the animals immunized with *S. cerro* and *Sh. flexneri* antigens. The same quantity of active cells was detected when examining the cells of the animals immunized with three antigens.

Comparable results were obtained for determination of the quantity of cells producing antibody to *S. cerro* antigen (0.6 per cent, 0.5 per cent and 0.5 per cent). Significant differences ( $P < 0.01$ ) were observed only for the *S. aberdeen* antigen. In other words, the response to each of the antigens seems to have an independent course. This observation has been confirmed by investigations of antibody content in blood serum.

In conclusion, the available results seem to indicate that lymphoid cells are predetermined to the reaction with a specific antigen or, more likely, with a group of antigens. This conclusion is supported by the following findings: (a) production of antibody of one kind by the majority of active cells; (b) correlation between the quantity of double producers and the number of antigens injected; and (c) relative independence of the responses to each of the antigens injected.

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## HAEMATOLOGY

### Stimulation by Leukaemic Sera of Colony Formation in Solid Agar Cultures by Proliferation of Mouse Bone Marrow Cells

Two papers have recently described techniques for stimulating cells from mouse haemopoietic tissue to proliferate *in vitro* in solid agar to form cell colonies. Pluznik and Sachs<sup>1,2</sup> used feeder layers of mouse embryonic cells or cell free extracts of embryonic tissues to stimulate colony formation by spleen cells, while Bradley and Metcalf<sup>3</sup> described similar colony formation by bone marrow cells when neonatal mouse kidney cells were used as a feeder layer. In the latter work the colonies were found to be aggregates of myelopoietic cells in various stages of differentiation, mixed with mononuclear cells containing varying amounts of metachromatic cytoplasmic granules.

Recently, we have found that serum from *AKR* mice with spontaneous lymphoid leukaemia is effective in stimulating colony formation by mouse bone marrow cells. Based on this observation, a survey was carried out on sera from individual normal and leukaemic *AKR* mice and on sera from other mouse strains to determine the frequency of sera with colony-stimulating activity. Single cell suspensions of bone marrow cells from pools of 2-3, two months old *C57BL* mice were prepared in equal volumes of double strength Eagle's minimal essential medium supplemented with foetal calf serum and 0.6 per cent agar in a concentration of 50,000 nucleated cells per ml. of nutrient agar medium. Volumes of 0.1 and 0.05 ml. of serum were placed in glass or plastic Petri dishes (diameter 35 mm) and immediately afterwards 1 ml. portions of the cell suspensions in nutrient agar, con-

taining 50,000 nucleated bone marrow cells, were added to each dish and allowed to gel. The dishes were then incubated at 37° C in a humid incubator gassed with 5 per cent carbon dioxide in air. Control cultures were prepared with no added mouse serum.

In cultures with added leukaemic serum, micro-colonies were visible at  $\times 40$  magnifications after two days of incubation and the colony size rapidly increased up to the sixth day of incubation, forming macro-colonies containing approximately 200 cells, after which colony growth appeared to proceed more slowly, reaching an average size of 600 cells by the tenth day. Colony counts were performed as a routine after 10 days of incubation. In addition to the macro-colonies, numerous micro-colonies were seen on all stimulated plates, but these were not counted. In control cultures, micro-colonies were observed early, but the majority of these failed to increase in size and many disintegrated as culture proceeded. Only occasionally were one or two macro-colonies observed to develop in control cultures and an arbitrary number of five macro-colonies with the 0.1 ml. serum dose was chosen as the minimum number of colonies required to be present to indicate stimulation of colony formation by added serum.

Tests of leukaemic and normal sera were run under double blind conditions, and the results obtained from these tests are shown in Table 1. Of 71 leukaemic *AKR* sera tested, 64 (90 per cent) were found to stimulate colony formation, and the average number of colonies formed with the 0.1 ml. dose was  $24 \pm 20$  ( $\pm S.D.$ ). With the 0.05 ml. dose of serum, the mean number of colonies stimulated by the active leukaemic sera was  $7 \pm 7$ . Of 30 sera from normal two months old *AKR* mice, 10 (33 per cent) were found to exhibit activity and stimulated the development of a similar number of colonies to that stimulated by leukaemic serum. Nine of 17 (53 per cent) *AKR* mice aged 7-9 months and showing preleukaemic morphological changes in the thymus were found to stimulate colony formation. In a group of 16 sera from two months old *AKR* mice carrying transplanted thigh tumours derived from primary leukaemias in four *AKR* mice, 13 (82 per cent) were found to exhibit colony stimulating activity.

Tests were also made on sera from *C3H* and *C57BL* mice. Of 42 sera from *C3H* mice aged 2-18 months, only two sera (5 per cent) exhibited colony stimulating activity. Of some interest was the fact that 16 sera from *C3H* mice with breast, lung or liver tumours were without colony stimulating activity. Of 28 sera from *C57BL* mice aged 2-20 months, 4 (14 per cent) were found to stimulate colony formation.

Table 1. ANALYSIS OF SERA FROM LEUKAEMIC AND OTHER MICE FOR COLONY STIMULATING ACTIVITY ON *C57BL* BONE MARROW CELLS

Strain	Type of mouse	Age in months	No. of mice tested	No. of sera	
				Positive	Negative
<i>AKR</i>	Normal	2	30	10 (33 per cent)	20
	Preleukaemic	7-9	17	9 (53 per cent)	8
	Leukaemic	7-12	71	64 (90 per cent)	7
<i>AKR</i>	Transplanted leukaemia	2	16	13 (82 per cent)	3
<i>C3H</i>	Normal	2-18	42	2 (5 per cent)	40
	Mammary tumours	11-15	10	0	10
	Miscellaneous tumours	12-18	6	0	6
<i>C57BL</i>	Normal	2-20	28	4 (14 per cent)	24

A detailed cytological analysis has been made of colonies stimulated by *AKR* leukaemic serum and will be reported elsewhere. In brief, the earliest colonies sampled (4-12 cells) at two days were composed mainly or entirely of large cells with ring-shaped or horseshoe-shaped nuclei, together with smaller ring or horseshoe-shaped nuclear cells and some polymorphs. Most of these cells were without metachromatic granules. From the third day, colonies also contained large mononuclear cells with a single round nucleus of mean diameter larger than  $10\mu$ .



and an irregular cytoplasmic content of metachromatic granules. After the fifth day an abrupt change occurred in the colonies which sharply differentiated them from the bone marrow cell colonies developing on cell feeder layers<sup>3</sup>. All polymorph cells and cells with ring-shaped or horseshoe-shaped nuclei disappeared and the colonies remained as pure populations of large and small mononuclear cells. The small mononuclear cells had round nuclei of mean diameter 4–5 $\mu$ , often in an eccentric location, and the cytoplasm was filled with a varying number of metachromatic granules.

From the fourth day the mononuclear cells began to develop cytoplasmic vacuolation and this vacuolation became more marked as the colonies aged. By the tenth day, when colony cell number ranged from 400 to 1,000 cells, many small mononuclear cells exhibited pyknotic nuclei or completely lacked a visible nucleus. Cellular disintegration became more marked when colonies were incubated for more than 10 days. Only the large cells with ring-shaped nuclei and the large mononuclear cells appeared to exhibit mitotic activity and the mitotic indices of both cell types were about 4 per cent between the second and sixth days of culture. Mitotic activity declined between the seventh and tenth days of incubation.

The present data suggest that a bone marrow colony stimulating factor is present in the serum of most *AKR* mice with spontaneous or transplanted lymphoid leukaemia. This may well not be an abnormal factor since a similar factor appears to be present in the sera of some normal *AKR* mice and less frequently in the sera of mice of other strains. Leukaemic sera, however, appear to contain more of this factor than does normal serum or serum from mice with other types of tumours. The myelopoietic nature of the early colony cell population is of interest since leukaemic *AKR* mice invariably show excessive myelopoiesis as evidenced by elevated levels of polymorphs in the blood, with a normal time sequence for labelling of blood polymorphs following tritiated thymidine labelling (Metcalf, unpublished data). The present serum factor may be responsible in part for this increased myelopoietic activity in leukaemic mice. In view of the development ultimately in the colonies of a pure population of mononuclear cells having some features of mast cells, however, the serum factor may not be simply a myelopoietic factor but a growth stimulant for a number of haemopoietic cell types.

Further studies are in progress to establish the precise nature of the cells in the bone marrow colonies, and to determine the chemical nature of the active factor in leukaemic serum.

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## CYTOLOGY

### Morphological Changes in the Parafollicular Cells of the Rat Thyroid Glands after Administration of Calcium shown by Electron Microscopy

THE parafollicular cells named by Nonidez<sup>1</sup> are the second type of glandular cells which make up the mammalian thyroid glands. They occur along the basal (interstitial) surface of the follicular epithelium, and never face the

colloid-containing follicular lumen. Electron microscopy has shown that this cell type differs completely in ultrastructure from the follicular cells<sup>2,3</sup>. Such a morphologically distinct cell type has been assumed to have a function distinct from that of the follicular cell, that is, the production of iodine-containing thyroid hormones.

A factor which lowers serum-calcium and which is called calcitonin or thyrocalcitonin is known to be in the second group of hormones secreted from the thyroid gland<sup>4,5</sup>. A morphological investigation with the light microscope suggested that this new found hormone could be secreted from the so-called mitochondrion-rich cells of the thyroid, but the identity of these cells as the parafollicular cells could not be established<sup>6</sup>. In order to clarify a possible relationship between the parafollicular cell and the hormone concerned with calcium metabolism, we tried electron microscopy of the thyroid glands of normal and calcium-injected rats. We also looked at the thyroids of hypophysectomized rats.

In a preliminary experiment, the influence of an intravenous injection of calcium solution on the serum calcium level was observed. Calcium chloride solution which contains 10 mg of calcium ion/ml. was injected in an amount of 1.0 ml/kg of body weight into the femoral vein of each experimental rat, under slight anaesthesia with ether. Blood samples from control and experimental animals were collected and the total calcium concentration was measured by means of a flame-spectrophotometer. Changes in total serum calcium content after the injection are shown in Fig. 1. It may be noted that the concentration of calcium in the serum which immediately rose, as a result of the infusion of exogenous calcium, soon afterwards decreased gradually and had a minimum value which was far less than the normal concentration 4 h after the injection.

The parafollicular cells in the osmium-fixed preparations have already been characterized by the presence of many clear vesicles which probably contain less dense secretory substances and could be transitional from the dark secretory granules exclusively found in the Golgi area<sup>2,3</sup>. We found that the secretory vesicles increase in electron density and appear as more or less dense granules after fixation with glutaraldehyde followed by osmium tetroxide (Fig. 2). This method of preparation is effective for the examination of the functional state of parafollicular cells by electron microscopy, because the secretory granules become easily distinguishable from vesicles of the other types, such as the Golgi vesicles, the vesicular elements of the endoplasmic reticulum, and pinocytotic vesicles.

The secretory granules of parafollicular cells diminished in number after injection of calcium and reached a minimum number 4 h after the injection (Fig. 3). The

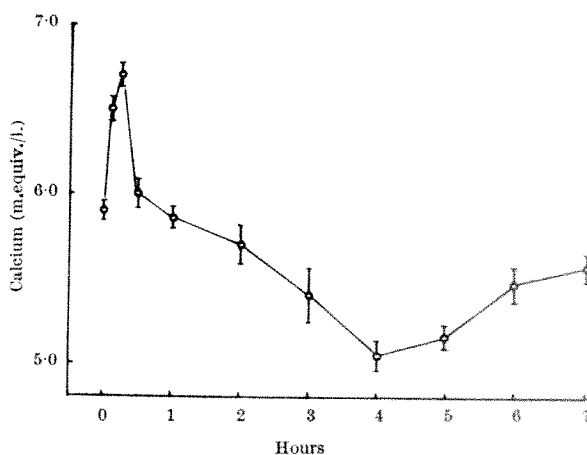


Fig. 1. Fluctuation of total serum concentration of calcium in the rats after intravenous injection of calcium chloride solution.

degree of degranulation varies considerably from cell to cell, but at this stage almost empty cells containing only two or three granules appear in a given section of the cell. Simultaneous observation of the thyroids of hypophysectomized rats indicated no morphological changes in the parafollicular cells, although the follicular cells showed a high degree of atrophy.

In Fig. 4 are histograms showing the distribution of density of the secretory granules of the parafollicular cells in each group of experiments. The population density of granules was indicated by the number of granules in a square micron of the cytoplasmic area, which was measured on fifty cells for each group by means of a planimeter on the electron micrographs magnified 10,000 times. The mean population density of normal parafollicular cells as well as those of hypophysectomized rats is about  $4.3/\mu^2$ , but it shifts to 3.4 at 2 h and 2.1 at 4 h after injection of calcium. This result indicates clearly the degranulation of parafollicular cells as a specific effect of the acute exogenous hypercalcaemia.

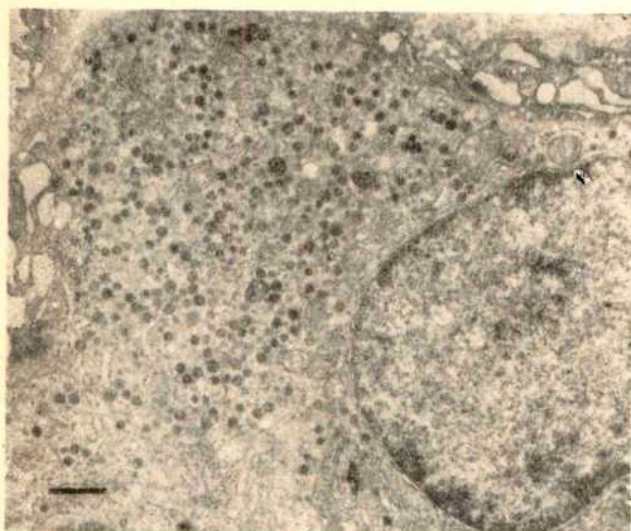


Fig. 2. A parafollicular cell of the normal rat thyroid fixed with glutaraldehyde followed by osmium tetroxide. A large number of dense secretory granules are contained in the cytoplasm. ( $\times 7,500$ .)



Fig. 3. A parafollicular cell of the rat 4 h after injection of calcium. The preparation procedure is the same as that shown in the above picture. Only a few secretory granules are contained, and the cytoplasm appears clear. ( $\times 7,500$ .)

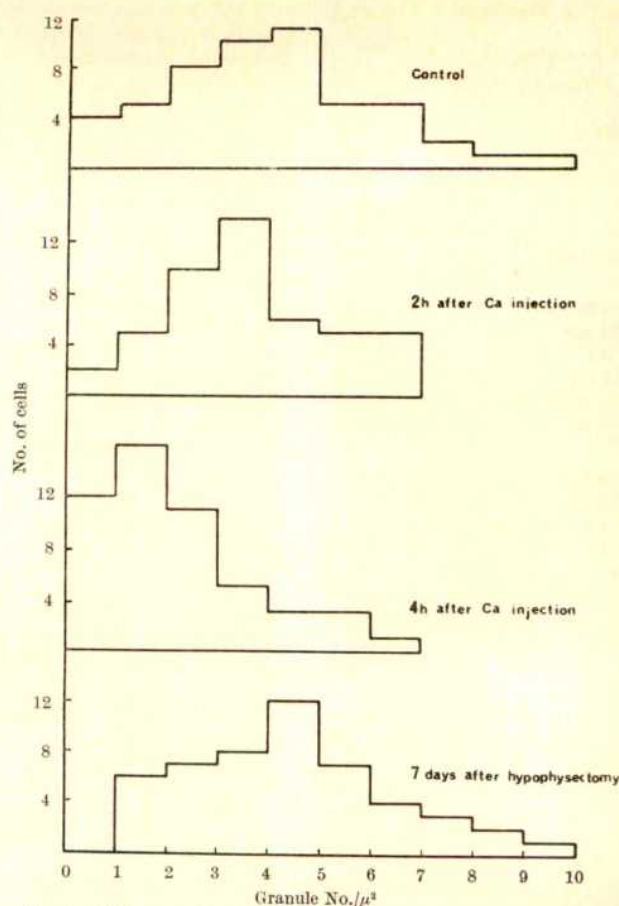


Fig. 4. Histograms showing the distribution of population density of secretory granules of the parafollicular cell in each experimental group: normal control, 2 and 4 h after calcium injection, and 7 days after hypophysectomy.

This experiment suggests that the parafollicular cells are the most intimately concerned with the function of the thyroid gland which lowers the serum concentration of calcium in response to the acute administration of calcium into the blood stream. It is possible that the second thyroid hormone, that is, calcitonin or thyrocalcitonin, may be produced from the parafollicular cell.

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### Pattern of DNA Replication of the Sex Chromosomes in Three Males, Two with XXX and One with XXYY Karyotype

INVESTIGATIONS of the pattern of DNA replication have shown that the X chromosomes in normal female cells exhibit marked asynchrony and one of them has a pronounced pattern of late replication. Other chromosomes differ also with respect to the time of DNA synthesis. In male cells it has been found that the Y chromosome replicates late compared with chromosomes of the 21-22 group<sup>1,2</sup>. This relatively late replication pattern of the



Table 1. MEAN GRAIN COUNTS ON Y AND CHROMOSOMES OF 21-22 GROUP

Individual	Mean Y	Mean of 21-22 group	Late replicating X chromosome
XY (A)	8.8	3.2	—
XY (B)	10.0	2.2	—
XXYY	8.2	1.4	22
XY (A)	9.1	1.0	—
XY (B)	8.2	2.0	—

Y chromosome has also been found in other mammals<sup>3-5</sup>. Investigations in patients with additional X chromosomes have shown that all X chromosomes in excess of one have pronounced late replicating patterns, similar to that of one of the X chromosomes in normal female cells<sup>6-10</sup>. As far as we know there have been only two previous references to the replication pattern of cells with two Y chromosomes. The presence of two heavily labelled presumptive Y chromosomes in an XY male was quoted as a personal communication from Castilla, Breg and Miller, in the review by Miller<sup>11</sup>. Kikuchi and Sandberg<sup>12</sup> studied replication patterns in two males with XY karyotype.

The two XY males were clinically normal on general physical examination, but above average in height; the XY male was mentally retarded and had small testes. Peripheral blood leucocyte cultures were made from these three patients by a modification of the method of Moorhead *et al.* Labelling was carried out by adding tritiated thymidine (specific activity 4.4 c./mmole), to give a concentration of 0.2 µc./ml. of culture medium, 6 h before termination. Colchicine was added 3 h before termination. Slides were stained with aceto-orcin and coated with Kodak 'AR 10' autoradiographic stripping film. When cells suitable for chromosome analysis had been photographed, the film was removed in 45 per cent acetic acid and the same cells were rephotographed. For XY individuals it was possible to distinguish morphologically the two Y chromosomes in twelve cells of one individual and in seven cells of the other. In the case of XXYY the two Y chromosomes could be distinguished in eight cells.

Grain counts were made on Y chromosomes, chromosomes of the 21-22 group, and in the XY individual on the late replicating X chromosome too. Grain counts for the Y chromosomes are reported as mean values for each individual, for the two Y chromosomes cannot be distinguished from each other in each cell. Grain counts for the 21-22 group are reported as the mean value for all four chromosomes of this group. Grain counts on the Y chromosome and 21-22 group of two normal males were used as controls. In each case ten cells were found to be suitable.

The results of grain counts in three males with two Y chromosomes and two normal control males are presented in Tables 1 and 2. In the three individuals with two Y chromosomes it was impossible to distinguish between the two Y chromosomes in each cell. It was noted, however, that in each cell the grain counts were nearly the same over both Y chromosomes, and it was therefore permissible to take a mean value for each cell. Two sources of variation contribute to the total variation encountered in the amount of labelling on the Y chromosome. Within an individual there may be variation between cells which results from differences in the stage at which they take up

labelled thymidine and from random variation. Between individuals there may be variation caused by a different pattern of labelling of their Y chromosomes.

To estimate accurately the significance of the variation between individuals the effect of different stages must be eliminated. The total grain count on the 21-22 group was used as a standard of the stage, because the size and shape of the chromosomes of group 21-22 are nearest to those of the Y chromosome. When the grain count of a Y chromosome (or mean of two Y chromosomes in the abnormal individuals) is plotted against the log of the total grain count on the 21-22 group a linear relationship emerges. It is therefore possible by analysis of covariance to eliminate stage differences from our comparison between individuals by using the log of the grain count over the group 21-22 as the correcting covariate.

When the corrected counts for Y grains were used it was found (Table 3) that the variance between individuals is greater than that within individuals (between cells), *P* between 10 per cent and 5 per cent. When, however, the individuals were divided into a group of normal and a group of abnormal individuals, their variance was not significantly different from the within group variance. From this we can conclude that, although individual differences exist in the labelling pattern of the Y chromo-

Table 2. RESULTS OF GRAIN COUNTS ON THE CELLS OF ABNORMAL AND NORMAL INDIVIDUALS

Individual	X	Y	Y	21-22 group			
XY (A)	4	7	3	3	2	3	
	15	14	5	5	11	5	
	5	4	3	0	0	2	
	13	11	10	6	4	3	
	15	7	10	2	0	2	
	5	2	1	0	0	1	
	17	4	2	2	0	4	
XY (B)	10	5	0	0	1	2	
	15	12	0	3	1	0	
	14	16	4	5	3	6	
	10	12	2	2	4	2	
	15	15	1	7	10	7	
	8	5	3	0	2	2	
	12	10	0	1	0	0	
	12	12	5	3	10	3	
	7	6	0	0	0	0	
	7	7	5	6	0	0	
	15	5	0	2	0	5	
	11	6	1	0	0	0	
XXYY	24	9	11	3	3	1	4
	20	8	9	0	0	2	1
	20	8	5	3	0	0	0
	30	3	4	1	1	0	0
	35	11	15	2	3	2	2
	9	4	6	0	0	2	1
	13	7	4	1	1	0	0
	23	15	12	6	1	3	2
XY (A)	12		1	2	0	0	
	11		2	1	0	0	
	7		0	0	0	0	
	12		0	2	6	0	
	2		0	0	0	0	
	9		1	0	0	0	
	13		5	2	1	6	
	7		1	0	0	0	
	8		0	0	2	1	
	10		3	1	5	3	
XY (B)	6		2	0	2	1	
	5		4	4	1	0	
	12		1	6	1	3	
	13		5	1	5	0	
	12		0	1	1	4	
	10		0	0	2	2	
	6		2	0	0	2	
	12		5	3	1	0	
	4		0	0	1	2	
	2		0	0	1	1	

Table 3. ANALYSIS OF COVARIANCE

Analysis of all data	df	SSD	SCPs	SSY	Corrected Y	df	MSS	VR	P
Between individuals	4	1.2073	0.67	42	70.6	4	17.65	2.34	0.05 > P > 0.01
Within individuals	42	7.1023	41.58	552	308.6	41	7.53		
Total variation	46	8.3096	42.25	594	379.2	45			
Further breakdown of between individuals variation									
Normal versus abnormal (A and B) XY (A)	1	0.33	1.98	11	12.58	1	12.58	< 1	Non-sig.
XY (B) and XXYY	3	0.88	-1.31	31	29.05	2	14.53		
Pooled within groups									
Total between individuals	4	1.21	0.67	42	41.63	3			

df, Degrees of freedom; SSD, sum of squares for  $\bar{D}$  values; SCPs, sum of cross products  $\bar{D}Y$ ; SSY, sum of squares for Y values; corrected Y, SSY corrected for covariance with  $\bar{D}$ ; MSS, mean sum of squares; VR, variance ratio; P, probability.

some, the differences do not appear to be related to the difference between normal and abnormal individuals.

We found that, in the three individuals with two *Y* chromosomes, both *Y* chromosomes replicate late compared with the other small acrocentric chromosomes. Counts of grains showed that this replication pattern does not differ significantly from that in normal male cells. It was found that grain counts on the two *Y* chromosomes in the three abnormal individuals fell into a homogenous group which could therefore be considered to come from one population. In addition the *Y* grain counts in each cell were homogenous. It seems, therefore, that the two *Y* chromosomes behave similarly with respect to DNA synthesis. Similar synchrony has been seen in other groups of chromosomes. Gilbert *et al.*<sup>14</sup> reported synchrony in the *A* group of chromosomes in normal and abnormal individuals, and Giannelli<sup>15</sup> reported similar findings in group *D* in a *D*<sub>1</sub> trisomic subject.

In individuals with additional *X* chromosomes, marked asynchrony and pronounced late replication of these chromosomes have been found. This late DNA synthesis of the *X* chromosomes is thought to be correlated with genetic inactivation of at least a large part of *X*, and is believed to take place during the prenatal life. It could have been suggested that extra *Y* chromosomes would also replicate late like the extra *X*. The data in this report show that both *Y* chromosomes behave in the same way and like the normal *Y*. This suggests that the mechanism of inactivation operating in the case of the extra *X* chromosomes does not apply in the case of the extra *Y*. Phenotypic effects such as the extra height and aggressiveness reported in patients with an extra *Y*<sup>15-17</sup> can therefore be related to the action of genes on the *Y* chromosome in pre- and post-natal life.

There are no comparative reports in the literature of grain counts on *XXYY* and *XXY* individuals. It was therefore impossible to draw conclusions on the basis of grain counts on the relationship between the late replicating *X* and the *Y* chromosome.

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## PATHOLOGY

### New Carcinogenic Naphthalene and Biphenyl Derivatives

As part of an extensive series of tests for carcinogenic activity a number of naphthalene and biphenyl derivatives of potential or actual commercial use were examined. The practicability of standardizing the methodology of large scale quantitative bioassay of many different compounds was also investigated.

The procedures consisted of oral administration by gavage five times in a week for 52 weeks (260 doses) of several concentrations of test compound to sixty male and female Fischer rats. The animals were observed an additional 26 weeks unless their condition required earlier killing. Chemicals were dissolved or suspended in "steroid suspending vehicle" (9 g of sodium chloride; 5 g of sodium carboxymethylcellulose 7 LP; 4 ml. of polysorbate 80; 9 ml. of benzyl alcohol, and water to 1 l.). The amount of the compound tolerated was determined in three to five rats/dose concentration for a 2 month period. The definitive tests were based on these results.

We report here the salient findings obtained at one dose concentration, one-third of the maximally tolerated, with four compounds (Table 1). In male rats 171 to 218 doses of 10 mg of 3-methyl-2-naphthylamine, originally proposed as a substitute for 2-naphthylamine, caused chiefly tumours as well as sessile adenomatous growths of the lower intestinal tract, in the ileum and the colon. In addition, papillomas of the skin and squamous cell carcinoma of the ear duct developed. In female animals, after 76 to 118 doses of 10 mg and a latent period from 126-191 days, adenocarcinoma of the mammary gland was found in virtually all animals<sup>1</sup>. This compound, thus, is potent for the breast, although the female Fischer rat is less susceptible than other albino strains as, for example, the Wistar or Sprague-Dawley rat<sup>2-5</sup>.

The related 3-nitro-2-naphthylamine was less active. Both males and females receiving doses of 10 mg had tumours in the forestomach after 555 days (males) and after about 420 days (females). Several other aromatic nitro compounds gave a similar result<sup>6,7</sup>. In females the mammary gland was also affected. 1,2-Dichloro-3-nitronaphthalene was still less active, because 260 doses of 30 mg led to two fibromas and one pituitary adenoma, possibly spontaneous lesions, in males, and four mammary gland tumours in females after 500 days.

3,3'-Dimethoxybenzidine, or dianisidine, after 260 doses of 10 mg gave tumours in male and female rats at a variety of sites (skin, gastrointestinal tract, breast, ear duct) as early as 293 days. Most of the lesions were seen in rats autopsied after 500 days of treatment. This compound, which is of industrial importance<sup>8</sup> as a dyestuff intermediate, is therefore definitely carcinogenic. Gehrmann *et al.*<sup>9</sup> found it inactive in dogs, while Pliss<sup>10</sup> noted that the dimethoxy derivative was less active than benzidine itself.

In the entire series, 660 simultaneous control animals were used. The tumour incidence in this group was recalculated for each fifteen male and fifteen female rats to permit ready reference to the experimental groups discussed here. Detailed findings and description of the pathology will be published elsewhere.

In conclusion, the carcinogenic activity of several naphthalene and benzidine derivatives was determined by quantitative tests in rats. 3-Methyl-2-naphthylamine readily evoked tumours of the lower intestinal tract in males and is therefore a new tool to produce and investigate this lesion, so important in man. In females this compound is a potent mammary carcinogen. 3-Nitro-2-naphthylamine gave mainly tumours of the forestomach. 3,3'-Dimethoxybenzidine induced tumours at several sites. 1,2-Dichloro-3-nitronaphthalene was only slightly tumorigenic.

Table 1. TUMOURS INDUCED BY 2,3-SUBSTITUTED NAPHTHALENES AND BY DIANISIDINE

Compound	Daily dose (mg)	Sex	Survival (days)	No. of lesions	No. of lesions*				
				No. of rats with lesions†	Skin	Gastro-intestinal tract	Mammary gland	Ear duct	Other‡
3-Methyl-2-naphthylamine	10	M	124-335	17/11	3	8§	—	3	3
		F	126-191	16/14	—	1	13	—	2
3-Nitro-2-naphthylamine	10	M	374-556	12/8	—	8	—	—	4
		F	153-555	12/7	—	4	6	—	2
1,2-Dichloro-3-nitronaphthalene	30	M	269-564	3/3	—	—	—	—	3
		F	374-564	5/5	—	—	4	—	1
3,3'-Dimethoxybenzidine, 2HCl	10	M	372-565	9/9	3	3	—	2	1
		F	177-548	7/6	1	—	3	1	2
Controls¶	—	M	540	0-8	0	0	0-1	0-1	0-7
		F	540	2-5	0	0	1-3	0	1-2

\* Only one lesion of a type is recorded for each rat.

† Each group initially included fifteen rats. In a few instances there was a single early death (less than 30 days) so that survival data shown relate to fourteen rats.

‡ Several sites to be described in detailed report. Interstitial cell tumours of the testis developed in many old male rats in all groups, and are not included.

§ Mostly in the caecum and ileum; the figure includes tumours and precancerous polyps due to treatment.

|| Fore stomach.

¶ The values for the controls are averages calculated from 370 simultaneous control rats surviving 540 days or longer. The total number of tumours of a group of fifteen rats is listed to permit comparison with the experimental groups.

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### Neoplastic Transformation of Rat Embryo Cells by Simian Papovavirus SV<sub>40</sub>

SV<sub>40</sub> virus manifests its oncogenic action only in hamsters<sup>1</sup> and in mastomys<sup>2</sup>. There are, however, reports of its transformation *in vitro* of cells of hamsters<sup>3</sup>, mice<sup>4</sup>, rabbits<sup>4</sup>, pigs<sup>4</sup>, cows<sup>5</sup>, monkeys<sup>6</sup> and humans<sup>7</sup>. The purpose of our investigation was to determine whether the SV<sub>40</sub> virus possesses the ability to transform rat cells *in vitro*.

A monolayer of Wistar rat embryo skin-muscle cells grown in Povitskaja bottles (100 ml.) was infected with SV<sub>40</sub> virus using large plaque (Rh-2 A-426) and small plaque (strains No. 128 and 130) variants of this virus<sup>8</sup>. Medium 199 supplemented with 10 per cent bovine serum was used as the maintenance medium. The medium in infected and control cultures was changed once or twice a week. Eighteen days later the cells were collected with versene solution and put into tubes. The tube tissue

cultures were maintained, as shown above, without subcultivation. The results obtained are presented in Table 1.

In test No. 1, beginning from the fourth week, foci of epithelium-like cells were found which were not sharply delimited from the surrounding fibroblast-like cells. The foci (1-3 per tube) soon became multilayers. By the end of the third month the fibroblast-like cells degenerated, but the foci remained and increased in size, acidifying the medium intensively. The subcultivation of such foci from individual tubes presented no difficulties; two cell lines—426/20 and 130/20—were established. Such phenomena were not observed in any of the control tubes.

In test No. 2 the features of cell transformation were different. On the fourth week multiple round-shaped cells appeared in the infected tissue cultures; they were disseminated in the monolayer or formed multilayer foci. The appearance of giant cells was characteristic of these cultures. The infected tissue cultures acidified the medium intensively. By the end of the second month of infection the number of round-shaped cells considerably decreased, only large multilayer aggregates of such cells remained, which could be recognized grossly. As a result of the subcultivation of such tissue cultures from individual tubes two cell lines were established—426/25 and 128/25. In control tissue cultures such changes were not seen, and the monolayer degenerated in the second or third month of the experiment.

The testing of culture fluids from infected cultures in green monkey kidney cell culture showed that the virus was present in trace amounts during the first 2-4 weeks and then disappeared.

The demonstration of the tumour antigen in the cells of established rat-cell lines formed important evidence of the specificity of described transformative changes of rat cells. Five per cent extract of cells 426/20 and 426/25

Table 1. TRANSFORMATIVE ACTIVITY OF SV<sub>40</sub> VIRUS IN SKIN-MUSCLE RAT EMBRYO CELL CULTURE

Test No.	Virus strain	Dose of virus (P.F.U. per tissue culture bottle)	No. of tubes with subcultures	Days after infection		Features of transformative foci
				30	60	
1	A-426	10 <sup>7-8</sup>	15	8/10*	10/10	Multilayer foci
	130	10 <sup>6-8</sup>	21	5/16	8/16	of epithelium-like cells
	Control	—	12	0/9	0/9	
2	A-426	10 <sup>7-8</sup>	15	13/15	15/15	Multilayer foci
	128	10 <sup>6-8</sup>	15	9/15	15/15	of round-shaped cells; giant cells
	Control	—	15	0/14	0/14	

\* Numerator—number of tube tissue cultures with phenomena of transformation.

Denominator—number of tissue cultures remaining till the end of the observation period.



reacted specifically in complement fixation test with an active pool of hamster sera from animals bearing tumours induced by  $SV_{40}$ , but did not react with hamster sera from animals bearing tumours induced by polyoma or Rous sarcoma viruses.

By means of the direct method of fluorescent antibody, using the conjugate of serum globulin (obtained from hamsters bearing  $SV_{40}$  tumours) with fluorescein isothiocyanate, the presence of  $SV_{40}$  tumour antigen was revealed in the nuclei of 100 per cent of line 426/20 cells (other lines were not tested). The nuclei of various control culture cells not infected with  $SV_{40}$  showed no distinct fluorescence.

Wistar suckling rats 3–5 days old were inoculated with the cells of lines 130/20, 426/20 and 128/25 (eighth to tenth passage) (using  $3 \times 10^6$ – $5 \times 10^6$  cells subcutaneously). None of the twenty animals developed tumours during an observation period of 4–6 months. When hamsters were inoculated into the cheek pouch with about  $10^6$  cells of line 426/20, nodules 0.5 cm in diameter were formed at the site of inoculation 6–8 days after inoculation, which disappeared 3–4 weeks later. A histological study of such nodules showed that they had the features of spindle-shaped cell sarcoma (I. S. Levenbook).

The data obtained point to the ability of  $SV_{40}$  to induce neoplastic transformation of rat cells. Morphological features of the initial stages of transformation were not identical in the two tests described above. This may be accounted for by different cell composition of the tissue cultures used in these experiments.

The absence of tumorigenicity of transformed cells in rats is correlated with the absence of oncogenic activity of  $SV_{40}$  in these animals. Similar data were obtained in this laboratory when the transformation of mouse embryonic cells of  $C_3HA$ ,  $CC_{57}Br$ , and  $C_{57}BL$  mice by  $SV_{40}$  virus was studied (N. N. Dodonova *et al.*, in the press).

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### Spontaneous Neoplastic Transformation of Mouse Heart Tissue *in vitro*

WE have examined the possibility that cells derived from heart tissue might undergo spontaneous neoplastic transformation less rapidly than other tissue, the heart rarely being a site of primary malignancy.

Fibroblasts from rat myocardium grown in a chicken plasma clot under nitrogen produced tumours when they were implanted in rats, although control cultures did not<sup>1</sup>. Cell strains derived from mammalian heart tissue are rare, and even the cells thought at first to have been derived from cynomolgous monkey heart<sup>2</sup> and reported to produce fatal tumours when injected into pretreated Wistar rats<sup>3</sup> were later thought to be a line of human heteroploid cells

similar to HeLa cells that had contaminated the culture and overgrown the monkey heart cells<sup>4</sup>.

In the present experiments, hearts were excised from anaesthetized strain A mice (20 days old and free from polyoma). Each heart was washed in warm saline, minced in a few drops of saline, and centrifuged and rewashed several times. The tissue was suspended in a variety of growth media: medium 199 and 10 per cent calf serum; Puck's medium and 15 per cent calf serum; and basal medium Eagle with 15 per cent foetal calf serum (BME). One heart was used with each medium. Equal portions of each heart were placed in each of four roller-tubes with 2 ml. of medium. Cells from trypsinized heart tissue were also used. The tubes were incubated at 37° C in a roller drum at 12 r.p.h.

The fragments attached within 12 h of inoculation and the volume of medium was increased to 3 ml. Cells obtained by trypsinization attached very poorly and failed to grow progressively. Outgrowths from the fragments began in 2–4 days and were typical fibroblasts, radial in appearance with a mixture of large, granular cells. The latter disappeared in about 2 weeks, but a number of isolated cells remained. Medium 199 and Puck's supported steady growth less successfully than BME and were discarded. The trypsinization of cultures grown in BME was only partially successful in that the majority of cells were lost and the growth of the surviving cells decreased.

The fragments in BME grew in all four test-tubes; the cultures were re-fed three times each week. Acidity caused the appearance of cellular processes and the more isolated cells died. When growth was allowed to continue, additional layers of cells formed; but the cells were for the most part arranged parallel to one another. After a month the fragments usually detached from the glass and, if small, reattached and flattened out. Extensive growth occurred in areas where the original fragment had been located. Definite networks, typical of fibroblast growth, were often seen where there were few cells. Within 4 months the cultures were growing steadily and rapidly and extended up the sides of the 8 oz. "gem oval" bottles. Growth became so heavy after 3 weeks that several layers of cells peeled off in sheets. Cells would survive mechanical or enzyme removal from the glass surface. For subculturing, a monolayer was mechanically dispersed and divided between as many bottles as needed. Although cloning was not tried, a colony of ten cells could survive and grow in a small culture vessel (Fig. 1).

Strain A male mice given 1.25 mg of cortisone acetate 24 h previously were injected with cultures 4–5 months old (fifteen subcultures). All ten animals injected subcutaneously with at least  $3 \times 10^6$  viable cells developed tumours. Tumours appeared approximately 3 weeks after inoculation and grew at a steady rate; the tumours afterwards grew in unconditioned mice. Six cortisonized mice which received  $10^6$  cells and ten untreated mice injected with  $10^6$ – $3 \times 10^6$  cells developed no tumours during the 5 months after injection. The failure of the cell culture to grow in unconditioned hosts may be associated with the acquisition of new antigens by the transformed cells or with the persistence of calf serum proteins on the cell surfaces.

Microscopic examination of tumour sections (Fig. 2) showed features consistent with a highly malignant tumour: solid masses of the polymorphous type of mesenchymal tissue with large and small spindle cells, and large and occasional giant round cells. Ordinarily fibroblasts from mice, if they have undergone malignant transformation, result in classic spindle cell sarcomata when reinjected into mice<sup>5</sup>. The mesenchymal tumours, although they contained giant cells possibly associated with rhabdomyosarcoma, are difficult to classify properly because of the lack of definite striations. Even tumours of human soft tissue, which have been intensively investigated, are not easily diagnosed<sup>6</sup>; and the proper identi-

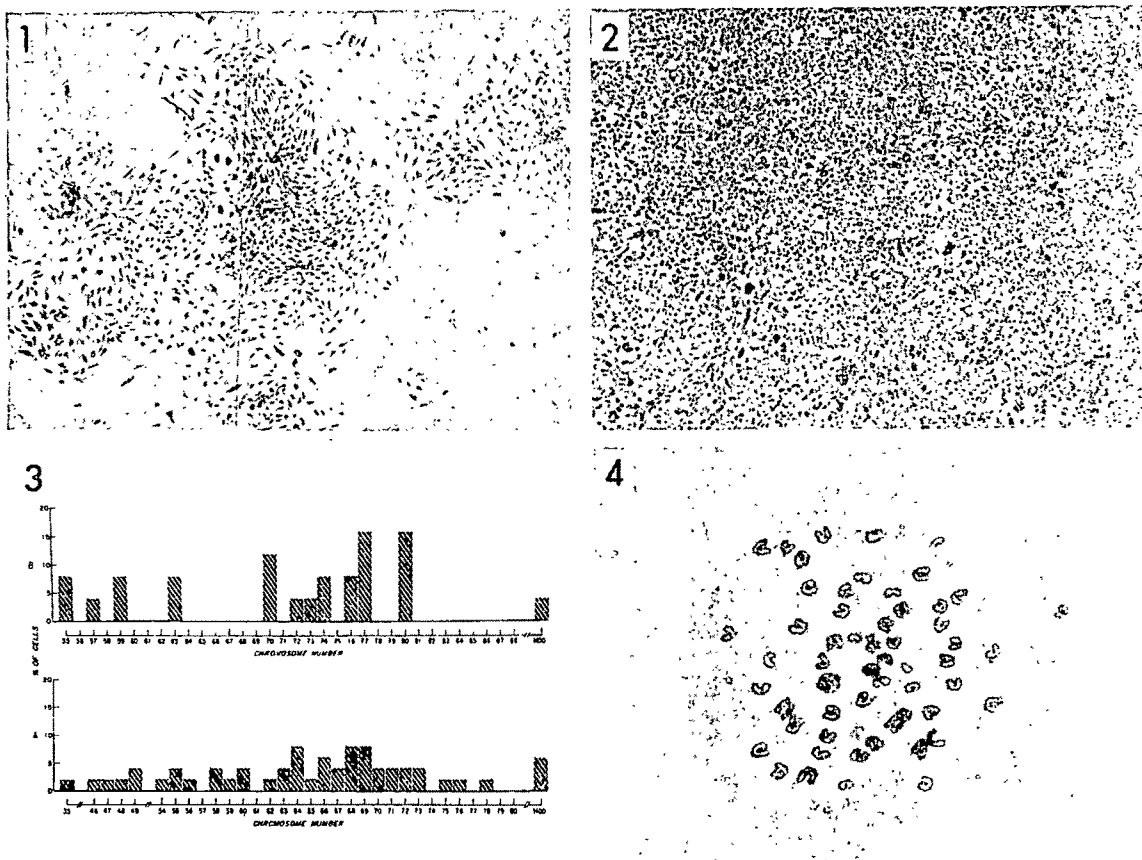


Fig. 1. Colonies of cells growing on a coverslip showing overlap and network typical of fibroblasts. ( $\times c. 17$ .)

Fig. 2. [Solid mass of polymorphous type mesenchymal tissue with large and small spindle cells, large round cells, and occasionally giant forms of the latter. ( $\times 70$ .)

Fig. 3. Chromosome distribution at 4 and 10 months based on fifty counts at each time interval.

Fig. 4. Metaphase plate of cell with fifty-four chromosomes, two of which are metacentric.

fication of these tumours must be considered difficult or even impossible.

When the tumours appeared 6 months later, chromosome preparations were made on cultured cells by the usual squash and air dry techniques; slides were stained with 0.5 per cent aceto-orcein<sup>7,8</sup>. Chromosomes of 100 complete metaphases were counted. At first the chromosome number varied from the middle fifties to more than one hundred with a dispersion of chromosome numbers and no significant peak, although there was an indication of a slightly larger number of cells with chromosome numbers in the hypotetraploid range (Fig. 3A). Chromosome readjustment continued (Fig. 3B), characterized by heteroploidy with a broad mode of 74–80 chromosomes. In 25 per cent of the cells, one or more metacentric (Fig. 4) or minute chromosomes were found. Most cells, however, had all telocentric chromosomes.

The chromosomal instability of mouse cultures in the early stages of *in vitro* manipulation is well known<sup>11</sup>. There is evidence that selection is still going on within this population, thus providing evidence for a gradual replacement of normal karyotypes with karyotypes containing alterations. It is still not clear whether this readjustment of the cells indicates transformation, because neoplastic transformation may or may not be associated with gross changes in chromosomes.

No conclusion can be drawn about the cause of the cytological and neoplastic transformation which occurred spontaneously within 4 months. Medium added to the cells with or without periodic transfer resulted in pulses of growth stimulation as indicated by the rise in number of dividing cells. Nevertheless, a period of cell damage may

have ensued as a result of the decomposition of dead cells and the formation of metabolic waste materials. Under some circumstances many purines and pyrimidines, including adenine, produce mutations in bacteria<sup>9</sup> and in other circumstances cause chromosome breakage<sup>10</sup>. As a result of cell degeneration or disintegration, abnormal purines and pyrimidines may be produced and may act as mutagens.

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### Activity of Aspartate Transcarbamylase in Mammary Tumours induced by 7,12-Dimethylbenzanthracene in the Rat

It has been shown that all biosynthetic reactions involved in the formation of ribonucleic acids (RNA), proteins and phospholipids, become accelerated in rapidly proliferating tissues. This requires, however, the presence of related precursors; in the case of an increased synthesis of RNA, the presence of pyrimidine bases and ribose. Beaconsfield *et al.*<sup>1</sup> demonstrated the existence of a direct correlation between the extent of RNA synthesis and ribose formation by way of the oxidative pentose phosphate cycle. Similarly, an increase of RNA synthesis must be accompanied by an increase in the synthesis of pyrimidine bases. The first step in the pyrimidine synthesis is the transformation of the carbamoyl group from carbamoyl phosphate to L-aspartic acid, forming ureidosuccinic acid. This reaction is catalysed by the irreversibly acting aspartate transcarbamylase (ATC).

Several authors suggested a correlation between ATC activity and the rate of cell growth and cell division. Calva and Cohen<sup>2</sup> observed an increase of ATC activity in the regenerating rat liver which became normal again when the phase of regeneration finished. In the rapidly growing Novikoff hepatoma the ATC activity is also higher than in the surrounding normal liver tissue<sup>3</sup>. Kim and Cohen<sup>4</sup> found that, compared with the adult liver, the ATC activity in the foetal rat liver is increased by a factor of between five and six. This value decreased to the level of adult liver within about 20 days after birth. These findings point to the existence of direct correlation between the ATC activity and the RNA synthesis and, consequently, the cell growth and cell division.

This correlation can best be demonstrated in tissues of the same origin which may show a tendency to positive or negative growth depending on the treatment given. The mammary carcinomata of the female rat induced experimentally by Huggins<sup>5</sup> are suitable models in this relation. Without any treatment these tumours usually grow very rapidly, but regress under the influence of hormones from the opposite sex. For the purpose of these experiments we induced mammary carcinomata in female Sprague-Dawley rats. At intervals of 10 days the animals were fed with three doses of 10 mg of dimethylbenzanthracene (DMBA) dissolved in sunflower oil<sup>6</sup>. Carcinomata developed in most cases within 60–80 days of the first application of DMBA. About 50 per cent of all carcinomata showed a rapid growth and this was followed up by continuous measurement of the size of the tumours. In estimating the size of the tumours two diameters were measured perpendicular to each other, multiplied together and plotted against time. A straight line drawn through the points for a period of 2 weeks gives a mean tumour growth rate for this time. The tangent of the ascending angle  $\alpha$  of this line is a measure of the rate of growth or regression. This is defined as the change of the product of the two tumour diameters obtained during a period of 2 days. A positive angle indicates a tendency to increased growth, a zero degree indicates stagnation, and a negative angle a regression of the tumour growth (Fig. 1).

Regression of mammary cancer can be achieved by the administration of appropriate dosages of testosterone. The animals were given doses of 3.0 and 6.0 mg of testosterone propionate/kg body weight twice a week, respectively. This dose regime inhibited the growth of the mammary carcinomata induced in rats by DMBA<sup>7</sup>. The therapeutic effect of testosterone must inevitably imply a reduction in the requirement for RNA, and thus for the corresponding precursors, which should result in a lower activity of ATC in stagnating or regressing carcinomata. We therefore investigated the activity of ATC in growing mammary carcinomata and such carcinomata as showed stagnation or regression when treated with testosterone.

Table 1. RELATIONSHIP BETWEEN THE TENDENCY OF GROWTH OF MAMMARY TUMOURS AND THE ACTIVITIES OF ATC AND GOT

Ascending angle (in degree)	ATC (nmoles/mg h)	GOT (nmoles/mg min)	Testosterone (mg/rat twice a week)
-76	48	-	0.5
-65	-	62.7	1.0
-64	220	-	0.5
-64	216	-	0.5
-63	228	68.6	0.5
-59	159	47.0	0.5
-56	232	-	0.5
-53	63	-	0.5
-40	177	-	0.5
-37	178	30.2	1.0
-25	77	56.7	0.5
± 0	124	35.7	1.0
+26	265	40.6	-
+27	463	74.3	-
+35	383	67.4	-
+36	-	58.3	-
+38	349	108.0	-
+38	463	43.1	-
+50	424	60.5	-
+50	-	58.0	-
+50	-	89.5	-
+54	-	28.2	-

As a parameter of the amino-acid and protein metabolism, the activity of glutamic-oxalacetic transaminase (GOT) was investigated and compared. The activity of the enzymes was measured on excised tumour tissue. The tissue was homogenized in 0.14 molar potassium chloride (1:3) three times for 20 sec in an 'Ultra-Turrax' and centrifuged for 30 min at 17,000*g*. The supernatant was diluted with a solution of potassium chloride corresponding to the expected enzyme activities.

The activity of ATC was determined according to the method of Koritz and Cohen<sup>8</sup>, except that a 0.5 per cent solution of sodium diphenylamine-*p*-sulphonate was used instead of a 1 per cent solution. The activity was expressed as the specific activity in nmoles/mg h. The GOT activity was determined spectrophotometrically according to Bergmeyer and Bernt<sup>9</sup> and recorded as the specific activity (nmoles/mg min). Measurements were also made of the increase of oxalacetate with time which was then reduced by an additional indicator reaction with reduced nicotinamide-adenine dinucleotide (NADH<sub>2</sub>) and malic dehydrogenase to malate. The consumption of NADH<sub>2</sub> was proportional to the amount of oxalacetate present, and the decrease was followed up at 340 nm. The protein content of the samples was determined according to the biuret method.

The results of the measurements are summarized in Table 1. All animals injected with 3.0 mg or 6.0 mg of testosterone/kg of body weight showed a stagnation or regression of tumour growth. The levels of ATC activity of these tumours are clearly lower than those of growing carcinomata. When the values for the activity of ATC were averaged for both testosterone-treated and untreated animals mean values of  $156.4 \pm 68.6$  and of

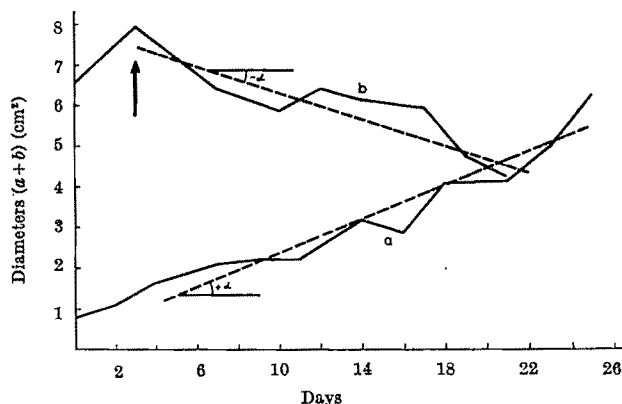


Fig. 1. Growth of mammary tumours. *a*, Growing tumour (without treatment with testosterone); *b*, regressing tumour (treatment with 3.0 mg testosterone propionate/kg of body weight twice a week). The arrow indicates the start of treatment with testosterone.

391.2 ± 76.5 nmoles/mg h, respectively, were obtained. The two results are significantly different with  $P < 0.0027$ , whereas no significant differences were detected in the GOT activities of both groups—the averages 50.2 ± 15.3 for animals treated with testosterone and 62.8 ± 23.7 nmoles/mg min for untreated animals. The results thus confirm the existence of a correlation between the activity of ATC and the extent of RNA synthesis, whatever the action of testosterone may be. Its mode of action may be interpreted in terms of the theory of Monod and Jacob<sup>10</sup> as a negative effector, but also according to Koshland<sup>11</sup> as a regulator of the active site of an enzyme.

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## RADIOBIOLOGY

### X-Radiation Sensitivity of DNA—Ability to form Specific Molecular Hybrids with Isologous mRNA

THE basic functions of cell DNA are priming of DNA synthesis and priming of RNA synthesis. The radiation damage of the first is believed to be closely related to mutant formation and cell reproduction, and that of the second to genetic determination and regulation of intracellular metabolic processes. Recent investigations of these basic functions of DNA resulted in profound changes in the existing research practice and directed the development of radiation biochemistry of DNA from an almost purely phenomenological ground to a more causal approach. Although a relatively clear picture of cell molecular biology does exist, conflicting results are still obtained about the activity of the DNA primer, so far as its radiosensitivity against ionizing radiation is concerned.

Some authors report a negligible effect of radiation on priming activity of DNA in a DNA-polymerase system up to 10 kr. X-rays<sup>1</sup>. Others consider DNA synthesis to be a process that, on the contrary, is not suppressed but rather, within certain X-ray dose limits, is stimulated by ionizing radiation<sup>2</sup>.

Recently Harrington published data showing remarkably high sensitivity to X-rays of DNA priming properties assayed by a DNA-polymerase system. A dose of 500 r. reduced the activity of the DNA to about 50 per cent<sup>3</sup>; the priming activity of DNA for RNA polymerase was much less affected.

We have investigated radiosensitivity of another macromolecular property of DNA, that is the ability to form genetically specified molecular hybrids with correspondent messenger RNA. We used the nitro-cellulose membrane filter technique of Nigaard and Hall<sup>4</sup>. Our experimental results reveal a very steep slope in the dose-response curve (Fig. 1). Obviously, "complementarity" remains unchanged for a certain increase in X-dose, until a critical point of chemical "defects" accumulation is reached and then there is a sharp drop in hybrid formation. This brings the values of irradiated samples to those of the unhybridized controls.

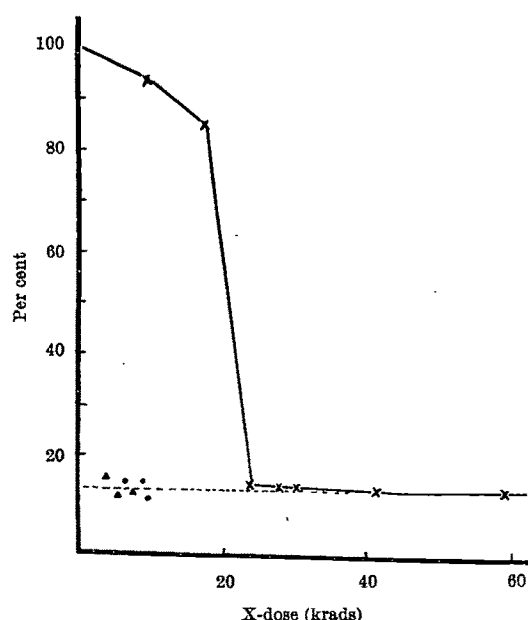


Fig. 1. Dose dependence of DNA/mRNA hybrid formation on X-irradiation of DNA. x, Irradiated DNA of *E. coli* B + mRNA of *E. coli* B; Δ, DNA of *E. coli* B + mRNA of *E. coli* B (non-hybrid); ●, DNA of *B. subtilis* + mRNA of *E. coli* B. Double stranded DNA from *E. coli* B was irradiated in citrate buffer (pH 7.8) at 4° C in a concentration of 1,200 μg/ml. After irradiation the DNA samples were re-precipitated with ethanol. The hybridization experiments were performed for 210 min at 78° C according to Nigaard and Hall<sup>4</sup>. RNA labelled with phosphorus-32 was obtained by means of a 5 min pulse with phosphorus-32 during the exponential stage of growth. The quantitative analysis of hybrid complex formed was carried out after alkaline hydrolysis and ribonucleotide separation on 'Dowex' 1 × 8. Irradiation: 200 kV, 20 m.amp, 0.5 aluminium 600 r./min. Two controls were used for background determinations: *E. coli* DNA + *E. coli* RNA without incubation at 78° C and *E. coli* RNA + *B. subtilis* DNA with incubation at 78° C for 210 min. In both these controls no more than 15 per cent incorporation was detected.

It is tempting to assume that the nearly horizontal part of the curve corresponds to a relative reversal of DNA-mRNA complex formation. It may be possible from these data to determine the number of breaks in the DNA which may be sustained without affecting the genetic capacity of the DNA. Once this damage has been exceeded there would theoretically be no chance for repair because of impossibility of complementation.

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## PHYSIOLOGY

### In vitro Investigation of Resting Muscle Membrane Potential in Prewanling and Weanling Rat

CHANGES have been reported in muscle electrolytes with age<sup>1</sup>, and several laboratories throughout the world have reported similar changes<sup>2-5</sup>. From birth to maturity the sodium and chloride content in a unit muscle mass decreases while the potassium increases. These changes are often interpreted as a function of increasing muscle

cell size and a decreasing extracellular space in the muscle.

The sodium and chloride content was shown to be greater than the potassium content of the rat gastrocnemius at 1 day of age and by 45 days this pattern was reversed<sup>6</sup>. Similar findings have been reported for the rat semimembranous muscle<sup>7</sup>. Both laboratories demonstrated that even with these changes in electrolyte content the intracellular potassium concentration is constant with age<sup>6,7</sup>.

The resting membrane potential was reported to be low in the muscles of newborn rats and fully developed by one month of age<sup>7</sup>. In view of this work, and the failure to find significant changes in intracellular potassium<sup>6,7</sup>, it seemed pertinent to determine the resting membrane potential in muscles taken from rats during the first month of life. It is the purpose of this paper to report our findings<sup>8</sup>.

The resting membrane potentials in 10–17 and 24–27 day old rats (Houston Cheek–Jones) were measured *in vitro* with conventional electronic equipment, silver–silver chloride electrodes and Ling–Girard micropipettes. The hind limb of the rat was removed, placed in a chamber and perfused with a buffered Ringer solution<sup>9</sup>, and aerated with 95 per cent oxygen and 5 per cent carbon dioxide. After 15–30 min incubation, penetrations were made at random in the gastrocnemius muscle. In the usual case twenty-five to fifty penetrations were made into each muscle. The mean obtained from these penetrations represented the resting membrane potential for that muscle.

Table 1

Age	No. of animals	Resting muscle potential $\pm$ S.E.	Total penetrations	P
10–17 days	7	36.63 $\pm$ 3.43	283	<0.001
24–27 days	7	67.76 $\pm$ 2.13	315	

Table 1 gives the data and statistical analysis. The gastrocnemius muscles of the younger rats show resting membrane potentials significantly lower than the older rats. The usual value of resting membrane potential for excised muscle from mature rats is about 70–75 mV<sup>8</sup>; the muscle resting membrane potential of 24–27 day old rats may not yet be at full potential. The change in resting membrane potential with age cannot be explained on the basis of potassium changes. It has already been reported that intracellular potassium does not change with age<sup>6,7</sup>, and in our investigation the external potassium was held constant. It is concluded that the resting membrane potential of the gastrocnemius muscle from young rats is significantly reduced relative to that from older animals.

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## Measurement of Angiotensin II in Blood

Khairallah and Page<sup>1</sup> have tried our method<sup>2</sup> for measuring angiotensin in blood and report almost complete loss of added angiotensin as well as the production of a new vasopressor substance during chemical extraction. Because these results differ from previous experience, the method has been reassessed to find out whether such differences are inherent in the technique, or are caused by their different application of the technique. Repetition of the previously reported control experiments<sup>2</sup> has confirmed our original results.

The criticisms by Khairallah and Page<sup>1</sup> can be summarized in two points as follows. (1) Losses of angiotensin occur sequentially in all steps of the extraction procedure with a cumulative loss of more than 80 per cent of the tritium label of tritium incorporated into angiotensin after its addition to blood. (2) Regardless of the losses occurring in the isolation procedure, a large part of the vasopressor material recovered is not angiotensin II, but is a lipid substance formed by hydrolysis of blood components by the hydrochloric acid used in the extraction procedure.

Khairallah and Page support their conclusions with evidence of recovery of a non-angiotensin lipid vasopressor material from all samples of blood, arterial and venous, as well as blood from an anephric patient (the latter case certainly being conclusive, because, theoretically, angiotensin should not be found in the absence of the kidneys). These observations are a dichotomy, which they note by quoting our categorical statement that no vasopressor material has ever been found in peripheral venous blood; nevertheless, they did not consider that their findings of vasopressor in all blood samples suggested a possible difference in their application of the same method.

Loss of angiotensin during isolation can occur with the multiple transfers of small volumes, but the loss by Khairallah and Page of 45 per cent of the tritium incorporated into angiotensin through trapping in the red cell mass during initial filtration is excessive and has not been our experience or that of Boucher<sup>3</sup> or Paladini<sup>4</sup>, who also precipitate the blood with ethanol and have a total recovery of 65 per cent to 95 per cent of added angiotensin. This difference could reflect too great a reliance on the stability of the tritium label by Khairallah and Page (our own experience has shown about 40 per cent spontaneous dissociation of the tritium incorporated into angiotensin), or it could be caused by inadequate washing of the precipitate during filtration.

The isolation of a lipid vasopressor substance by Khairallah and Page is a new finding; they say that this substance is probably formed by protein hydrolysis with the hydrochloric acid used in the extraction. In their investigations they titrated the concentration of hydrochloric acid in their solutions and found it to be 12.0 moles/l. We cannot explain this concentration, because the method calls for the addition of 0.5 ml. of concentrated hydrochloric acid with a minimal solution volume of 5.0 ml. Our calculation of 0.5 ml. of concentrated hydrochloric acid (37 per cent, specific gravity 1.192) in a 5.0 ml. volume is 1.219 moles/l., and the measurements of the acid molarity of extracting solutions in our laboratory have varied from 0.8 to 1.4 molar hydrochloric acid. It is difficult to know how Khairallah and Page achieved a concentration of 12.0 moles/l. of acid if the details of the technique are followed, for a concentration of 12.0 molar hydrochloric acid would require 4.95 ml. of acid in a 5.0 ml. volume, or more than eight times the amount of acid that is added.

To determine the effect of hydrochloric acid on angiotensin, we have repeated experiments with addition of hydrochloric acid to angiotensin solution to equal 1.2 molar and 12.0 molar concentration when reduced to a 5.0 ml. volume by evaporation on a steam bath at 80° C. The angiotensin recovery from the 1.2 molar solution was



98 per cent while only 11 per cent was recovered from the 12.0 molar acid solution. From this work, we agree with Khairallah and Page that 12.0 molar acid will destroy angiotensin, and could well hydrolyse other substances to produce many products, any of which could have vasopressor activity, but this point seems unrelated to the present problem because the technique calls for a maximum concentration of 1.2 molar hydrochloric acid which has not been found to destroy angiotensin.

The constancy of our own results in over 1,500 assays as well as duplication of the method by other groups (personal communications from H. G. Langford and E. Yendt) suggest that the differences found by Khairallah and Page are due to variations in their application of the technique.

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### Metabolic Differentiation of the Anterior and Posterior Latissimus Dorsi of the Chick during Development

FAST and slow contracting mammalian muscles differ considerably in their biochemical properties, for example, in their enzyme activity, reflecting especially different quantitative proportions of glycolytic (extramitochondrial) and respiratory (mitochondrial) systems<sup>1</sup>, ATPase activity of myosin<sup>2</sup>, ionic content<sup>3,4</sup> and many other biochemical characteristics. Avian muscles are especially suitable for the investigation of biochemical differences of fast and slow muscle fibres, as whole muscles consist exclusively of each type of fibre<sup>5,6</sup>. Little is known of the metabolic differences between fast and slow muscle fibres of birds in which, unlike mammalian muscle fibres, there are also basic structural and electrophysiological differences of fast (phasic) and slow (tonic) muscle fibres, which differ as in frogs according to "fibrillen" or "felderstruktur"<sup>6,7</sup>, focal or multiple innervation, sensitivity to acetylcholine<sup>8</sup> and capacity for long-term contraction<sup>9</sup>. Both fast and slow contracting mammalian muscles are phasic and do not show such basic structural and electrophysiological differences.

The investigation of metabolic differences in the fast posterior latissimus dorsi and the slow anterior latissimus dorsi and their ontogenetic development should suggest whether the metabolic differences reveal a uniform pattern. In order to obtain some basic data on differences in energetic and protein metabolism in these two types of muscles, the differences in the amount of glycogen, proteins of the myofibrillar-nuclear and sarcoplasmic fractions, ribonucleic acid content and proteolytic activity in anterior and posterior latissimus dorsi of chicken (Leghorn) were examined during ontogenetic development.

The muscles were homogenized in 0.1 molar potassium chloride, and RNA<sup>10</sup> and proteolytic activity<sup>11</sup> were determined in the homogenate. The content of proteins in the myofibrillar-nuclear fraction (10 min at 1,000g) and the sarcoplasmic fraction (containing also mitochondria and sarcoplasmic reticulum) was determined by the biuret method. Glycogen was determined by a modification of the anthrone method<sup>12</sup>.

Fig. 1 shows that by the first day after hatching the glycogen content is greater in the posterior latissimus dorsi. Later the glycogen content somewhat decreases in

this muscle, but it remains at greater concentrations during development. The sarcoplasmic fraction is the same percentage of the total nitrogen in anterior and posterior latissimus dorsi of a 20-day-old embryo; its relative amount decreases later in both muscles. Anterior and posterior latissimus dorsi of older animals differ considerably in their degree of proteolytic activity; the anterior exhibits greater proteolytic activity in both sarcoplasmic and structural protein fractions<sup>13</sup>. Proteolytic activity in 20-day-old embryos and in 1-day-old animals is high in both muscles. It decreases later in both muscles, but more so in the fast posterior latissimus dorsi (Fig. 1). It can be seen from Fig. 1 that (a) reciprocal relations exist between concentrations of glycogen on the one hand and of RNA and proteolytic activity of muscles of older animals on the other, and (b) the differences in glycogen content are already established on the first day of life, whereas the differences in RNA content and proteolytic activity are established progressively during later development. RNA content of the anterior latissimus dorsi is greater by 40 per cent, and proteolytic activity greater by 200 per cent, than that in the posterior latissimus dorsi of 1-month-old animals. These differences, however, are established relatively late.

The relatively larger concentrations of RNA and proteolytic enzymes in the slow anterior latissimus dorsi would then suggest a faster turnover of proteins in slow muscles, probably related to the long-term maintenance metabolism

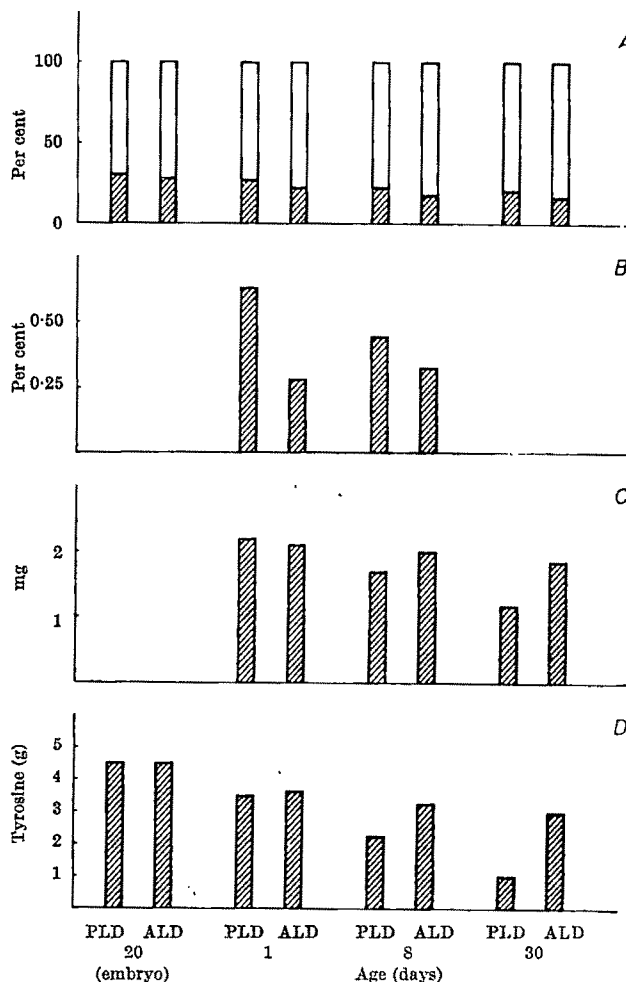


Fig. 1. Developing chick muscles. (A) Contribution of myofibrillar-nuclear (white columns) and sarcoplasmic (hatched columns) fractions to total proteins in muscles; (B) content of glycogen (percentage wet weight); (C) content of RNA (mg/100 mg of protein); (D) proteolytic activity (μg of tyrosine liberated/mg of protein). Each value is the mean from six animals. PLD, Posterior latissimus dorsi; ALD, anterior latissimus dorsi.

required for posture and maintenance of tension. A quicker rate of incorporation of amino-acids into proteins has indeed been found in both, the slow mammalian and the slow (tonic) avian muscles (work to be published by Gutmann and Hájek).

Glycogen concentrations are higher in the adult fast mammalian<sup>4</sup> and the fast (phasic) muscle fibres of birds and this apparently corresponds to the adaptation of the fast muscles, utilizing especially glycolytic mechanisms. Contrary to chick muscles, glycogen contents in fast and slow muscles of rats are equally high post-natally; differentiation occurs between 11 and 14 days after birth<sup>4</sup>. The differences in development of glycogen concentrations between fast and slow mammalian and fast (phasic) and slow (tonic) avian muscles are apparently related to the relatively later maturation of the motor system in rats.

The data suggest that differentiation may involve two different mechanisms, one essential for the fast muscle and reflecting a predominance of glycolytic processes, the other essential for the slow muscle and reflecting a faster turnover of proteins. It appears that the basic pattern of the two types of muscles is of a general nature and applies to the muscle of both birds and mammals.

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### Electrolyte Imbalance as the Mechanism for Inert Gas Narcosis and Anaesthesia

THE mechanism by which the volatile anaesthetics induce narcosis and anaesthesia remains the subject of speculation. Similarly, there has been much controversy concerning the mechanisms by which raised pressures of air or the chemically inert noble gases xenon, krypton, argon, neon and helium produce euphoria or loss of consciousness. Among the possibilities considered which could interfere with cerebral electrical activity have been a histotoxic hypoxia, depression of metabolism, membrane stabilization or block, interference with the sodium pump mechanism, increased production of inhibitor substance, interference with adenosine triphosphate production<sup>1</sup> and the formation of clathrates<sup>1-6</sup>.

There is, however, agreement that the site of action is at polysynaptic sites in the central nervous system, caused by interference with synaptic transmission rather than on propagated action potentials<sup>6,7</sup>.

McIlwain<sup>8</sup> has suggested a hypothesis by which the biochemical and physical theories of interference with synaptic transmission can be linked. It suggests that anaesthetics block ionic movement by affecting the cell membrane which in turn causes a fall in oxygen consumption and a rise in energy stores. Paton and Speden<sup>7</sup> support the hypothesis provided that the ionic movements considered are those concerned with the release and action

of chemical transmitters at the synapse. Mullins's theory<sup>9</sup> also supports an ionic block. The hypothesis is that the molecules of the inert gas occlude pores in the cell membrane, thereby preventing the passage of sodium during the initial phase of the generation of an action potential.

As a result of investigations on the action of 'n' alkyl alcohols, chloroform and ether on the film pressure or surface tension and the cation permeability of phospholipid model membranes, it was suggested that, on the contrary, "narcosis represents a transient reversible increase in membrane permeability to cation"<sup>10</sup>. The increased permeability is considered to be a result of the adsorption of narcotic agents into the cellular interfaces affecting the dielectric constant and changing the state of the membrane causing increased cation permeability.

An affinity of narcotic agents, including nitrous oxide, for interfacial films has been reported<sup>11</sup> and it was concluded that "inert gases at partial pressures, sufficient to bring about a standard effect in a biological system, act on a lipoprotein-water interface to cause a standard decrease of 0.39 dyne/cm in the interfacial tension".

Other experiments<sup>12</sup>, also with phospholipid model membranes, have established that increased pressures of nitrogen and a number of the noble gases also penetrate lipid membranes; the extent of the adsorption for the production of narcosis is in agreement with the rule of Clements and Wilson. Evidence was also obtained from these experiments that such penetration of lipid membranes may cause increased permeability of cations.

In the present experiments, the action of inert gases on sodium, potassium and chloride ions in the cerebral spinal fluid of cats exposed to narcotic and non-narcotic inert gases at raised pressures was investigated in order to determine the effect of such narcosis on ionic permeability *in vivo*. Under chloralose anaesthesia (55-60 mg/kg) a 3/8-in. hole was trephined in the skull of an adult cat and the dura reflected. A short, thin gauze wick from the hole led to a small plastic cup, in which was collected the cerebral spinal fluid. In addition, two stainless steel electrodes were screwed into the skull. One was placed over the frontal sinus as a reference and one a 0.5-in. lateral to the midline and a 0.25-in. posterior to the frontal-parietal suture. Natural auditory stimuli were produced by sharp raps on the side of the pressure chamber into which the cat was placed after the initial surgery. The resulting evoked potentials at the cortex were amplified and recorded on a Beckman 'Dynograph'.

For the first hour the animal remained at atmospheric pressure breathing air while auditory stimuli were presented every 3 min and the cerebral spinal fluid was collected. At the end of the hour, the full plastic cup was replaced and the chamber compressed to 11 absolute atm. with either 20/80 oxygen and nitrogen, oxygen and argon or oxygen and helium for a second hour. Again evoked potentials were presented and the cerebral spinal fluid was collected. After 1 h at the increased pressure, the cat was rapidly decompressed and the cup containing the cerebral spinal fluid was removed. In addition, control experiments were carried out where the second hour was also at atmospheric pressure.

Sodium and potassium ions in the cerebral spinal fluid were determined by flame photometry using dilutions of 1 in 20 for potassium ion and 1 in 500 for sodium ion against appropriate standards. Chloride ion was determined by the mercuric nitrate titration method of Schales and Schales<sup>13</sup>. The measurements were made by an individual who was unaware of the pressures or gases to which the cats had been subjected. The degree of narcosis produced in the cat by the inert gas was determined by measurement of the initial negative potential of the evoked response as described elsewhere<sup>14</sup>.

The mean electrolyte values in five cats during the first hour at atmospheric pressure are shown in Table I. The mean changes, during the second hour, while the cats were

exposed to various inert gases at increased pressures, are shown in Table 2. The control values are in agreement with existing data<sup>15</sup>. The only electrolytes to show a significant change from the controls were sodium and chloride ion in the cats exposed to either the nitrogen and oxygen or argon and oxygen mixture. These ions showed a significant depression in the cerebral spinal fluid. Potassium was not significantly affected throughout.

Table 1. MEAN VALUES FOR SODIUM, POTASSIUM AND CHLORIDE IONS IN CEREBRAL SPINAL FLUID OF FIVE CATS AT ATMOSPHERIC PRESSURE

	m.equiv.	S.E.M.
Sodium	177.8	± 4.5
Potassium	3.42	± 0.25
Chloride	147.6	± 4.6

As can be predicted from earlier work, narcosis was present only in cats exposed to either nitrogen or argon<sup>8,14,16</sup>. The evoked potentials were depressed by 18.6 per cent for nitrogen and 44.7 per cent for argon. In cats exposed to either helium or air at atmospheric pressure during the second hour there was no significant change in the evoked potentials. A similar electrolyte imbalance has been reported in guinea-pigs exposed to 6.6 absolute atm. oxygen during oxygen toxicity<sup>17</sup>. A significant increase in brain sodium ion occurred but potassium ion remained unchanged. In the present experiments the oxygen partial pressure at 2.2 absolute atm. is too low to be the cause of the electrolyte imbalance. Furthermore, that helium, with the same oxygen-partial pressure as the nitrogen and argon mixtures, did not affect the electrolytes further implicates the inert gas constituents as the cause.

Neurophysiological<sup>8</sup> and pharmacological investigations<sup>18</sup> have suggested that both oxygen and nitrogen or other inert gases can produce their toxic effects by similar mechanisms. In both conditions a hyperexcitability of the neurones develops, caused presumably by the influx of sodium ion. In the case of oxygen toxicity, however, the increased excitability eventually develops into oxygen convulsions whereas with the inert gases it develops into a depression of neurone activity and euphoria or anaesthesia.

The reasons for this difference remain to be elucidated, but it could be significant that as regards oxygen toxicity there is a reduction of the inhibitory transmitter  $\gamma$ -aminobutyric acid in the brain<sup>19</sup>.

Adrenalectomized animals similarly show increased brain excitability with an increase in brain sodium ion but no change in potassium ion<sup>20,21</sup>. The reason for this difference was resolved with the demonstration of a change in "exchangeable" potassium after adrenalectomy<sup>22</sup>. In normal animals only 80 per cent of brain potassium is "exchangeable" possibly because of membrane impermeability at either the cellular or intracellular level, or perhaps because potassium is chemically bound by brain lipids. After adrenalectomy, however, 100 per cent of the brain potassium becomes exchangeable.

It is therefore concluded that inert gas narcosis and anaesthesia are caused by the adsorption of the narcotic agent on cell membranes, which affects their permeability to cations and causes a reversible increase in intracellular ions. In addition there may be a modification of the distribution of intracellular potassium in brain neurones.

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## BIOLOGY

### Finger-prints in Schizophrenia

THE investigation of epidermal ridges (dermatoglyphics) by prints of the fingers, palms and soles has found increasing application in genetic research. Dermatoglyphic investigations in schizophrenia<sup>1-7</sup> have been largely confined to the qualitative analysis of the finger-prints into pattern types. The results have been markedly inconsistent. The present investigation has attempted to resolve these inconsistencies by using a quantitative method of finger-print analysis, ridge counting. The sampling methods and diagnostic criteria, not described in the previous investigations, have been defined.

The 485 subjects were in-patients from four mental hospitals and all were of British ancestry. A one in three random sample was taken of patients, who had been continuously in hospital for more than 5 yr. They fulfilled the clinical criterion of being classifiable, according to Leonhard<sup>8</sup>, as chronic systematic schizophrenics. There were 155 males and 157 females in this chronic group. The acute patient group comprised seventy-seven males and ninety-six females, seen within 2 days of admission to hospital, and with K. Schneider's first rank symptoms of schizophrenia<sup>9</sup>. "Rolled" finger-prints were taken by an inkless method. The technique and rules for ridge

Table 2. MEAN CHANGE FROM CONTROLS OF SODIUM, POTASSIUM AND CHLORIDE IONS IN CEREBRAL SPINAL FLUID OF CATS EXPOSED TO 10 ABSOLUTE ATM. OF INERT GAS/OXYGEN

	Air at atmospheric pressure		20/80 Oxygen and helium		20/80 Oxygen and nitrogen		20/80 Oxygen and argon	
	Mean (m.equiv.)	S.E.M.	Mean (m.equiv.)	S.E.M.	Mean (m.equiv.)	S.E.M.	Mean (m.equiv.)	S.E.M.
Sodium	+7.6	± 4.4	+4.8	± 2.1	-14.2	± 3.1*	-11.2	± 3.4*
Potassium	-0.1	± 0.22	-0.1	± 0.25	-0.54	± 0.18	+0.14	± 0.29
Chloride	+0.6	± 1.4	+0.6	± 0.7	-9.6	± 3.0†	-6.0	± 2.7‡
No. of cats	5		5		5		5	

\*  $P > 0.01$

†  $P 0.05 - 0.02$

‡  $P 0.05$

counting<sup>10</sup>, the enumeration of the ridges between the triradius and core of the finger-print pattern, were followed. The sum of the ridge counts from all ten fingers is designated the total finger ridge-count. Its genetics and distribution have been extensively investigated in the normal British population<sup>11</sup>. This source provides the normal values for total finger ridge-counts cited here.

The means and standard deviations of the total finger ridge-counts, for the schizophrenic and normal samples, are given in Table 1. The measures of skewness ( $g_1$ ) and kurtosis ( $g_2$ ) of the distributions are also given, with their standard errors.

Table 1. DISTRIBUTION OF TOTAL FINGER RIDGE-COUNT IN SCHIZOPHRENIC AND NORMAL SAMPLES

Subjects	No.	Mean	S.D.	Skew ( $g_1$ )	Kurtosis ( $g_2$ )
Male					
Normal	825	144.98	51.08	-0.290 ± 0.085	-0.274 ± 0.171
Schizophrenic	232	132.24*	45.01	-0.152 ± 0.161	+0.112 ± 0.322
Female					
Normal	825	127.23	52.51	-0.230 ± 0.085	-0.451 ± 0.171
Schizophrenic	253	134.20	49.07	-0.217 ± 0.154	+0.110 ± 0.308

\* Significance of difference from normal mean  $P < 0.001$ .

The difference between the means of normal and schizophrenic males is highly significant ( $t = 3.695$ ,  $P < 0.001$ ), but the difference between females does not reach a significant level. There is a highly significant difference between the means of the normal males and females. This sex difference, which holds for most racial groups<sup>12</sup>, is abolished in the schizophrenic sample. Unlike the normal samples, the schizophrenic distributions are not platykurtic, nor is the negative skew significant.

These results are similar to the findings of the earlier investigators<sup>1-3</sup>, who found that the normal sex difference in the frequency of finger-print patterns was absent in their schizophrenic samples.

Analysis of variance of the schizophrenic total ridge-counts in terms of the diagnostic sub-categories, and acuteness, or chronicity, does not reveal any significant differences. The means of the diagnostic sub-categories, hebephrenic, catatonic, and paranoid schizophrenia, however, are noteworthy, because these results show a consistent pattern in both sexes. These means, with their standard errors, are given in Table 2.

Table 2. MEAN TOTAL FINGER RIDGE-COUNT IN SCHIZOPHRENIC SUB-CATEGORIES AND DIFFERENCE FROM NORMAL MEANS

Subjects	No.	Mean	Difference from normal	Significance of difference
Male*				
Normal	825	144.98 ± 1.78		
Hebephrenic	121	133.11 ± 4.56	-11.87	$P < 0.05$
Paranoid	85	136.24 ± 4.38	-8.74	N.S.
Catatonic	26	115.08 ± 9.98	-29.90	$P < 0.01$
Female†				
Normal	825	127.23 ± 1.78		
Hebephrenic	93	139.11 ± 4.91	+11.88	
Paranoid	127	134.80 ± 4.16	+7.57	
Catatonic	33	117.25 ± 8.42	-9.98	

\* Analysis of variance of means, differences significant,  $P < 0.01$ .

† Analysis of variance of means, differences not significant, therefore comparison with normal not made.

The means of paranoid and hebephrenic schizophrenics deviate towards the normal mean of the opposite sex. The deviations of the means of the hebephrenics, clinically the more severely affected, are greater. The means for catatonics of both sexes are lower than normal and are of the same order. These findings suggest that previous discrepant results could be a result of variation in the proportions of the three sub-categories in the total schizophrenic samples.

An explanation of the results of this investigation must be sought in genetic terms, for the total finger ridge-count is almost wholly genetically determined<sup>11</sup>. Current theories of schizophrenia, recently summarized<sup>13</sup>, favour a monogenic mode of inheritance, with environmental and other genetic factors modifying the expression and penetrance of the major gene. It is suggested that there is an association between the total finger ridge-count and

genes modifying the expression of the schizophrenic phenotype.

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## Salivary Secretion in the Cattle Tick as a Means of Water Elimination

MANY observations have indicated that the engorgement of bloodsucking arthropods is accompanied by the elimination of excess water resulting in the concentration of the blood-meal. This water may be rapidly eliminated during or after feeding by the Malpighian tubules of mosquitoes<sup>1</sup>, tsetse flies<sup>2</sup> and bugs<sup>3</sup>, or by the coxal gland of argasid ticks<sup>4,5</sup>. This results in the ingested blood protein concentration being trebled, as in *Culex*<sup>6</sup>, or doubled as in the case of *Ixodes* and *Ornithodoros*<sup>5</sup> and *Argas*<sup>7</sup>, while the data of Kitaoaka<sup>8</sup> and Maddrell<sup>9</sup> also suggest a doubling for *Boophilus microplus* and *Rhodnius prolixus*.

During the final rapid engorgement of the cattle tick, *Boophilus microplus* (Canestrini), the weight of an adult female tick increases from 10 mg to more than 200 mg in less than 12 h, and recent work has confirmed that some concentration of the ingested blood occurs in this period. The degree of concentration is determined by the concentration of the haemoglobin of the host's blood (the relationship to the host haematocrit, although obvious, is less well marked because of variations in the erythrocyte haemoglobin levels). This was demonstrated by quantitative spectrophotometric analyses at 577 mμ of ingested oxyhaemoglobin from recently detached ticks. Fig. 1 shows that ticks from host animals with a low haemoglobin concentration had more than doubled the concentration of the ingested blood, whereas those from

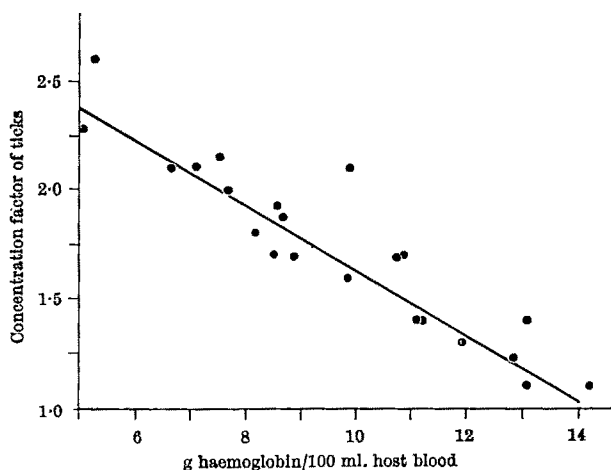


Fig. 1. The relationship between the degree of concentration by the tick of its blood meal and the host blood haemoglobin concentration.

hosts with a high haemoglobin concentration had produced a lower degree of concentration.

It was established, by enclosing engorging ticks on the host in small polythene sheaths, that neither excretion through the Malpighian tubules nor transpiration across the cuticle was responsible for the water loss. In any case, the cuticular water loss from a fully engorged adult was shown to be no more than 10–15 mg for the time of the final 12 h period of engorgement. A possible mechanism suggested by Mr. J. D. Gregson (personal communication), however, was that the dilute clear oral secretion, which he had observed in *Dermacentor andersoni*<sup>10</sup>, was responsible for returning excess water to the host.

To test this hypothesis, eight ticks, 5–6 mm in length, which were already attached to a 16 month old calf (weight, 150 kg) were each injected at 16.00 h with 4.4  $\mu$ l. of 5 c./ml. (Code No. TRS. 1, Radiochemical Centre) tritiated water (sodium chloride added to 1 per cent). Any material which exuded from the puncture was absorbed with filter paper and immediately stored in water to enable the measurement of this loss of radioactivity from the tick. Under natural conditions these ticks would have engorged and detached by 09.00 h the next day. The ticks were therefore enclosed in open boxes with gauze coverings (to allow free exchange of gases and water vapour) stuck to the hide of the host. The following morning three ticks had engorged fully and detached, while the remainder were unchanged. Table 1 shows the recovery of 75 per cent of the injected radioactivity, of which 26 per cent was obtained from the calf, either from the urine or from a blood analysis extrapolated to an estimated total body water of 70 per cent. Tritium losses can be expected in expired water vapour, perspiration and faeces, which were not examined. It can also be seen that those ticks which did not engorge retained a considerable amount of the injected radioactivity. It is therefore concluded that during engorgement large amounts of water which have passed across the gut epithelium into the haemolymph are returned to the host in the salivary secretion of the tick. In the case of a tick engorging to a weight of 250 mg on a host with a haematocrit of 33 per cent it can be seen that this would be in excess of 200  $\mu$ l. of water, representing 60–70 per cent of the host blood water.

Table 1. PERCENTAGE RECOVERY OF 176 MC. TRITIATED WATER INJECTED INTO EIGHT TICKS (LIQUID SCINTILLATION COUNTING USING PPO IN ETHANOL-TOLUENE)

Source	Tritium (mc.)	Per cent of total injected
Five unengorged ticks	73.3	41.6
Three engorged ticks	3.8	2.2
Exudate from punctures	9.1	5.2
Host body water	23.9	13.6
Host urine	21.3	12.1
Total	131.4	74.7

Further confirmation of the water excretory function of the saliva has been provided by determinations of the chloride concentrations of oral secretion (collected by Gregson's technique<sup>10</sup>), haemolymph and whole engorged ticks.

The chloride levels of the oral secretion and haemolymph were found to be approximately equal at 110–130 m.equiv./l. Haemolymph chloride concentration will be maintained by Donnan equilibrium at a higher level than that of the gut contents. In consequence, the secretion of isotonic chloride in the saliva will result in the progressive elimination of chloride from the tick and the avoidance of the osmotic imbalance which would result from the loss of water alone.

A variety of other substances of probable host origin such as urea and histamine are also found in the oral secretion and it seems likely that they, too, are being eliminated by this method.

Thus it would seem that in *B. microplus* and probably other Ixodidae the blood meal is concentrated, and electrolytic and osmotic imbalance avoided by a remark-

able adaptation of the salivary secretion. The effect of host haemoglobin levels on the degree of concentration of the blood ingested by the cattle tick is interesting and may be significant in the feeding of other blood-sucking arthropods. The general similarity in the proportion of water eliminated from the blood meals of different blood-feeders, despite their different metabolic and ecological requirements, may be significant also. It perhaps indicates that this phenomenon is controlled by physical factors which limit the ability of water to leave the blood mass and cross the gut epithelium.

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### Differences in Light Reactions of Larvae of the Armyworm, *Leucania separata* Walker, in relation to their Phase Status

THE coloration, rate of development and behaviour of the larvae of the armyworm, *Leucania separata* Walker, differ when the larvae are subjected to different degrees of crowding during development. Active black larvae appear in crowded conditions and develop more rapidly, feed more voraciously, and tolerate starvation and less palatable food-plants better than the sluggish pale larvae that appear in uncrowded conditions. This phenomenon has much in common with phase polymorphism in locusts<sup>1</sup>.

The armyworm is normally a nocturnal feeder in the latter half of its larval stage, but hordes of larvae often feed and move by day as well. We have investigated whether this change in daily rhythmic activity is related to internal changes associated with phase transformation.

Pale larvae reared in isolation, and black larvae reared in crowds of twenty individuals/container, were used. Tests were carried out in the dark at 30° C and relative humidity about 80 per cent. Each larva tested was released 70 cm from a 5 W lamp on a 90 cm x 60 cm black board. A considerable number of mid- and late-instar larvae travelled towards the lamp. The time required for each larva to crawl from the starting point to the line on which the lamp was placed was recorded. Table 1 shows that black larvae from crowded cultures travelled more rapidly to the line than the pale larvae reared in isolation. Several larvae, especially pale ones, never reached the goal line; they either moved in another direction or remained motionless. This difference in reaction became clearer as the larvae developed (Table 1, instars 3, 4 and 5).

The light reaction of larvae of the fifth and sixth instars was investigated. The larvae made six kinds of tracks (Fig. 1). Tracks A and B were regarded as photopositive reactions, although some larvae changed their route after

Table 1. TIME REQUIRED BY PALE AND BLACK LARVAE OF *L. separata* TO TRAVEL TO A POINT SOURCE OF DIM LIGHT

Instar	Type of larva	No. of larvae tested	Time required (min)*				
			0-1	1-2	2-3	3-4	Not reached†
Third	Pale	20	0	5.0	50.0	15.0	30.0
	Black	29	0	24.1	44.8	10.3	20.7
Fourth	Pale	30	0	30.0	33.3	10.0	26.7
	Black	39	0	48.7	38.5	5.1	7.7
Fifth	Pale	30	3.3	13.3	20.0	6.7	56.7
	Black	20	15.0	45.0	15.0	5.0	20.0

\* Numbers of larvae are shown as percentage frequencies.

† This column includes larvae that remained motionless and those that moved elsewhere than in the direction of the lamp.



Table 2. REACTION TO LIGHT OF PALE AND BLACK LARVAE OF *L. separata* DURING THE FIFTH AND SIXTH INSTARS

Instar	Type of larva	No. of larvae tested	A	B	C	D	E	F†
Fifth : first day	Pale	27	0	7.4	0	33.3	18.5	40.7
	Black	23	47.8	17.4	0	13.0	0	21.7
Sixth : first day	Pale	41	2.4	14.6	0	31.7	22.0	29.3
	Black	49	14.3	24.5	14.3	34.7	8.2	4.1
Sixth : second day	Pale	43	0	14.0	2.3	30.2	11.6	41.9
	Black	35	34.3	5.7	28.6	20.0	2.9	8.6
Sixth : third day	Pale	9	0	11.1	0	55.6	0	33.3
	Black	11	18.2	18.2	9.1	54.5	0	0

\* Shown as percentage frequency.

† Letters refer to types of tracks illustrated in Fig. 1. A, B, and C are photopositive, D probably and E always are photonegative, and F indicates virtual immobility.

reaching the vicinity of the light source and then passed by it (B). Some photopositive individuals circled for a time on their way to the light or compassed around it (C). Some larvae left the side of the arena (D), and many of these showed photonegative behaviour when placed near the lamp. Track E shows a more typical, initially photonegative reaction, and track F shows the larvae essentially motionless in the arena, with or without a brief period of movement.

Table 2 shows that black larvae were consistently more active and more photopositive than pale larvae. In the sixth instar, however, the percentage of black larvae that took track C increased, and by the third day in that instar the percentage that could be termed truly photopositive had decreased considerably. This change could be associated with the onset of the period of prepupal travel. On the other hand, the percentages of pale larvae that remained immobile or reacted photonegatively were large in both instars.

The response of black larvae to light is not always a simple photopositive reaction, but they do respond to the position of a point source of dim light while they move. On the other hand, those pale larvae that were not photonegative seem to be disorientated. Both types of larvae were repelled by a brighter light, so that daytime activity of the larvae in outbreak conditions could be related to some other factor(s), such as starvation. This difference in response to light indicates, however, that there is some internal difference between pale and black larvae and that it may be an underlying cause of the differential daily rhythms of larvae at low and high population densities.

The greater activity of black larvae and their greater ability to orientate to a light source suggest that they have better developed exploratory behaviour than pale larvae. This seems compatible with Kennedy's hypothesis concerning locust phases<sup>2</sup>, in the sense that black larvae (corresponding to ph. *gregaria* in locusts) are more specialized in a "sensorimotor" direction.

It is also interesting that the differences in the efficiency of orientation of both the high-density (black) and the low-density (pale) phases of the larvae (see tracks A and B, track F and possibly track C, dotted, in Fig. 1) are similar to those observed between the active and sluggish larvae of species of *Malacosoma*<sup>3,4</sup> in which maternal

nutrition is partly responsible for the determination of types of the progeny larvae<sup>5</sup>. Table 2 illustrates this resemblance. This indicates that in *L. separata*, and possibly in some other species, the behavioural differences as seen in *Malacosoma* can be brought about by different degrees of crowding during larval development. At the same time, the differences in efficiency of orientation within the larvae of each phase suggest that some other factor, such as nutritional conditions or the degree of crowding in the parent generation or a genetic factor, could also be involved in the partitioning of larvae into a series of behavioural types.

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## Fallopian Tube and Early Cleavage in the Mouse

TECHNIQUES have been perfected for the culture *in vitro* of late two cell mouse ova into blastocysts in simple chemically defined conditions<sup>1,2</sup>. Attempts to obtain development from earlier stages have failed, unless the ova were cultured in organ cultures of the fallopian tube<sup>3,4</sup>. These results suggested that the fallopian tube provides some critical contribution necessary for the development of the mouse zygote into the late two cell stage. Nevertheless, it has been a common experience that when the mouse zygote is cultivated independently of the fallopian tube a few cleave to the two cell stage and then cease development<sup>5</sup>. It has now been shown<sup>6</sup>, however, that a high percentage of zygotes cleave to two cell stages and then cease development when cultured in Waymouth<sup>7</sup> medium supplemented with ATP, deoxynucleosides and a feeder layer of irradiated HeLa cells. These experiments suggest that the initial development of the mouse zygote is dependent on a supply of exogenous factors, additional to those required by later stages.

This report presents evidence that zygotes can develop *in vitro* into two cell ova, independently of the fallopian tube, in the same simple culture conditions sufficient to support the development of late two cell ova, and, in addition, that the two cell ova produced in this way are viable and capable of further development only if they are exposed to the environment of the fallopian tube between the first and second cleavage divisions.

Fertilized one cell ova were obtained from the ampullary regions of fallopian tubes of superovulated 7-8 week old random bred Swiss mice on the morning the vaginal plugs were found. The mice were superovulated by an intraperitoneal injection of 10 IU of pregnant mare serum gonadotrophin ('Gestyl', Organon) followed 48 h later by an intraperitoneal injection of 10 IU of human chorionic gonadotrophin ('Pregnyl', Organon). The cumulus cells were removed with hyaluronidase (300 U/ml. of Dulbecco's phosphate buffered salt solution<sup>8</sup> (pH 7.2) to which 1 mg/ml. of polyvinylpyrrolidone was added). The ova were then washed in several changes of a pyruvate : lactate medium<sup>9</sup>, and finally set up in cultures according to the previously described method of Brinster<sup>1</sup>. Twenty-four hours later the number of ova which had undergone the

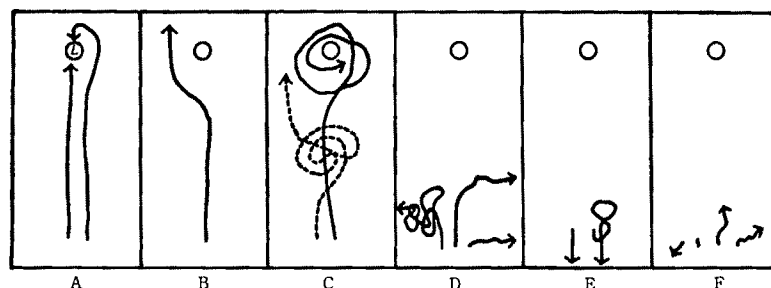


Fig. 1. Schematic representation of the paths of larvae of *L. separata* in relation to a point source of light (L).

Table 1. DEVELOPMENT OF TWO CELL OVA FROM MOUSE ZYGOTES AFTER CULTURING *in vitro* FOR 24 H

Experiment	No. of one cell ova cultured	No. of two cell ova 24 h later	Percentage of two cell ova
1	120	74	61.7
2	136	90	66.2
3	298	206	69.1
4	504	320	63.5
5	540	330	61.1
Total	1,598	1,020	63.8

Ova were cultured in a medium containing both lactate and pyruvate\*.

\* One cell ova collected 20-22 h after the injection of human chorionic gonadotrophin.

Table 2. DEVELOPMENT OF TWO CELL OVA FROM MOUSE ZYGOTES AFTER CULTURING *in vitro* FOR 24 H WITH AND WITHOUT AN ENERGY SOURCE

Medium	No. of one cell* ova cultured	No. of two cell ova 24 h later	Percentage of two cell ova
No energy source	192	0	0
Pyruvate : lactate	192	143	74.5

\* One cell ova collected approximately 24 h after injection of human chorionic gonadotrophin.

first cleavage division was noted. In five experiments (Table 1) 60 per cent to 70 per cent of the uncleaved ova proceeded through the first cleavage division. This yield was consistent between all experiments, and is of the same order as that observed by Cole and Paul<sup>6</sup>.

In another two experiments one cell ova were cultivated simultaneously with and without an energy source in the medium. The pooled results (Table 2) demonstrate unequivocally that development through the first cleavage division is dependent on an exogenous source of energy.

Two cell ova from the first three experiments (Table 1) were transferred into organ cultures of the ampullary regions of fallopian tubes, which were obtained from 7-8 week old random bred Swiss mice in early metoestrus I. The oestrous cycles were synchronized by the intra-peritoneal injection of 5 IU of pregnant mare serum gonadotrophin ('Gestyl', Organon) and 5 IU of human chorionic gonadotrophin ('Pregnyl', Organon) after 48 h. Twenty to twenty-two hours after the second injection the fallopian tubes were removed, carefully uncoiled and transected in the approximate region of the ampullary isthmal junction. The unfertilized ova within the cumulus mass were then flushed out of the ampulla. This ampullary region was explanted on Ham F10 medium<sup>9</sup> supplemented with 100 U/ml. of penicillin G (potassium salt) and 50 µg/ml. of streptomycin sulphate, which had previously been found to be the best medium for maintaining the fallopian tubes in organ culture (unpublished work of Whittingham). Chen's method<sup>10</sup> of organ culture was used. Each ampulla was cultivated on a raft of tea-bag paper floating on 0.5 ml. of medium. The medium was renewed after 48 h by transferring the tea-bag paper plus ampulla to a fresh culture chamber. The cultures were incubated at 37° C in a desiccating cabinet through which humidified 5 per cent carbon dioxide in air flowed continuously. Twelve ova were transferred to each ampulla approximately 1 h after the ampullae were placed in organ culture and left for 72 h. The ampullae were then flushed and the number of blastocysts and morulae developing from these two cell ova counted (Table 3). The results show clearly that the two cell ova produced from zygotes *in vitro* are capable of further development when transferred to the ampulla of the fallopian tube.

The viability of blastocysts obtained by the procedures just described was examined by transferring them into uterine foster-mothers. Albino Swiss blastocysts were

Table 3. DEVELOPMENT OF BLASTOCYSTS FROM CULTURED TWO CELL OVA TRANSFERRED INTO EXPLANTED AMPULLAE FOR 72 H

Experiment	No. of two cell ova transferred	No. cleaved*	No. of morulae	No. of blastocysts	Percentage of blastocysts
1	60	33	2	27	45.00
2	60	38	5	21	35.00
3	120	65	11	40	33.3
Total	240	136	18	88	36.7

\* Number of two cell ova which have cleaved at least once; this includes those ova which have developed to morulae and blastocysts. Degenerated or fragmented ova were discarded.

transferred into pseudopregnant nulliparous *F*<sub>1</sub> hybrids (*C3H* female × *DBA* male) 2.5 days after ovulation, using methods already described<sup>11</sup>. Seven blastocysts were transferred into the left uterine horn of each of thirteen recipients. On the seventeenth day the uterine contents were examined and four were found to be pregnant. Altogether six apparently normal fetuses and five resorption sites were found. These results are comparable with those obtained by the transfer of blastocysts cultured from the late two cell stage. Thus embryos which have undergone their first division *in vitro* independently of the fallopian tube are capable of development into normal fetuses.<sup>6</sup>

These results demonstrate that the development of the mouse zygote to the two cell stage *in vitro* is dependent on an exogenous source of energy, as are the later stages leading to the development of the blastocyst. Presumably, in natural conditions this energy supply is provided by the mother. The notion<sup>12</sup> that the cytoplasm of mammalian ova contains sufficient nutrients to sustain zygotic development cannot be upheld. Whether the development of the two cell ovum requires an exogenous nitrogen source, as do later cleavage stages<sup>13</sup>, has still to be determined. Nevertheless, the results show that this early stage of development does not require any factors additional to those of late two cell stage. The results also show that the environment of the fallopian tube is only required to promote development in the earlier phase of the period between first and second cleavage. The nature of the contribution of the fallopian tube is unknown.

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### Dose Rate Effect of Radiation on Spermatogonia of the Silkworm

IN mice the mutation rate in irradiated spermatogonia is less at low dose rates (0.009 r./min) than at high dose rates (90 r./min and more)<sup>1,2</sup>. Tazima *et al.*<sup>3</sup> have observed that the mutation frequency for chronic irradiation is higher than that for acute irradiation when the silkworms are irradiated about 7 days after hatching. To explain such a peculiar dose rate effect, various hypotheses<sup>3,4</sup> have been proposed. In this communication, the effect of dose rate on death and repopulation of spermatogonia is cytologically analysed with particular reference to the possibility of selective elimination of the cells after

exposure to different dose rates of radiation. The spermatogonia of the silkworm are readily killed by acute irradiation<sup>5,6</sup>.

For the chronic irradiation, cobalt-60  $\gamma$ -rays were administered continuously for 122 h from 5 to 10 days post hatching, with a total dose of 1,000 r. (0.136 r./min). The acute irradiation was carried out 7 days after hatching with a dose rate of 200 r./min for 5 min. Every 24 h after the initiation of chronic irradiation or the completion of acute irradiation, the testes of the larvae in each experimental group were fixed with PFA-3 fixative, embedded in paraffin, and sections 7  $\mu$  thick were stained with Mayer's acid haemalum. Both pycnotic and non-pycnotic spermatogenic cells in the testes were scored on serial sections of the specimens under the microscope. As indicated in Fig. 1, in the group which received chronic irradiation pycnotic figures of the spermatogonia began to appear 24 h after the initiation of exposure and increased with time, showing a maximum at 72 h. Thereafter, pycnosis decreased and disappeared almost completely before the exposure was terminated. In the testes which were acutely irradiated, however, pycnotic spermatogonia had already reached a maximum level 48 h after irradiation. Afterwards they decreased rapidly. On the other hand, the number of non-pycnotic spermatogenic cells (surviving or newly formed cells) was reduced remarkably after both acute and chronic irradiations. In both cases, however, the apical cells, primordial germ cells and some very young spermatogonia were left intact. The number of such surviving cells remained constant for a while, indicating inhibition of cell division. After several days spermatogonia began to regenerate steadily at both dose rates. The cells then increased in number rapidly and reached the control level by the end of the fourth instar. Throughout the experiment, it can be said that the extent of spermatogonial death and the number of surviving or

newly formed cells is approximately similar at the two very different dose rates used in this experiment, except for the initial cell degeneration after the exposures.

We conclude that cell selection is not involved in the dose rate effect on mutation frequency in the spermatogonia of the silkworm. The same conclusion was reached for the dose rate effect in mouse spermatogonia<sup>3,7-9</sup>. It should, however, be noted that in the silkworm the surviving spermatogonia remain in a state of arrest for several days after irradiation. Possibly, the surviving cells are arrested in a stage of development in which they are particularly sensitive to mutation by radiation. In this respect Sado<sup>10</sup>, using the uptake of <sup>3</sup>H-thymidine, reported that surviving cells were accumulated at G2 of the division cycle after irradiation. More detailed experiments, however, are necessary before any firm conclusion can be drawn.

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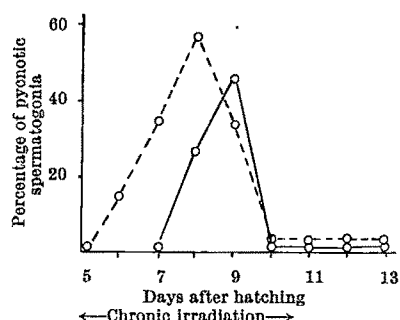


Fig. 1. Changes in percentage of pycnotic spermatogonia after acute (—) and chronic (---) irradiations. Acute irradiation was carried out on the seventh day after hatching.

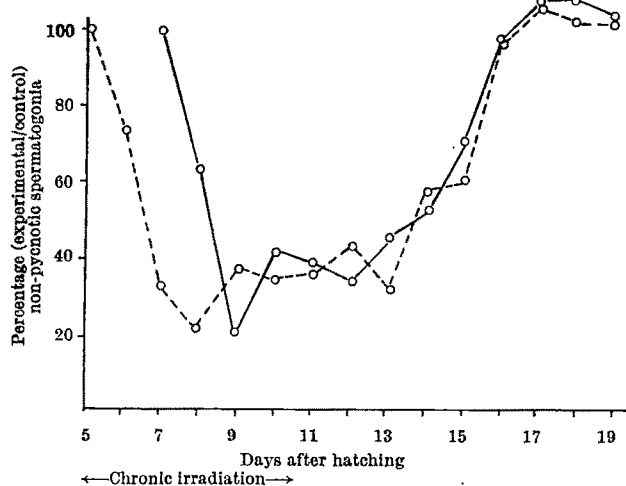


Fig. 2. Changes in experimental/control percentages for non-pycnotic spermatogonia after acute (—) and chronic (---) irradiations. Acute irradiation was carried out on the seventh day after hatching.

## Culture of Tomato Callus Tissue

REPORTS on the growth and division of plant cells in culture media have had various objectives: to establish the totipotency of the diploid cell<sup>1-4</sup>; to grow the whole plant from an isolated single cell, in a defined medium without neighbouring cells or tissue, a goal very recently achieved<sup>5,6</sup>; to use a defined medium, as distinct from a conditioned medium, that is a medium on which cells have previously been grown<sup>5-7</sup>; to explore the nature and mechanism of factors inherent in coconut milk<sup>1,8,9</sup> or pea<sup>10</sup> which promote cell division.

In our case, the purpose is to extend the time available for investigation of changes in pigmentation in the fruit of the ripening tomato, which can be short. This is especially true of fruit containing the gene *lutescent* (*l*). On one day it may appear a normal ripe yellow; two days later it may have turned red. Chlorophyll synthesis has been noted in masses of endive callus<sup>4</sup>. Accepting the view that the cell is totipotent, we believe that it should be possible, with masses of callus, to investigate the nutritional requirements and physical environment for carotenoid synthesis in tomato fruit.

Attention is drawn to three points of interest: an unusually rapid growth rate; the effect of pea extract, as contrasted with that of kinetin; differences in genotypes. With the fastest growing strain, a 200-fold increase in 2 weeks has been achieved, so that with four or five pieces, each about 30–40 mg in weight, sub-cultured on a nutrient medium containing agar, 20–30 g of cells can be obtained as needed (Fig. 1). We tested first a modification of White basal medium<sup>11</sup> with 2 per cent sucrose and vitamin and trace element supplements, and the following additions: casamino-acid (2 g), *L*-tryptophan (0.02 g), kinetin (80  $\mu$ g), indolyl-3-acetic acid (0.01 g) and 2,4-dichlorophenoxyacetic acid (3 mg)/l. As shown in Table 1, there was little or no growth.

Table 1. FRESH WEIGHT OF CALLUS, IN GRAMS, 14 DAYS, 28° C

Genotype		Medium			
		Basal	Basal + pea extract		
		1	1*	2	3
Red	<i>r<sup>+</sup>t<sup>+</sup></i>	0.083	1.09	1.18	0.56
Red lutescent	<i>r<sup>+</sup>t<sup>+</sup>l</i>	0.056	0.80	0.94	—
Yellow	<i>rt<sup>+</sup></i>	0.094	1.42	1.41	—
Tangerine	<i>rt<sup>+</sup></i>	0.044	0.35	0.46	—
Red ghost	<i>r<sup>+</sup>t<sup>+</sup>gh</i>	0.019	0.16	0.42	—
Tangerine ghost	<i>r<sup>+</sup>t<sup>+</sup>gh</i>	0.028	0.11	0.18	0.17
Yellow ghost	<i>rt<sup>+</sup>gh</i>	0.045	1.13	2.69	5.64

\* Values for run No. 1 are the averages of five samples each; for runs 2 and 3, four samples; the inoculum varies from 0.02 to 0.03 g.

With pea extract, however, results were dramatically different. The medium was prepared as follows: 250 g of frozen peas from the market were blended in 1 l. of distilled water and autoclaved for 10 min at 120° C and centrifuged. White solution, with 2 per cent sucrose, was then added to the supernatant. To ensure a clear solution (for liquid media) it was passed through a thin layer of 'Hyflo supercel', and the vitamins and growth factors were then added. The medium was then autoclaved at 120° C for 12 min, with the addition of agar (0.8 per cent) when a solid medium was desired.

Other additions to the medium, singly or in combination, included yeast RNA alkaline hydrolysate, cholesterol, and thiourea, at concentrations of 200 µg (based on the weight of unhydrolysed yeast RNA), 10 µg and 10 µg/l. respectively. No unequivocal effect was noted on growth, although thiourea kept the callus mass light in colour.

The starting material consisted of about 1 mg of hypocotyl tissue taken from seed which had been immersed for 30 min in 1 per cent sodium hypochlorite and washed three times in sterile water. The pieces of hypocotyl were placed on slants and changed to callus in 1–2 weeks. After several transfers to fresh medium, growth rates increased. The following tissue cultures were grown: red *r<sup>+</sup>t<sup>+</sup>*, yellow *rt<sup>+</sup>*, tangerine *rt<sup>+</sup>*, lutescent *r<sup>+</sup>t<sup>+</sup>l*, red ghost *r<sup>+</sup>t<sup>+</sup>gh*, tangerine ghost *r<sup>+</sup>t<sup>+</sup>gh*, and yellow ghost *rt<sup>+</sup>gh*.

Data in Table 1 permit comparison between the basal medium alone and the medium supplemented with pea extract. Results for the latter are averaged, and include combinations of thiourea, cholesterol and the RNA hydrolysate. Probably the most critical requirement is that the inoculum be in its most active phase. This seems to be the best explanation for the very rapid development for yellow ghost, *rt<sup>+</sup>gh*, as a result of numerous transfers; by contrast red, *r<sup>+</sup>t<sup>+</sup>*, run three, may have had unintentionally a less active inoculum.

Cultures involving *t*, whether alone or in conjunction with *gh*, have so far invariably shown slow growth. Cells

were characteristically spherical in shape, about 40µ (red) and about 100µ (yellow and tangerine) in diameter, but some of the latter were elongated, about 130 × 50µ. The cultures are not initially of isogenic stock, so that such differences cannot be ascribed to effects of the known gene differences. These differences in cell size are not reflected in dry matter content. After 14 days, dry matter was uniformly between 3.7 per cent and 3.9 per cent. It rose to between 5 per cent and 6 per cent for 1-month-old cultures. These figures may be compared with values between 4 per cent and 5 per cent<sup>10</sup> calculated at the period of the maximum growth rate for tobacco callus.

Nitrogen content was determined by micro Dumas and micro Kjeldahl procedures. Again no significant differences between strains were observed. By micro Dumas, the average value for four strains was 0.2 per cent nitrogen, that is about 5 per cent on a dry weight basis. Kjeldahl nitrogen was slightly lower, three samples yielding values of 3.61 per cent, 3.78 per cent and 3.70 per cent on the dry weight basis.

Total nucleic acid was run on the fast-growing *rt<sup>+</sup>gh* by the method of Ogur and Rosen<sup>12</sup>. The nucleic acid spectrum was identical in shape, from 230 mµ to 280 mµ, with that reported for carrot explants<sup>1</sup>. Based on an average E value of 25 for a concentration of 1 mg/ml., the total nucleic acid content was almost exactly 1 per cent of the dry weight. Comparisons are difficult, because for bacteria a much higher content, about 10 per cent, is not unusual, and the analysis may well have been made, at 14 days, past the peak (see ref. 1).

It can be emphasized that a rapid growth rate requires that the inoculum itself be highly active, and that the rate can be enhanced by repeated transfers.

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### Changes in the Shoot Apex during the Early Development of the Fern *Marsilea vestita*

*Marsilea* is an aquatic fern which can be grown in sterile culture and which passes through a juvenile phase before reaching adulthood. It is therefore an ideal plant to use in examining the events at the shoot apex which lead up to the production of juvenile and, later, adult leaves. Allsopp<sup>1</sup> has suggested that in *Marsilea* a small apical meristem initiates primordia with a relatively limited capacity for development, and Cutter<sup>2</sup> states in her review: "It is thus held that there is an important correlation between the size of the shoot apex and the extent of development of the leaf primordia to which it gives rise, leaf primordia formed on a large apex being capable



Fig. 1. Twenty-eight-day culture of tomato callus (yellow, *rt<sup>+</sup>*).

of developing into adult leaves, whereas those formed on a small apex are considered to have only a limited capacity for growth and development. Apical regions of well-nourished sporelings with adult leaves are seen by inspection to be larger than those of less-well-developed sporelings with juvenile leaves, but no detailed study of changes in size of the apex in *Marsilea* has yet been undertaken". The object of this communication is to find out whether there is any correlation between the size of the shoot apex and the production of juvenile or adult leaves.

Sterile sporelings of *M. vestita* Hook. and Grev. were obtained from surface-sterilized sporocarps and grown in sterile White nutrient fortified with 3 per cent sucrose and left under continuous fluorescent light (150 ft.-candles) or placed in darkness. Twenty-five plants were sampled at seven different times and their apices were fixed, sectioned at  $7\mu$ , and stained<sup>3</sup> to yield median longitudinal apical sections. These were drawn with the aid of a camera lucida and the apex was delineated by an imaginary base line drawn immediately above any recognizable leaf primordium at  $90^\circ$  to the axis of the shoot (Fig. 1). The parameters measured by means of a planimeter from the drawings of the apices were: the area, and the total cell number of each section. Each point on the graph in Fig. 2 represents ten median sections.

Juvenile leaves of *Marsilea* have unifid, bifid or trifid laminae, and the first leaf to appear which has a quadrifid

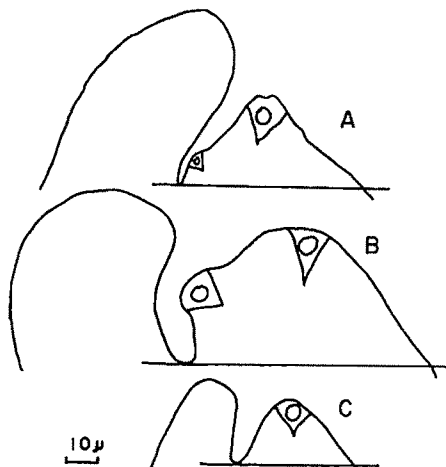


Fig. 1. Drawings of shoot apices of *Marsilea vestita*: (A) six day old plant grown in the light; (B) 30 day old plant grown in the light; (C) 30 day old plant grown in the dark. The large triangular apical cell is drawn at the top of the apex of the shoot. The apical cell to the left in A and B belongs to a group of cells which are about to become a leaf primordium.

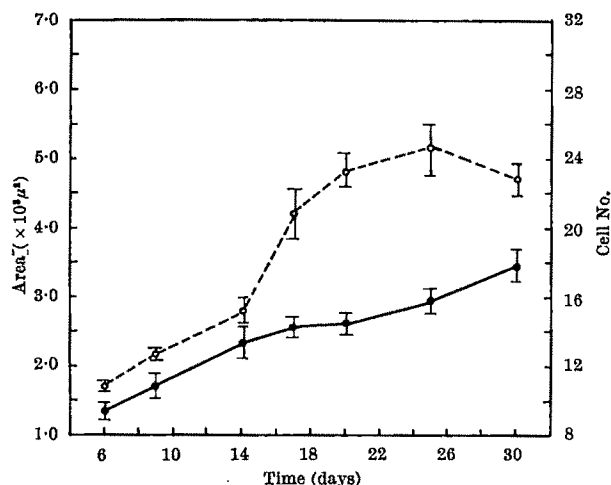


Fig. 2. Graph of sectional area (—) and cell number (---) of shoot apex of *Marsilea*. Standard errors indicated by horizontal lines.

Table 1. EFFECT OF DARKNESS ON SHOOT APEX OF 6 DAY OLD *Marsilea* PLANTS

Treatment	Sectional area shoot apex $\times 10^4 \mu^2$	Cell number in section of shoot apex
None	1.31 (0.14)	10.90 (0.15)
Darkness (24 days)	2.15 (0.34)	11.60 (0.61)
Light (24 days)	3.46 (0.25)	22.70 (0.90)

Standard error of each mean in parentheses.

lamina is arbitrarily designated as the first adult leaf<sup>4</sup>. At 17–20 days the plant ceases to produce juvenile leaves and begins to produce adult leaves. This transition to adulthood can be correlated with a change in the size of the apex, for all parameters increased gradually (Fig. 2). There is a plateau in the graph of the area of the apex just before adult leaves begin to appear. Most of the increase in size of the apex was the result of an increase in cell division, because cell number increased considerably with age (Fig. 2). From Sossountzov's cytological examination of the shoot apex of *Marsilea* we know that the majority of cell divisions occur in those areas below the apical cell and prismatic layers. These areas correspond to "sub-distal" and "organogenic" regions in fern apices<sup>5</sup>. It is evident here that adult leaves were produced on an apex of increasing size and that further increase in size of the apex occurred while further adult leaves were being produced. This raised the question of whether or not adult leaves can be produced on a small apex.

It has been shown<sup>6</sup> that the shoot apex of *Marsilea* is smaller in plants grown in darkness (Fig. 1). Average measurements of seven median longitudinal sections from plants grown in darkness (Table 1) show that etiolation caused a marked reduction in the area and cell number of the shoot apex. Etiolation does not affect the diameter of the apical cell or its nucleus as shown by actual measurement with an ocular micrometer. If *Marsilea* is supplied with a carbon source it will grow and produce adult leaves in total darkness (although the leaves do not expand as in plants grown in the light). This production of adult leaves occurred when the dark-grown plants possessed shoot apices which were as small as the apices of 12 day-old juvenile plants. Thus, if there is a correlation between the size of the shoot apex and the production of adult leaves, it was not evident here.

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## AGRICULTURE

### Variation in Concentration and Composition of Toxic Alkaloids among Strains of *Phalaris tuberosa* L.

In recent years there has been an increasing number of deaths in flocks of sheep which have grazed pastures dominated by the Australian commercial strain of phalaris (*Phalaris tuberosa* L.)<sup>1</sup>. There were many cases of acute phalaris poisoning on the tablelands of New South Wales in 1965. In some flocks losses were as high as 20 per cent.

Several dimethyltryptamine alkaloids, which are known to occur in phalaris herbage<sup>2</sup>, have been implicated as the toxic substances<sup>3</sup>. The syndrome of nervous and heart malfunctions induced by injecting these alkaloids



closely resembles that observed in cases of field poisoning.

To determine whether strains with low concentrations of these alkaloids occur in nature, ecotypes from the entire geographic range of the species in the Mediterranean region have been assayed. These ecotypes are referred to by their Commonwealth Plant Introduction numbers. Four breeding populations and the Australian commercial strain were also assayed. Twenty-two strains were grown with little nutrient or moisture stress in a controlled environment chamber at day/night temperatures of 21°C/16°C and 8-h day length. Herbage was also collected from swards of six of these strains grown in the field near Canberra. At the time of collection in mid-winter, nearby stands of the commercial strain were highly toxic. At each harvest, the plants were cut approximately 0.5 in. above the level of the root medium. The fresh herbage was frozen rapidly and stored in vacuum flasks with dry ice. The alkaloids were extracted in 0.1 normal hydrochloric acid and purified by solvent partitioning. After separation by paper chromatography, the alkaloids were assayed colorimetrically. The extraction and assay procedures used were those of Bogdanski *et al.*<sup>4</sup> and of Weissbach *et al.*<sup>5</sup>, respectively, as modified by Gallagher, Koch and Chia (personal communication).

The accuracy of the chromatographic and colorimetric techniques was high, the variation between duplicate samples being about 1 mg of alkaloid/100 g of dry matter. Large sampling errors occurred earlier in the overall procedure, probably at the time of sampling the herbage from the swards or pots.

There was a twenty-fold variation in the concentration of the dimethyltryptamine alkaloids among the twenty-two strains (Tables 1 and 2). The strain means appear to be continuously distributed. In the field samples the total alkaloid concentration was higher in the commercial strain than in any of the other five strains. Among the wider range of strains grown in the controlled environment, several were found with high alkaloid concentrations. On the other hand, a number of strains were considerably lower in alkaloid concentration than was the commercial strain.

Three compounds were common to all strains, *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine and 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine).

Table 1. CONCENTRATION OF DIMETHYLTRYPTAMINE ALKALOIDS IN THE HERBAGE OF SIX PHALARIS STRAINS GROWN IN THE FIELD AND IN A CONTROLLED ENVIRONMENT AT 21°/16° C AND 8 H DAY LENGTH

Strain	Origin	Field Total alkaloids	Field Dimethyl- tryptamine	Controlled environment Total alkaloids	Controlled environment Dimethyl- tryptamine
Australian commercial	Italy?	59 (1.76)*	46 (2.65)†	40 (1.57)*	27 (2.42)†
CPI 19299	Algeria	44 (1.63)	22 (2.30)	31 (1.49)	23 (2.28)
General select	Breeding population	26 (1.39)	7 (1.82)	33 (1.42)	5 (1.19)
CPI 19305	Morocco	25 (1.37)	3 (0.84)	62 (1.77)	1 (0.84)
Special select	Breeding population	20 (1.09)	2 (1.20)	41 (1.59)	2 (0.91)
CPI 14498	Algeria	17 (1.22)	5 (1.53)	3 (0.51)	2 (1.25)
Least significant differences (5 per cent)		(0.50)	(0.86)	(0.39)	(0.74)

Concentrations are in mg/100 g of dry matter.

\* Values for concentrations of total alkaloids ( $\bar{x}$ ) were transformed to  $\log_{10} \bar{x}$  for statistical analysis.

† Values for concentrations of dimethyltryptamine ( $y$ ) were transformed to  $1 + \log_{10} y$  for statistical analysis.

Table 2. CONCENTRATION OF DIMETHYLTRYPTAMINE ALKALOIDS IN SIXTEEN PHALARIS STRAINS GROWN IN A CONTROLLED ENVIRONMENT AT 21°/16° C AND 8 H DAY LENGTH

Strain	Origin	Total alkaloids	Dimethyl- tryptamine	Strain	Origin	Total alkaloids	Dimethyl- tryptamine
S184	Crete	178 (2.25)*	9 (1.97)†	CPI 14057	Israel	41 (1.60)*	30 (2.44)†
PX12	Breeding population	105 (2.00)	54 (2.57)	CPI 14071	Turkey	37 (1.45)	7 (1.87)
CPI 19344	Portugal	96 (1.98)	87 (2.93)	CPI 19357	Italy	31 (1.49)	27 (2.44)
CPI 14279	Greece	70 (1.82)	53 (2.71)	CPI 15110	Italy	22 (1.34)	12 (2.07)
S190	Crete	60 (1.74)	Trace (0.54)	CPI 15022	Turkey	12 (1.09)	6 (1.96)
CPI 19264	Israel	51 (1.69)	6 (1.78)	CPI 15220	Spain	8 (0.91)	5 (1.62)
CPI 14419	Portugal	45 (1.65)	38 (2.58)	CPI 19268	Lithuania	7 (0.82)	1 (1.10)
WBC	Breeding population	43 (1.63)	4 (1.44)	CPI 19351	Greece	5 (0.67)	1 (1.08)
		Least significant differences (5 per cent)				(0.37)	(0.40)

Concentrations are in mg/100 g of dry matter.

\* Values for concentrations of total alkaloids ( $\bar{x}$ ) were transformed to  $\log_{10} \bar{x}$  for statistical analysis.

† Values for concentrations of dimethyltryptamine ( $y$ ) were transformed to  $1 + \log_{10} y$  for statistical analysis.

Traces of unidentified related compounds also occurred in some strains. The concentration of bufotenine was small in all strains, and there were marked differences between the strains in the proportions of *N,N*-dimethyltryptamine and 5-methoxy-*N,N*-dimethyltryptamine (Tables 1 and 2).

The ranking of six strains with respect to total alkaloid concentration was not consistent when these were grown in the field and in a controlled environment chamber ( $r=0.20$ ,  $P \gg 5$  per cent) (Table 1). The total alkaloid concentration in the commercial strain is known to be affected markedly by many environmental factors, particularly soil nitrogen level and light intensity<sup>6</sup>. The differences which existed between the field and the controlled environments had a consistent effect on the concentration of *N,N*-dimethyltryptamine in the six strains ( $r=0.94$ ,  $P < 1$  per cent), but their effects on the concentration of 5-methoxy-*N,N*-dimethyltryptamine varied considerably among these strains ( $r=0.78$ ,  $P > 5$  per cent). Despite this genotype-environment interaction, the total alkaloid concentration in one strain was much lower than in the commercial strain in both environments.

Neither the concentration nor the composition of the alkaloids appears to be related to the area of origin or to any agronomic character of the strains. It would therefore seem feasible to breed agronomically acceptable strains of phalaris which have low concentrations either of any particular dimethyltryptamine alkaloid or of all such compounds. The requisite investigation of the inheritance of the alkaloid differences is in progress.

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## PSYCHOLOGY

### Positive and Negative Reinforcement from Intracranial Stimulation in Pigeons

SINCE the discovery of reinforcement by intracranial stimulation in rats<sup>1</sup>, neural systems of reward and punishment have been investigated in a number of mammalian species (for example, in monkeys, cats, dogs, goats, dolphins, and man<sup>2-7</sup>), but only one study<sup>8</sup> using a non-mammalian species (goldfish) has been published. At least one theoretical account of self-stimulation<sup>9</sup> relies on the notion that it depends on the simultaneous excitation of two functionally distinct, but anatomically contiguous, neuronal systems. Comparative studies might be expected to throw light on this hypothesis, because the functional systems involved need not necessarily be contiguous in all species. From this point of view, the

restriction of previous investigations to the class of mammals is regrettable, because the areas that appear to be primarily involved in reward and punishment, the limbic system and the hypothalamus, have developed structurally very little through the evolution of mammals<sup>10</sup>.

The development of the avian forebrain from reptilian structures has followed a very different course from that taken by the mammals, the most striking differences being the absence, in birds, of any distinct neo-cortical layering and the greater development of the striatal regions<sup>11</sup>. Despite these anatomical contrasts, few studies of the functions of the avian forebrain areas, and none of the reward value of intracranial stimulation, have been published.

A pair of bipolar twisted silver electrodes was implanted in the telencephalon of each of twenty-three adult domestic pigeons (*Columba livia*) while they were anaesthetized with 'Nembutal' supplemented with ether, and held in a modified rat stereotaxic apparatus, using a holder, adapted from Gogan<sup>12</sup>, that held the lachrymal ducts at the same height as the ears. Following tests, animals were perfused with 10 per cent formol saline, and serial sections in the plane of the electrode tract were cut at 15  $\mu$ . The slides were stained with haematoxylin and eosin, and those in the proximity of the tip silver-stained by the Glees method<sup>13</sup>.

The method used to evaluate the reward value of intracranial stimulation is similar to that used by Wilkinson and Peele<sup>3</sup> in their study on cats, and is made necessary by the difficulty of establishing a satisfactory operant level of any response that can be used to operate a micro-switch. The technique adopted ensures a large number of pairings of the operant response (pecking a key) with delivery of intracranial stimulation. Subjects were deprived of food, and trained to obtain wheat rewards in a Skinner box on a fixed ratio schedule of one reward to ten responses. They were then given a series of acquisition and extinction sessions until stable, rapid extinction was achieved after an acquisition period of a fixed length (200 responses, yielding twenty food rewards). A 0.4 sec train of intracranial stimulation was then introduced for every response throughout a session, the current being raised in 10  $\mu$ amp steps from 10 to 60  $\mu$ amp, and thereafter in 20  $\mu$ amp steps up to 160  $\mu$ amp. It is reasoned that aversive stimulation should slow down the rate of responding for food reward, and that rewarding stimulation should sustain responding in the absence of food reward.

Fifteen subjects met the reward criterion (more than 50 responses in each of two successive extinction periods), four met the punishment criterion (failure to complete the 200 response food-rewarded acquisition period within 10 min), and four, meeting neither criterion, were classified as neutral. Subjects meeting the reward criterion were given eight sessions at threshold current strength (thresholds ranged from 10 to 120  $\mu$ amp); mean rates of response in excess of 3,000/h were recorded during 20 min periods following cessation of food delivery. Further analysis of the performances of these subjects revealed that, in some cases, the rate of responding for food reward was lowered by intracranial stimulation at the same current levels that sustained extinction responding, although not to such an extent that the punishment criterion was met. Subjects in which this phenomenon was demonstrated were classified as ambivalent, this behaviour being interpreted as due to the simultaneous activation of rewarding and punishing pathways.

Histological analysis of the sites of the electrode tips showed: (a) of five placements in the hyperstriatum, two were rewarding, one ambivalent, one punishing, and one neutral; (b) four of seven neostriatal placements were rewarding, one was ambivalent, and two neutral; (c) three palaeostriatal electrodes were rewarding and two were ambivalent; (d) the single archistriatal placement was neutral; (e) three septal electrodes were punishing

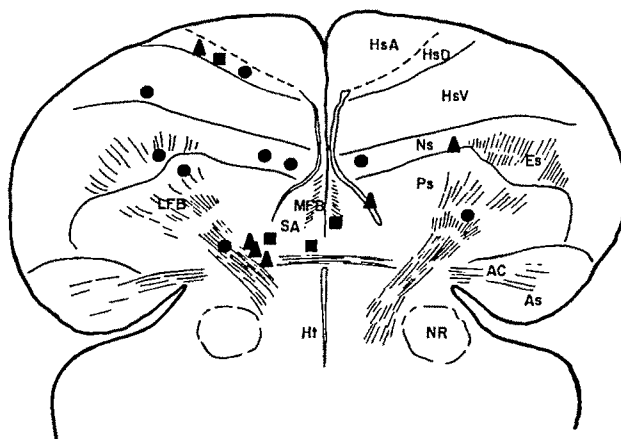


Fig. 1. Frontal section through pigeon brain, at the level of the anterior commissure. AC, Anterior commissure; As, archistriatum; Es, actostriatum; HsA, hyperstriatum accessorium; HsD, hyperstriatum dorsale; HsV, hyperstriatum ventrale; Ht, hypothalamus; LFB, lateral forebrain bundle; MFB, medial forebrain bundle; NR, nucleus rotundus; Ns, neostriatum; Ps, palaeostriatum; SA, septal area. ●, "Pure" reward; ▲, ambivalent; ■, aversive.

and two were ambivalent. Fig. 1 is a diagram showing the sites of all the electrodes classified as rewarding or punishing, distinguishing ambivalent responders from "pure" reward subjects; for the sake of clarity, all loci are shown at the same rostro-caudal level. From Fig. 1 it can be seen that, in general, placements affecting the lateral forebrain bundle and palaeostriatum are rewarding, those influencing the medial forebrain bundle and septal area are aversive, and electrodes capable of influencing both structures are ambivalent; in particular, it can be seen that the four ambivalent electrodes in the palaeostriatum and septal area fall in areas where the two structures are in close proximity. Because the septal area and medial forebrain bundle of mammals are generally accepted as part of the reward system in mammals, a surprising contrast between avian and mammalian organization will have emerged if subsequent results confirm the predominantly aversive effects of stimulation in these loci in birds. Phillips<sup>14</sup> found that electrical stimulation of the septal area in six mallard ducks yielded "escape" responses, and this provides good support for the present findings.

The demonstration of self-stimulation in three such distantly related classes of animal as osteichthyes, aves and mammalia provides evidence for the generality of the phenomenon, and investigations of other functions of pathways in which it occurs may help to clarify the significance of this type of reward.

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## Effect of Magnetic Fields on Reaction Time Performance

In previous investigations<sup>1,2</sup> we indicated some significant empirical relationships between selected geophysical parameters and gross measures of human behaviour. The present investigation attempts to demonstrate the effects of artificially produced magnetic fields on a standard, relatively uncomplicated, psychomotor task, simple reaction time.

In our initial attempt, twin Helmholtz coils, 14.5 in. in diameter, were mounted in a concealing wooden frame to provide an 11.5 in. coil interspace. The frame, vertically movable, was attached to a wooden chair so that seated subjects, using a chin rest, could have the cerebrum approximately at the centre of the transverse magnetic fields. A gaussmeter probe was mounted in the wooden frame so as to monitor constantly the magnetic field at 1-2 in. above the centre of the subject's head. In a darkened room, each subject was instructed to press and promptly release a telegraph key, mounted on a lapboard, as quickly as possible after the appearance of an eye-level red light 7 ft. away. Three experiments were conducted: the first with equipment as described and visual read-out of a timer, the second with equipment as described but with an automatic print-out timer and an added oscillator, and the third with more efficient, commercially fabricated coils and heavier power supply. It should be cautioned that all gauss levels mentioned in these experiments reflect not absolute levels of magnetic intensity to which a subject is exposed, but those levels over and above the naturally occurring magnetic field intensity already extant in the experimental area. Further, because of the lack of field homogeneity in such a large interspace with 14 in. coils and the need to keep the probe 1 or 2 in. above the subject's head, the centre of the force field tended to give field strengths approximately one third greater than was read from the gaussmeter.

In the first experiment, steady state fields of 5 and 17 gauss, levels considerably greater than those required in natural geomagnetic activity for the designation of "severe" storm, were used with eighteen male hospitalized schizophrenic subjects, 22-49 years of age. No statistically significant effects on reaction time performance by application of steady state magnetic fields could be demonstrated, and so this approach was abandoned in favour of modulated fields.

In the second experiment, thirty male clinically normal subjects, 19-32 years of age, were randomly placed in one of three groups of ten subjects each: a control group; a group subjected to a sinusoidally modulated field of 5-11 gauss at 0.1 c/s; or a third group exposed to a similarly modulated field, but at 0.2 c/s. The three groups did not differ significantly in chronological age. The upper limit of 11 gauss was set by the capacity of the equipment used. The modulating frequencies selected were based on observations of (a) naturally occurring periods of oscillation in the cerebral direct-current potentials of animal and human subjects undergoing changes in levels of consciousness<sup>3</sup>, and (b) the enhancement of physiological effect by low frequencies (0.1-10 c/s) when alterations of consciousness have been induced by application of exogenous direct currents<sup>4</sup>. Each subject had two practice reaction time trials followed by fifty trials with 5 sec between them. Modulated fields were obtained by coupling a low frequency sine wave generator to the coil system through a solid state mixer-follower circuit.

In the initial analysis of the findings of the second experiment, the fifty reaction time trials of each subject were divided into five blocks of ten trials each and the median for each block of trials determined. Thus, there were three groups of ten different subjects each, with each subject providing five median scores. The data were submitted to analysis of variance in accordance with designs for multifactor experiments with repeated meas-

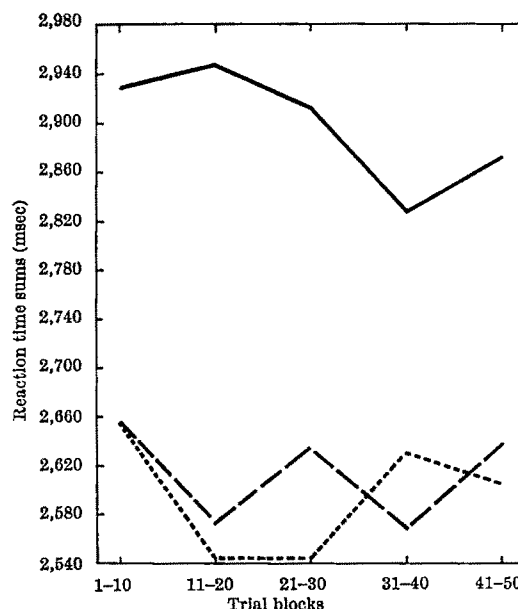


Fig. 1. Reaction time as a function of blocks of trials in male subjects. —, 0.2 c/s; ---, 0.1 c/s; ····, control.

urements<sup>5</sup>. Fig. 1 plots the sum of reaction time medians for all subjects in a group as a function of trial blocks. An *F*-test applied to all fifty trials, the last thirty trials, and the last twenty trials, gave values of 3.26, 2.90 and 2.62, respectively (d.f. 2, 27), which indicated differences approaching significance ( $P < 0.10$ ). For the last forty trials and the last ten trials the *F*'s calculated between groups were 3.45 and 3.58 (d.f. 2, 27); they were statistically significant ( $P < 0.05$ ). The Scheffé method<sup>6</sup> was applied to test the differences between the means of the three conditions and revealed that the 0.2 c/s condition was significantly different ( $P < 0.05$ ) from a combination of the other two. The findings encouraged the use of a more sensitive design using each subject as his own control. Twelve subjects were called back and two additional fifty trial reaction time performances were obtained from each so as to provide a counter-balanced design with two subjects in each of the possible six sequences of conditions. A minimum of 24 h elapsed between each condition in a sequence for every subject. The median of each block of ten reaction time trials was obtained for each subject. The sum of the medians provided a single figure indicating each subject's performance in each condition. Fig. 2 shows the performance of the twelve subjects, as a group, in each of the three conditions during each block of trials. The findings were subjected to analysis of variance in accordance with designs for single-factor experiments with repeated measures<sup>5</sup>. For the last thirty trials, last twenty trials, and last ten trials, each combined, *F*'s of 3.75, 4.21 and 4.66, respectively (d.f. 2, 22), indicated statistically significant differences ( $P < 0.05$ ). Again, the Scheffé method indicated that reaction time performance means in the 0.2 c/s condition differed significantly ( $P < 0.05$ ) from a combination of the means of the other two conditions. For all fifty trials and for the last forty trials combined, *F* values (d.f. 2, 22) of 3.06 and 3.36 approached statistical significance ( $P < 0.10$ ).

The third experiment replicated the design of the second experiment, but with thirty female subjects, 17-40 years of age. A complete wooden booth held the concealed commercially fabricated Helmholtz coils so as to provide an 11 in. coil interspace. A fixed wooden chair, movable chin rest, and seat platforms of various thicknesses enabled the subject's head to be close to the horizontal centre of the magnetic field. The magnetic probe, concealed in a wooden container in the roof of the booth, was about 2 in. from the subject's head. The coils were

powered by a d.c. power supply of 1 kW input and capable of 50 amp, 22.5 V output. Desired modulations could be accomplished by connecting an externally powered oscillator directly to the power supply.

Analysis of the data derived from assigning the subjects randomly to the three groups of ten each indicated that although differences were in the expected direction, they were not statistically significant. Fig. 3 shows the sum of the reaction time medians for all subjects in a group as a function of trial blocks. As previously, the more sensitive design was used by calling back twelve subjects and exposing each to the other two conditions of the experiment. Again, this provided a completely counter-balanced design using each subject as her own control. Fig. 4 shows the performance of the twelve female subjects as a group in each of the three conditions during each block of trials. When the data were subjected to analysis of variance all fifty trials, as a block, yielded an  $F$  of 3.49 (d.f. 2, 22) which was statistically significant ( $P < 0.05$ ). The Scheffé method indicated that the effects of the 0.2 c/s condition were significantly different ( $P < 0.05$ ) from those in the 0.1 c/s condition.

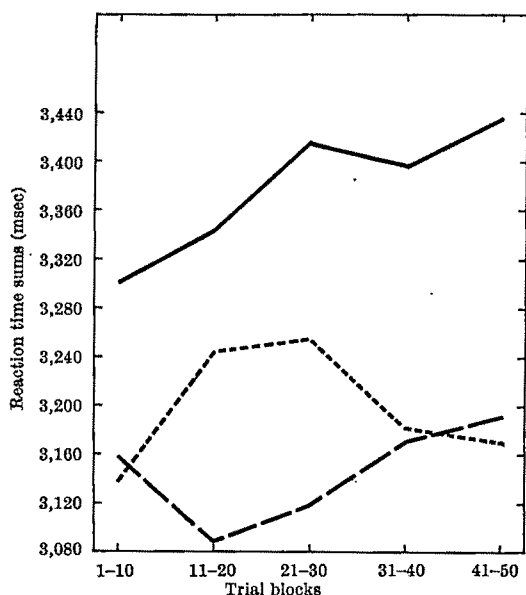


Fig. 2. Reaction time as a function of blocks of trials in male subjects used as their own controls. Symbols as in Fig. 1.

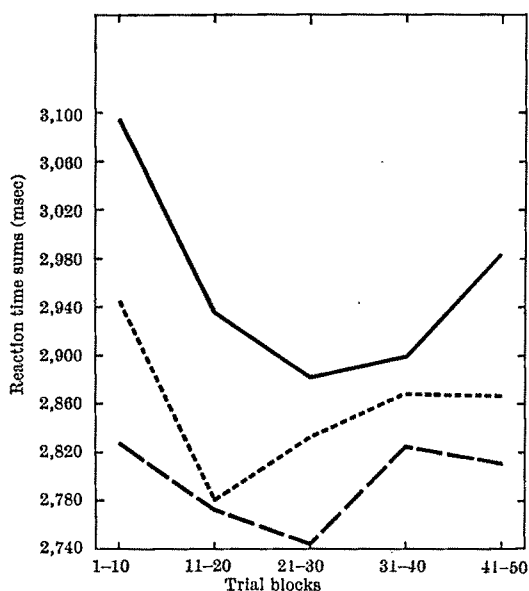


Fig. 3. Reaction time as a function of blocks of trials in female subjects. Symbols as in Fig. 1.

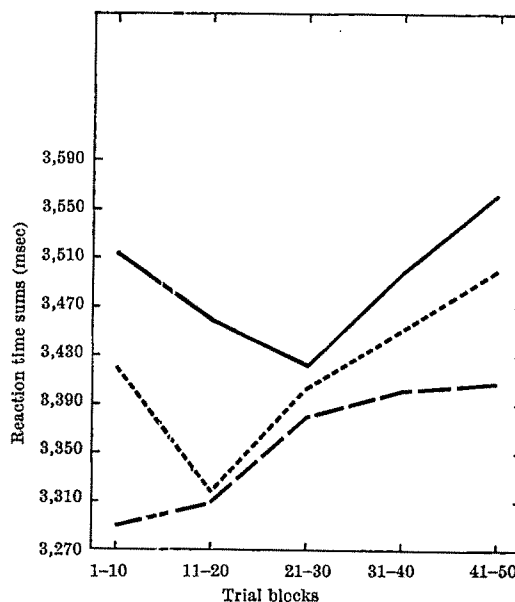


Fig. 4. Reaction time as a function of blocks of trials in female subjects used as their own controls. Symbols as in Fig. 1.

In general, then, the findings indicate that experimentally produced modulated magnetic fields can significantly affect reaction time performance.

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## GENERAL

### Polyplanar Hip Joint for Use in Lower Limb Bracing

CHILDREN with nervous or muscular disorders affecting the movements of the trunk and lower limb sometimes have great difficulty in walking or learning to walk. External support may be provided by long callipers fitting into the heels of the boots and extending upwards to a padded metal strap around the waist. A simple hinge joint at the level of the hip (joint) allows the legs to swing in one vertical plane only. The complexity of normal walking is highlighted when a child attempts to move in such an apparatus, for the pelvis cannot rotate around a vertical axis through the hip joint of the supporting leg during the swinging phase of the other leg. The patient therefore progresses by pivoting his body around an axis passing through the ball of the supporting foot. This produces the abnormal foot positions relative to the line of march and the pelvis, which are shown in Fig. 1a and b. Fig. 1b shows that the foot bearing the weight ( $A$ ) must pivot through an angle equal to that of the pelvic swing ( $BAC$ ). In normal walking (Fig. 1a) the position of the foot remains unaltered relative to the line of march, because the necessary rotation of the lower limb has taken place at the hip joint.

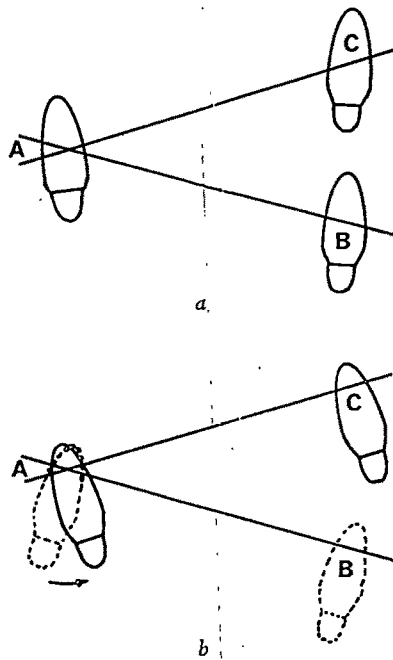


Fig. 1. AB and AC indicate the frontal plane of the pelvis.

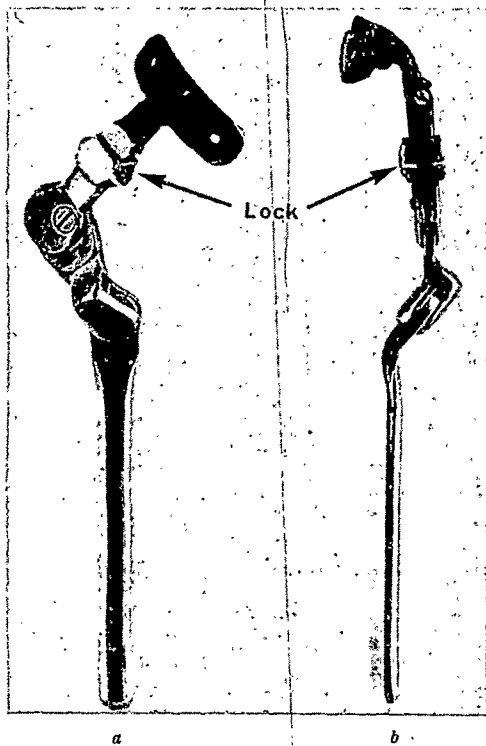


Fig. 2. a, Sitting hinge unlocked and flexed to 90° (right side); b, sitting hinge locked for walking (left side).

The anatomical movements relevant to the subsequent discussion take place at the hip joint and can be summarized as follows.

(1) Movement in an antero-posterior plane: a, forwards—flexion; b, backwards—extension.

(2) Movement in a coronal or transverse plane: a, away from the mid-line—abduction; b, towards the mid-line—adduction.

(3) Movement taking place about a vertical axis: a, rotation causing the kneecap to turn inwards—internal rotation; b, rotation causing the kneecap to turn outwards—external rotation.

## Design

Many handicapped children have a limited capacity to forget established skills and replace them with new skills. It is therefore important that they should learn to walk in the easiest and most natural way. We concluded that a near-normal rotation of the leg during forward and backward movement could be induced by using an additional joint the axis of which was directed outwards and downwards, instead of being horizontal. This design also allows a small amount of movement away from the line of march, so that the walking sequence approximates more closely to the normal pattern. To allow a normal sitting position, the standard (horizontal axis) hinge is fitted above the walking hinge joint (Fig. 2), but the former is locked during standing and walking.

## Kinematic Analysis

The action of the hinge used in forward and backward movement was analysed by considering the system of axes defined in Fig. 3. The hinge axis is  $X$ , and the axes perpendicular to it are designated  $Y$  and  $Z$ . Axes representing swing, rotation and abduction ( $x$ ,  $y$  and  $z$ , respectively) are obtained from  $X$ ,  $Y$  and  $Z$  by a negative rotation of magnitude  $\alpha$  about  $Z$ .

The co-ordinates of the point  $P$  are

$$\left. \begin{aligned} X &= -l \sin \alpha \\ Y &= -l \cos \alpha \cos \theta \\ Z &= -l \cos \alpha \sin \theta \end{aligned} \right\} \quad (1)$$

where  $\theta$  is the angular displacement of the hinge. In general, co-ordinate transformation by a rotation  $-\alpha$  about  $Z$  gives:

$$\left. \begin{aligned} x &= X \cos \alpha - Y \sin \alpha \\ y &= Y \cos \alpha + X \sin \alpha \\ z &= Z \end{aligned} \right\} \quad (2)$$

and in this case, the co-ordinates of  $P$  in terms of  $x$ ,  $y$  and  $z$  are obtained by substituting equations (1) in equations (2).

$$\left. \begin{aligned} x &= l \sin \alpha \cos \alpha (\cos \theta - 1) \\ y &= -l (\sin^2 \alpha + \cos^2 \alpha \cos \theta) \\ z &= -l \cos \alpha \sin \theta \end{aligned} \right\} \quad (3)$$

Consider the angular displacements about  $x$ ,  $y$  and  $z$  as defined for this configuration in Fig. 4 (a-c). From this diagram and equations (3)

$$\left. \begin{aligned} \varphi_x &= \tan^{-1} \frac{z}{y} = \tan^{-1} \frac{\cos \alpha \sin \theta}{\sin^2 \alpha + \cos^2 \alpha \cos \theta} \\ \varphi_y &= \tan^{-1} \frac{x}{z} = \tan^{-1} \frac{\sin \alpha (1 - \cos \theta)}{\sin \theta} \\ \varphi_z &= \tan^{-1} \frac{x}{y} = \tan^{-1} \frac{\sin \alpha \cos \alpha (1 - \cos \theta)}{\sin^2 \alpha + \cos^2 \alpha \cos \theta} \end{aligned} \right\} \quad (4)$$

It can be seen that  $\varphi_x$  and  $\varphi_y$  change signs when  $\theta$  changes sign, but  $\varphi_z$  remains positive for all values of  $\theta$ . Physically, this means that hip flexion is accompanied by

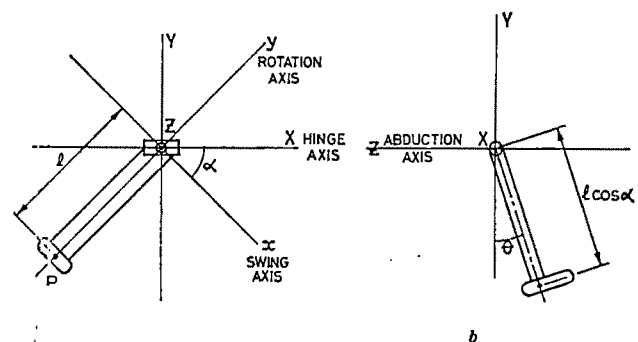


Fig. 3.



external rotation, and extension by internal rotation; adduction never occurs.

To minimize abduction, an appropriate value for  $\alpha$  needs to be selected by setting the derivative of  $\varphi_z$  with respect to  $x$  equal to zero, which leads to the condition

$$\tan^2 \alpha = \cos \theta$$

For small values of  $\theta$ ,  $\cos \theta$  is almost unity and, ignoring the negative root,  $\alpha = \pi/4$ . With this value of  $\alpha$ , equation (4) for the angular displacement becomes

$$\left. \begin{aligned} \varphi_x &= \tan^{-1} \frac{\sqrt{2} \sin \theta}{1 + \cos \theta} = \tan^{-1} \sqrt{2} \tan \frac{\theta}{2} \\ \varphi_y &= \tan^{-1} \frac{1 - \cos \theta}{\sqrt{2} \sin \theta} = \tan^{-1} \frac{1}{\sqrt{2}} \tan \frac{\theta}{2} \\ \varphi_z &= \tan^{-1} \frac{1 - \cos \theta}{1 + \cos \theta} = \tan^{-1} \tan^2 \frac{\theta}{2} \end{aligned} \right\} \quad (5)$$

Using these expressions,  $\varphi_x$ ,  $\varphi_y$  and  $\varphi_z$  are plotted against  $\theta$  in Fig. 5.

In practice, a swing of  $\pm 15^\circ$  should be associated with  $\pm 5^\circ$ – $10^\circ$  of rotation, while abduction or adduction should be negligible. The graph (Fig. 5) shows that with the axis of rotation angled downwards and outwards by  $45^\circ$ , a  $15^\circ$  swing produces  $8^\circ$  of outward rotation, and a  $-15^\circ$  swing  $8^\circ$  of inward rotation, whereas abduction, being minimal for low values of  $\varphi_x$  is only  $2^\circ$  during the same amount of movement. The practical value of such joints is at present being investigated using assessment techniques evolved at the Cerebral Palsy Physical Assessment Centre, Guy's Hospital.

A polyplanar joint, the movement of which approximates more closely to that of a hip joint, should show a number of advantages over the conventional simple hip joint currently used in long leg braces. The application

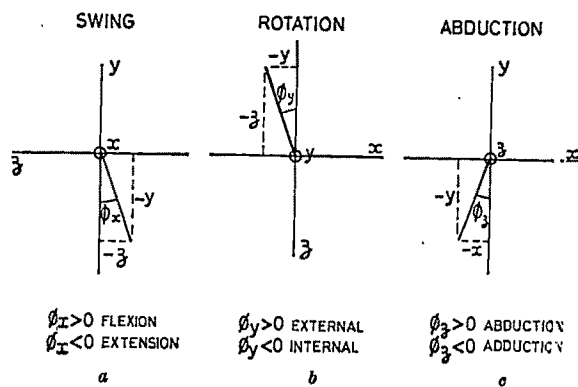


Fig. 4.

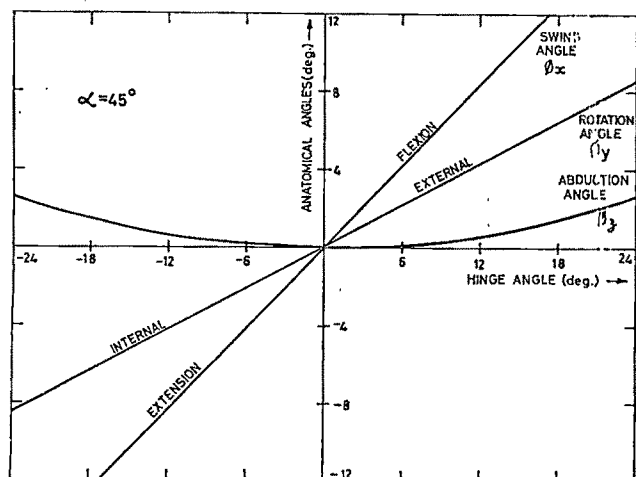


Fig. 5.

of engineering theory to the problem demonstrates how valuable co-operation between university departments can be in reducing the time spent on physical trials of such apparatus.

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## Negentropy, Information and the Feeding of Organisms

IN a recent reply to Büchel, concerning entropy and information in the universe, Popper<sup>1</sup> persists in the use of a biological example which appears to be incorrect. This concerns the developing birds' eggs which "appear to produce structural negentropy by increasing their structural organization; they are, as far as we know, "fed", in Schrödinger's sense, exclusively on heat (that is, on entropy)". This does not constitute a counter example to Schrödinger's views<sup>2</sup> because the developing embryo in the egg "feeds" on the negentropy of the yolk; this conversion process, in common with most biological energy conversion processes, is not very efficient and in the course of it entropy (as heat) is generated. The purpose of incubation is to reduce the rate of flow of heat away from the egg which is poorly insulated, no doubt inevitably so as a consequence of the structural requirements for gas exchange. Thus over the whole period of incubation there is a net loss of radiant energy from the egg and it is therefore misleading to suggest, as Popper does, that the egg has fed on entropy. To have asked of Schrödinger (as Popper requires) that he say something of organisms which distinguishes them from heat engines was scarcely possible in the strictly thermodynamic context of Schrödinger's famous remark, because it would have required going beyond the then available limits of thermodynamics to say something about the form or information content of the received energy.

Photosynthetic autotrophic organisms are entropy feeders which canalize the entropy of sunlight into the thermodynamic and structural negentropy of their own structures. The entropy feeding of these organisms can be said to be achieved ultimately by the agency of the informational (that is, structural) negentropy represented in the replicative and renewal templates within their cells. Büchel<sup>3</sup>, however, makes extravagant claims about the relationships of this structural negentropy to the thermodynamic entropy increase of a system, raising to the level of a general principle the proposition that "whenever structural negentropy is produced, the thermodynamic entropy must increase by at least the same amount". Even if it is accepted as proved that in the chosen example of a watchmaker assembling a watch, putting in as he does so  $n$  bits of information, the attendant increase in thermodynamic entropy will be at least  $Kn \log 2$  cal/grad, there is no evidence that a similar relationship will hold for the structural negentropy generated by a living organism. Taking the case of even the simplest organisms growing on defined media we do not have sufficient knowledge of the direction and magnitude of radiation and chemical energy exchange with the environment or of how to approach the problem of a valid estimate of their information content, even to embark on the measurement of this relationship, much less to claim its probable general validity.

HAROLD W. WOOLHOUSE

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Received January 25, 1967.

<sup>1</sup> Popper, K., *Nature*, 213, 320 (1967).

<sup>2</sup> Schrödinger, E., *What is Life?* (Cambridge University Press, 1944).

<sup>3</sup> Büchel, W., *Nature*, 213, 319 (1967).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, March 6

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. M. K. McPhun: "U.H.F. Tunnel-Diode Amplifier"; Dr. D. L. Hedderly, Mr. J. Hooper and Mr. M. K. McPhun: "Short-Hop Radio-Relay Systems Using Tunnel-Diode Repeaters".

UNIVERSITY COLLEGE LONDON (in the Chemistry Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. S. Bosanquet: "The Sum of an Unconvergent Series". (Inaugural Lecture.)\*

UNIVERSITY OF LONDON (in the Main Lecture Theatre, Physics Building, Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. Sir Vincent Wigglesworth, F.R.S.: "Insect Hormones" (further lectures on March 7 and 8).\*

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. D. A. Dowden: "The Coherence of Pure and Applied Heterogeneous Catalysis".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. R. A. Hibbert: "Mongolia Today".

## Tuesday, March 7

UNIVERSITY OF LONDON (in the Wolfson Lecture Theatre, Royal Postgraduate Medical School, Du Cane Road, London, W.12), at 5 p.m.—Prof. Sir Dugald Baird: "The Prevention of Perinatal Death".\*

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Inst. of Mech.E., at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. J. Spurgin and Mr. R. L. Carstairs: "Overall Station Control at Hunterston".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. King: "Heat" (Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on March 8, 14 and 15.)

UNIVERSITY OF ASTON IN BIRMINGHAM (in the Great Hall of the University, Gosta Green, Birmingham 4), at 5.30 p.m.—Prof. Holmes: "The Advancement of Knowledge in Civil Engineering" (Inaugural Lecture).\*

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 6 p.m.—Sir Henry Jones: May Lecture.

PLASTICS INSTITUTE, LONDON SECTION (at Imperial Chemical House, Millbank, London, S.W.1), at 6.30 p.m.—Dr. H. J. Sharp: "Plastics in Domestic Appliances".

## Tuesday, March 7—Thursday, March 9

INSTITUTE OF METALS (at Church House, Great Smith Street, Westminster, London, S.W.1)—Spring Meeting and Meeting of the British Joint Corrosion Group.

## Wednesday, March 8

PLASTICS INSTITUTE (at the Institution of the Rubber Industry, 4 Kensington Palace Gardens, London, W.8), at 2.30 p.m.—Mr. D. R. Reid: "Impact Testing and Brittle Failure".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Captain Lionel Munk: "The Value of Inland Waterways".

UNIVERSITY OF LONDON (at the Institute for Diseases of the Chest, Brompton Hospital, London, S.W.3), at 5 p.m.—Dr. Lynne Reid: "The Clinical Diagnosis and Classification of Emphysema".\*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. P. Penfield: "Thermodynamics of Electrical Networks".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. E. I. Lowe: "Computer Control of Chemical Processes".

UNIVERSITY OF LONDON (at Senate House, London, W.C.1), at 5.30 p.m.—Dr. P. Gaasstra (Wageningen): "The Relation Between Photosynthesis and Crop Growth".\*

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, W.C.1), at 5.30 p.m.—Dame Honor Fell, F.R.S.: "The Effect of Environment on Bone in Culture". (Second of three lectures on "The Scientific Basis of Dentistry" organized by The British Postgraduate Medical Federation.)\*

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Mr. T. N. Reynolds: "Acoustics in the Underwater Environment".

## Wednesday, March 8—Thursday, March 9

ZOOLOGICAL SOCIETY OF LONDON AND THE MALACOLOGICAL SOCIETY OF LONDON (at the Zoological Society of London, Regent's Park, London, N.W.1), at 10 a.m. daily—Symposium on "Studies in the Structure, Physiology and Ecology of Molluscs".

## Thursday, March 9

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.20 p.m.—Mr. B. C. Brookes: "Scientific Information and the Computer".\*

MINERALOGICAL SOCIETY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. J. P. MacCarthy: "Recent Developments in Home Heating".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "The Synchronization and Resynchronization of Alternators" opened by Mr. B. J. Cory.

UNIVERSITY COLLEGE LONDON (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Kensei Cheng (New York University School of Medicine): "Fast and Slow Fibres in Mammalian Extraocular Muscles".\*

## Thursday, March 9—Friday, March 10

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Dr. T. E. Calverley: "High-Voltage D.C. Transmission" (Silvanus P. Thompson Lecture).

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m. daily. Discussion Meeting on "The Origin and Treatment of Noise in Industrial Environments" organized by Mr. E. J. Richards.

## Friday, March 10

SOCIETY FOR WATER TREATMENT AND EXAMINATION (at the Royal Hotel, Woburn Place, London, W.C.1), from 9.30 a.m. to 5.30 p.m.—Symposium on "Water Filtration".

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 10.50 a.m.—Symposium on "Selective Phytotoxicity".

UNIVERSITY OF LONDON (in the Beveridge Hall, Senate House, London, W.C.1), at 12 noon—Memorial Meeting to commemorate the life and work of the late Prof. Frank Cunliffe Ormerod.\*

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Dr. R. B. Cundall: "Photochemistry of Some Olefin Containing Systems".

BRITISH SOCIETY FOR INTERNATIONAL HEALTH EDUCATION (at the Goldsmith's Hall, Foster Lane, London, E.C.2), at 5.30 p.m.—Sir John Wolfenden, C.B.E., and Dr. Victoria Garcia: "Education—Health's New Dimension".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. W. White: "Broadband Transmission by Radio and Cable".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. G. D. Sims and Dr. B. H. Venning: "The Future Education of Electronic Engineers".

UNIVERSITY OF LONDON (at Royal Holloway College, Englefield Green, Surrey), at 8.15 p.m.—Dr. T. F. Gaskell: "Looking for Gas in the North Sea".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. L. F. Boyd: "Space Science and the Astronaut".

## Saturday, March 11

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. Michael H. Day: "Man or Ape?—Research into *Homo habilis*".\*

## Monday, March 13

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP (joint meeting with the Food Group and Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, S.W.1), at 2.30 p.m.—Meeting on "Emulsions and Emulsifiers in the Food Industry".

UNIVERSITY OF LONDON (at the School of Oriental and African Studies, London, W.C.1), at 5 p.m.—Prof. Jean-Luc Chambard (Paris): "Atlas of an Indian Village. 1, The Village: Society and Economy".

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. Mothes (East Berlin): "On Cytokinins" (further lectures on March 14 and 15).\*

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Coachmakers Arms, 88 Marylebone Lane, London, W.1), at 6.30 p.m.—Mr. J. Barnes: "Industrial Design as Applied to Machinery".

## Monday, March 13—Friday, March 17

INSTITUTION OF CIVIL ENGINEERS, THE BRITISH NUCLEAR ENERGY SOCIETY AND THE JOINT BRITISH COMMITTEE FOR STRESS ANALYSIS (at Church House, Great Smith Street, Westminster, London, S.W.1)—Conference on "Prestressed Concrete Pressure Vessels".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2)—Conference on "Air Traffic Control".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (zoologist, physiologist or pharmacologist, preferably with an interest in comparative endocrinology or general physiology) in the DEPARTMENT OF BIOLOGICAL SCIENCES—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, 4, quoting Ref. 537/2 (March 8).

LECTURER IN CIVIL ENGINEERING—The Principal, Lanchester College of Technology, Priory Street, Coventry (March 13).

RESEARCH ASSISTANT in the DEPARTMENT OF GEOLOGY to work under the direction of Mr. F. J. Fitch in the field of geochronology—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (March 13).

RESEARCH ASSISTANT (with an honours degree in geography or a related field with some experience in field surveying and mathematical geography, and preferably a knowledge of sediment analysis) in the DEPARTMENT OF GEOGRAPHY, to work on the bathymetric survey of Loch Leven, Kinross—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (March 13).

LECTURER (experienced in the fields of solid mechanics, dynamics and design) in AGRICULTURAL ENGINEERING—The Registrar, The University, Newcastle upon Tyne, 2 (March 15).

POSTGRADUATE RESEARCH ASSISTANT IN STRUCTURAL CHEMISTRY to work on a Science Research Council supported project entitled "A Preparative and Kinetic Study of the Halogenation of Co-ordinated Ligands"—The Registrar, University of Bradford, Bradford, 7, Yorkshire (March 15).

ASSISTANT LECTURER IN BIOCHEMISTRY—The Secretary, Queen's College, Dundee, Scotland (March 18).

LECTURER (with working experience in the techniques of radiometric rock-dating) in the SUB-DEPARTMENT OF GEOPHYSICS—The Registrar, The University, Liverpool, quoting Ref. RV/406 (March 18).

CHAIR OF MATHEMATICAL STATISTICS AND CHAIR OF THEORETICAL PHYSICS—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (March 20).

ASSISTANT LECTURER IN PSYCHOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (March 24).

SENIOR LECTURER (graduate of an internationally recognized veterinary school with considerable experience and interest in teaching and research at university level, and preferably tropical or sub-tropical experience) in the DEPARTMENT OF ANIMAL SCIENCE, University of Ghana—The Assistant Registrar, Universities of Ghana Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (March 27).

EXPERIMENTAL OFFICER in the DEPARTMENT OF BOTANY for duties which will include upkeep of the Departmental Herbarium and assistance with teaching in taxonomy and research—The Secretary, Academic Council, The Queen's University, Belfast, Northern Ireland (March 31).

LECTURER (preferably with experience of West African or tropical conditions) in AGRICULTURAL BIOCHEMISTRY AND NUTRITION, with particular reference to carbohydrate and energy metabolism studies, in the DEPARTMENT OF AGRICULTURAL CHEMISTRY AND SOILS, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 31).

RESEARCH FELLOW (graduate with a research degree or equivalent experience) in the SEDIMENTOLOGY RESEARCH LABORATORY, GEOLOGY DEPARTMENT, to conduct an N.E.R.C. sponsored investigation into the sedimentology (including geochemistry, physical properties and clay mineralogy) of facies variation in the Great Oolite Series of the Malmesbury area—The Assistant Bursar (Personnel), The University, Reading (March 31).

SENIOR LECTURER (veterinary surgeon with experience of modern methods for the control and development of animal health and animal production, and preferably some teaching experience) in ANIMAL HEALTH in the DEPARTMENT OF ANIMAL HUSBANDRY AND HYGIENE—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (March 31).

LECTURER (with a good degree in forestry or engineering with post-graduate experience in wood science, and preferably some experience in industry) in WOOD SCIENCE in the DEPARTMENT OF FORESTRY—The Registrar, University College of North Wales, Bangor, North Wales (April 1).

ASSISTANT LECTURERS or LECTURERS in the DEPARTMENT OF PHYSICS (lines of research include photo electronics and image intensifiers; low temperature physics; magnetic alloys; dielectrics and semi-conductors, experimental and theoretical)—The Registrar, University College of North Wales, Bangor, North Wales (April 3).

LECTURER (graduate with some experience of tropical agriculture) in CROP HUSBANDRY at the University of Malawi—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 3).

SENIOR LECTURER or LECTURER in the DEPARTMENT OF PREVENTIVE AND SOCIAL MEDICINE, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 3).

UNIVERSITY LECTURER IN BIOCHEMISTRY—The Administrator, Department of Biochemistry, University of Oxford, South Parks Road, Oxford (April 3).

LECTURER (preferably with an interest in mycology, plant ecology or plant physiology) in BOTANY at University College, Dar es Salaam, University of East Africa—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 5).

SENIOR LECTURER and a LECTURER in ANATOMY—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1 (April 14).

RESEARCH DEMONSTRATOR (with a good honours degree in geology with special interests in stratigraphy and structural geology and/or petrology) in the DEPARTMENT OF GEOLOGY—Prof. J. G. C. Anderson, Department of Geology, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (April 15).

ASSISTANT LECTURER (science graduate in microbiology) in BACTERIAL AND VIRAL GENETICS—The Professor of Bacteriology, The University, Bristol.

DEMONSTRATORS or ASSISTANT LECTURERS in the DEPARTMENT OF HUMAN BIOLOGY AND ANATOMY—Prof. R. Barer, The University, Sheffield.

MASTER to teach CHEMISTRY to all levels—The Headmaster, The King's School, Canterbury, Kent.

MYCOLOGIST (with good taxonomic knowledge and physiological interests) for work on production of ergot alkaloids and other biologically active substances of fungal origin by fermentation methods—The Secretary, Biochemistry Department, Imperial College of Science and Technology, London, S.W.7.

British Antarctic Survey (formerly Falkland Islands Dependencies Survey). Scientific Reports. No. 49: A Magnetic Survey of North-East Trinity Peninsula, Graham Land. 2: Mount Bransfield and Duse Bay to Victory Glacier. By Dr. A. Allen. Pp. 32 + maps 1 and 2. 28s. net. No. 55: Seismic Refraction Investigations in the Scotia Sea. By Dr. A. Allen. Pp. 44 + 2 plates. 24s. 3d. net. (London: British Antarctic Survey, 1966.) [212]  
Fabian Society. Fabian Tract No. 369: Labour's Social Plans. By Prof. Brian Abel-Smith. Pp. 20. (London: Fabian Society, 1966.) 2s. 6d. [512]

### Other Countries

National Medical Library of Czechoslovakia. The Annual of Czechoslovak Medical Literature 1964. Edited by the National Medical Library of Czechoslovakia. Pp. xvi + 701. (Prague: Czechoslovak Medical Press, 1966.) [1312]  
American Chemical Society. Chemical-Biological Activities: Word Guide. Pp. v + 825. Chemical Titles: Word Guide. Pp. iv + 397. (Publications of the Chemical Abstracts Service, The Ohio State University, Columbus, Ohio 43210.) (Washington, D.C.: American Chemical Society, 1966.) [1312]

Colony of Mauritius. Meteorological Observations and Climatological Summaries. April, 1965. Pp. 31. May, 1965. Pp. 30. June, 1965. Pp. 34. (Port Louis: Government Printer, 1966.) [1512]

Population Reference Bureau, Inc. Population Bulletin, Vol. 22, No. 4, (November 1966): Truth and Consequences in a New Era. Pp. 81-104. (Washington, D.C.: Population Reference Bureau, Inc., 1966.) [1012]

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 109: Étude des Variations de la Résistance de la Fibre chez *Gossypium hirsutum* L.—Application à la Zone Cotonnière Septentrionale du Congo. Par Dr. J. Demol. Pp. 91. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1966.) 175 francs. [1912]

The Australian Mineral Development Laboratories. Sixth Annual Report, 1965-66. Pp. 41. (Adelaide: The Australian Mineral Development Laboratories, 1966.) [1912]

The Australian National University. Report of the Council for the period 1 January 1965 to 31 December 1965. Pp. v + 121. (Canberra: The Australian National University, 1966.) [1912]

Commonwealth of Australia. Department of Supply: Defence Standards Laboratories. Annual Report, 1965-66. Pp. 66. (Melbourne, Victoria: Defence Standards Laboratories, 1966.) [1912]

Transactions of the American Philosophical Society. New Series. Vol. 67, Part 7: Medieval Arabic Toxicology—The Book on Poisons of Ibn Wahshiya and Its Relation to Early Indian and Greek Texts. By Martin Levey. Pp. 130. (Philadelphia: The American Philosophical Society, 1966.) \$3.50. [1912]

Annals of the South African Museum. Vol. 48, Part 19: Hydroids of the Vema Seamount. By N. A. H. Millard. Pp. 489-496. 20 c. Vol. 48, Part 20: The Validity of *Raja rhizacanthus* Regan and *Raja pullopinetata* Smith, Based on a Study of the Clasper. By P. A. Hulley. Pp. 497-514. 40 c. Vol. 50, Part 1: The Girdles and Limbs of the Dicyonodontia of the *Tapinocephalus* Zone. By L. D. Boonstra. Pp. 1-11. 25 c. Vol. 50, Part 2: The Dinoccephalus Manus and Pes. By L. D. Boonstra. Pp. 13-26. 35 c. (Cape Town: South African Museum, 1966.) [2112]

Republic of Botswana. Annual Report of the Geological Survey Department for the year ended 31st December 1965. Pp. iv + 46. (Lobatse, Botswana: Director, Geological Survey, 1966.) [2112]

Sveriges Geologiska Undersökning Ser. Aa. Nr. 198: Geologiska Kartblad. Halmstad, Skala 1:50,000. kr. 8. Årsberättelse för År 1965. (Tillhör Årsbok 59, 1965.) Pp. 15. kr. 2. Ser. Aa., Nr. 198: Beskrivning till Kartbladet Halmstad. Av Carl Caidenius, Walter Larsson, Erik Mohrén, Gunnel Linnman och Helge Tullström. Pp. 138. kr. 25. Serie C. No. 604: Berggrund och Malmer i Svappavaaraområdet, Norra Sveridge. (Geology and Ores of the Svappavaara Area, Northern Sweden.) Av Rudyard Frietsch. Pp. 282 + 5 plates. kr. 48. Serie C. Nr. 606: Kartering av Bergbällar med Hjälp av Flygbildtolkning och Metodstudie. (Mapping of Bedrock Outcrops by Aerial Photo Interpretation: a Methodological Study.) Av Lelf Wastenson. Pp. 44 + 3 plates. kr. 8. Serie C. No. 608: Lake Hummeln: a Possible Astrobleme in Southern Sweden. 1: The Bottom Topography. By Nils B. Svensson. Pp. 18. kr. 4. Serie C. No. 611: Botanical Investigations in the Fossil Flora of Eriksdal in Fyledalen, Scania. By Hans Traaen. Pp. 36. kr. 5. (Stockholm: Sveriges Geologiska Undersökning, 1966. Distribueras genom Generalstabens Litografiska Anstalts Förlag, Stockholm 1.) [2212]

State University College of Forestry at Syracuse University. Technical Publication. No. 90: Polyporaceae of North America—The Genus *Poria*. By Josiah L. Lowe. Pp. 183. (Syracuse, New York 13210: Department of Forest Extension, State University College of Forestry, 1966.) \$1.50. [2212]

The Regional Research Centre of the British Caribbean at the Imperial College of Tropical Agriculture, Trinidad, W.I. (University of the West Indies). Soil and Land-Use Surveys No. 16: St. Kitts and Nevis. By D. M. Lang and D. M. Carroll. Pp. 50. (Trinidad, W.I.: The Imperial College of Tropical Agriculture, 1966.) 10s. [2212]

Australia: Commonwealth Scientific and Industrial Research Organization. The National Standards Laboratory. Pp. 52. (Chippendale, N.S.W.: The National Standards Laboratory, University of Sydney, 1966.) [2212]

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Discovery Reports, Vol. 34, Pp. 163-198: The Distribution of *Parathemisto gaudichaudii* (Guér.) with Observations on Its Life-History in the 0° to 20° E Sector of the Southern Ocean. By Jasmine E. Kane. (London: Cambridge University Press, 1966.) 30s. net. [112]

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 770, Vol. 251 (24 November 1966): The Late Quaternary History of the Cumberland Lowland. By D. Walker. Pp. 1-210 + plates 1 and 2. 80s.; \$12. No. 771, Vol. 251 (1 December 1966): Respiratory Adaptations of the Pupae of Beetles of the Family Psephenidae. By H. B. Hinton. Pp. 211-245 + plates 3-6. 31s. 6d.; \$4.70. (London: The Royal Society, 1966.) [112]

Fibres and Life. By Prof. A. Robson. (An Inaugural Lecture.) Pp. ii + 22. (Leeds: Leeds University Press, 1966.) 2s. 6d. [212]

British Engine, Boiler and Electrical Insurance Co., Ltd. Technical Report, New Series, Vol. 7, 1966. Pp. 98. (Manchester: British Engine, Boiler and Electrical Insurance Co., Ltd., 1966.) 12s. 6d. [212]

Glasshouse Crops Research Institute. Annual Report 1965. Pp. 158. (Rustington, Littlehampton: Glasshouse Crops Research Institute, 1966.) 12s. 6d. [212]

General Register Office. Census 1961—England and Wales. Household Composition Tables. Pp. xxxiv + 352. (London: H.M. Stationery Office, 1966.) 67s. net. [212]

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## MACHINE AT THE READY

THE British Government must now have finished the job of assembling the kind of machinery necessary for the administration of science and technology. This, at least, is what everybody must hope, for there is always a danger that reorganization, for all the inconvenience which it causes, will seem preferable to constructive activity. But now the old Ministry of Aviation is fast losing its identity within the Ministry of Technology. The Central Advisory Council on Science and Technology under Sir Solly Zuckerman is said to have met around a table on two occasions. On another stage, the Select Committee of the House of Commons on Science and Technology has begun to function. One way and another, the months ahead are likely to be full of bustle. It would be foolish as well as churlish to complain that nothing much has happened yet.

That said, it is only reasonable to keep in mind the doubts which conspire to suggest that the new machinery may prove to be deficient. Within the Ministry of Technology, it will take courage as well as good sense to bring about a rapid devolution of activity from government establishments to industry and the universities. From what the ministry is now saying in public, it can see what needs to be done. It remains to be seen whether it will be able to act as sensibly as it talks. The advisory committees will also need watching in the months ahead. The Council for Scientific Policy under Sir Harrie Massey has done so well in fighting for funds for the research councils that complacency may be the biggest danger. The Central Advisory Council will have more difficult battles on its hands, and its procedures are deficient in at least two important ways. For one thing, its proceedings will be private, which means that it will be less able than it should be to enlist informed opinion in its support. The fact that the Royal Society as such will not now be represented on the council is wise, at least as far as the society is concerned, but yet another reason for fearing that the advisory council will be working in a vacuum. Then it is not clear whether the council is strong enough, and well placed enough, to bring defence research and development fully under the same umbrella as civil work. The transfer to the Ministry of Technology of the Ministry of Aviation, traditionally the procurement agency for the services, will help, but the single representative of the Ministry of Defence on the new council may be a sufficient assurance that the services will accept the recommendations of the Central Advisory Council without further argument.

As luck will have it, the House of Commons committee may help to make good some of these deficiencies. It is bound, of course, to be some time before the committee has won such a high reputation for itself

that government departments will wait for its observations before making new policies, but the way in which the Select Committee on the Nationalized Industries seemed last week to have become one architect of the new policy on the organization of the Post Office is something to work towards. But, from the beginning, the committee on science and technology will be able to do valuable work by uncovering the arguments by which government departments and their committees suggest new policies or justify the old. Ministers and officials, who in Britain consistently seek to still criticism with secrecy, only ensure that it is often ill-informed. So everybody will be better off if the new committee of the House of Commons can be an instrument for enlightenment about the roots of policy. Certainly it should not fall in with the expectation of some officials that it will stick to uncontroversial matters. It should also take a particular interest in the machinery of government, which means that Sir Solly Zuckerman should be a frequent witness.

## PLAN FOR OCEANOGRAPHY

THE committee of the National Academy of Sciences on oceanography has produced a disappointing report (see page 957). Although it may give pleasure and profit to readers looking for new branches of research in which to work, it will not do much to help decide how the United States administration should seek to spend money on the development of oceanography. The chief reason for complaint is that the committee set out to formulate a set of principles intended to keep some kind of balance between oceanographic research of various kinds. There is, unfortunately, a danger that the rules which have now been suggested may become an obstructive precedent not merely in oceanography but in other fields as well.

The essence of the scheme put forward by the committee is an apparently tidy distinction between research of different kinds. The committee would like to see the National Science Foundation shoulder responsibility for what is called "discipline-oriented research", and the other agencies of the United States Government made responsible for short-term and long-term research which is "mission-oriented". The choice of short-term projects should be determined by "external considerations" including cost-benefit analysis, and, to make sure that the agencies do not put all their energy into a search for short-term benefits, the committee would like there to be an agreement on the ratio of short-term and long-term work sponsored by the

agencies. All this is put forward as a new "rationale of budget making". The trouble is that it is not a rationale but the opposite—an attempt to avoid thinking hard about individual proposals for research.

The most immediate difficulty is that of making a meaningful distinction between largely academic research sponsored by the National Science Foundation and the long-term work which agencies such as the Navy Department are encouraged to support in the belief that it will ultimately bring practical advantages. Thus the U.S. Navy is just as likely as a university research unit to decide that a long-term study of the movement of ocean currents would be worthwhile. This, indeed, is why the U.S. Navy has such a splendid record in supporting long-term research. Presumably there was once a committee paper setting out just how the navy would in the long run benefit from the successful studies of X-ray emission from the Sun which have been sponsored by the Office of Naval Research.

There is a real danger that the artificial distinction of the kind now proposed would turn out to be cramping either for the mission-oriented agencies or for the National Science Foundation. It is even more unrealistic, however, to suppose there can ever be a predetermined ratio of the cost of long-term and short-term research supported by one agency. This is bound to be an exceedingly arbitrary business and open to abuse as well. What the committee should have asked itself is how arrangements should be made to see that long-term research programmes and grant-giving are properly co-ordinated, and what arrangements there should be for stabilizing the scale of financing for long-term work against the fluctuations of enthusiasm for short-term goals. It would be an important step in this direction if the National Science Foundation were given a greater share of the money for oceanography. The councils created by the White House in July last year should help with co-ordination. But simple rules of thumb will create more problems than they solve.

## MARKING TIME AT GENEVA

It is entirely welcome that the nuclear powers have decided to wait for further discussions before tabling their draft treaty on the non-proliferation of nuclear weapons at the United Nations Disarmament Committee at Geneva. Three weeks ago (see *Nature*, **213**, 641) the nuclear powers seemed unreasonably optimistic about the chances of a quick agreement. Events have clearly taught them a little caution—and have set in train a flurry of diplomatic activity. Sir Solly Zuckerman has been in Bonn, doing his best to allay German anxieties about the Treaty. Lord Chalfont, the minister of state responsible for disarmament at the Foreign Office, has been trying to do the same in Brussels, where Euratom is at once offended and threatened by the suggestion that some other agency—the International Atomic Energy Agency at Vienna—

should assume international responsibility for safeguards and inspection. Other nuclear powers are engaged on the difficult discussions with India about the kinds of political guarantees which would at once be practicable and feasible. This is one reason why there was a welcome for the announcement a week ago that the United States and the Soviet Union have agreed to hold discussions on measures to limit their own deployment of strategic weapons. A tacit agreement between two similarly placed powers is much more easily arrived at than an agreement affecting everybody.

That, however, is no reason for giving up hope of what will happen at Geneva. The first need is to create a sense of realism there. It seems now to be acknowledged that a treaty to prevent the spread of nuclear weapons must be backed up by the most rigorous forms of international inspection, and the United Nations Assembly made it plain last year that the IAEA should be the instrument of choice. But the agency is only now beginning to stretch its wings. Although there are now more than fifty reactors on the books of the agency's inspectors, only a handful of these are power reactors. So far, they only cover one plutonium separation plant, and rules to cover diffusion plants, fabrication plants and uranium mines are not yet in operation. Nobody is to blame for this, but it is bound to be several years before comprehensive arrangements are worked out. That time scale must be reckoned with by the nations at Geneva. If they are lucky and get a quick agreement, they may have to put up with a provisional safeguards system. The delay will only be an insuperable obstacle if they do not know it in advance.

The political problems at Geneva are just as daunting, although it is pleasing that much less is now heard of the loss of the alleged uncovenanted benefits which could be expected to flow from a military programme of nuclear development. This view, of course, is nonsense. The argument about commercial secrecy, much heard in West Germany, will have less force when it is more widely recognized that research and development facilities would be less open to inspection than power plants. The argument about the place of Euratom in a system of international safeguards is less tangible, but in the long run it is unthinkable that the international agency should delegate its responsibilities for inspection to what is essentially a private organization. (That said, the existence of Euratom controls could make the task of international inspection lighter.) But the real difficulty about the treaty is its asymmetry. Whatever language may be used, the draft as it stands must seem to the non-nuclear powers to be a means of perpetuating the nuclear status of the others. Until the nuclear powers find some way of modifying this impression, they are not likely to get a treaty. Their insistence on rigorous inspection will only make it easier for the non-nuclear powers to retort by asking for a cut-off of production. On the face of things, the nuclear powers will be hard pressed to give a convincing reason why this should not be combined with the provisions on non-proliferation.



# NEWS AND VIEWS

## Post Office Research

THERE is some sharp criticism of the research programme of the British Post Office in the report of the House of Commons Select Committee on the Nationalized Industries, published a week ago (*The Post Office*, Volume 1, *Report and Proceedings*, Cmnd 340, H.M.S.O., 16s.). The select committee considers that the time is ripe for a "major review" of the scale on which research and development is being carried out. Although it acknowledges that the Post Office is spending 2.5 per cent of its revenue from telecommunications on research and development (adding up to £4.9 million a year with another £0.49 million for research on the postal services), and that the ratio of the spending at the Bell Telephone Laboratories in the United States is a similar proportion of the total revenue of the Bell System, the committee nevertheless suspects that research by the British Post Office may be no more than adequate. It draws particular attention to the need for "long-term research and development on telecommunications systems", and hopes that the transfer of the Post Office Research Station from London to Suffolk will provide an opportunity for basic research on new devices and on the mathematical aspects of communications theory. Although the select committee has clearly listened sympathetically to the view of industry that research and development should be even more closely linked with the production of equipment, it would prefer a closer link with the operation of the telecommunications network. As things are, the value of research and development carried out by industrial companies amounts to £10 million a year, and roughly half this cost is met by the Post Office. The select committee also exhorts the Post Office to develop closer links with universities by letting more research contracts to them.

The most ominous passage in the report may well be that concerning the recruitment of staff. The committee says that there has been a shortage of graduates, particularly good ones, for several years. One consequence was that men had to be taken off the long-term development of microwave equipment to help with the design of the satellite communications station at Goonhilly in Cornwall. The select committee says that the salaries offered by the Post Office to postgraduate scientists and to graduate engineers are not competitive with those obtainable in industry.

## A Select Committee

THE new Select Committee on Science and Technology met in the House of Commons for the first time last week. The committee has a Labour chairman, Mr. Arthur Palmer, and 13 members; 7 Labour, 5 Conservative, and 1 Liberal. The Labour members are Mr. Norman Atkinson, Mr. Tam Dalyell, Dr. E. A. Davies, Mr. D. Ginsburg, Mr. R. L. Howarth, Dr. David Owen, and Mr. B. S. Parkyn, and the Conservatives are Mr. Stephen Hastings, Sir Harry Legge-Bourke, Mr. Airey Neave, Sir Ian Orr-Ewing, and Mr. David Price. The solitary Liberal is Mr. Eric Lubbock.

Several of the members have qualifications, experience, or both, in science and science-based industry. Mr. Palmer is a chartered engineer and a chartered fuel technologist; Mr. Atkinson is a design engineer; Mr. Dalyell was secretary of the Labour Party Standing Conference on the Sciences, 1962-4; Dr. Davies gained his doctorate at Cambridge for work on superconductivity, and worked as a research scientist for Associated Electrical Industries, Ltd.; Dr. Owen is a fellow of the Royal Society of Medicine, and Mr. Parkyn a plastics chemist. Of the Conservatives, Mr. Hastings is a director of Handley-Page and the author of a book which criticizes the cancellation of the TSR 2 project; Mr. Neave has many years' experience of scientific and technical committees, and is a governor of Imperial College; Mr. Price is the Conservative front bench spokesman on science and technology. Sir Ian Orr-Ewing is an Oxford graduate in physics, and the Liberals are represented by another Oxford graduate, Mr. Lubbock, who read engineering.

For a House of Commons which is thinly populated with scientists, this is a fair showing. The committee intends to meet once weekly, and the sessions will be open to the public. Deliberations started with a discussion of the British reactor programme. What the committee does not intend to do is to conduct post mortems in the manner of the Public Accounts Committee; the intention is to discuss policy before it is made, and for this purpose it can call in evidence anyone it wishes. The Select Committee on the Nationalized Industries, with a similar constitution, established a convention of never calling ministers to give evidence, but the new committee has made it clear that it will not feel bound to do the same. The committee will make its recommendations in the first place to the House of Commons, although the intention is that they will filter through and influence policy-making.

If the committee does establish a reputation and begins to influence policy in an open way, it will be welcome. The new Central Advisory Council under Sir Solly Zuckerman will be operating in private, and all that is seen of the Council for Scientific Policy is an annual report. What these committees are thinking becomes apparent only when it is too late to influence them. The new select committee has the opportunity of showing that it is not necessary to retreat behind closed doors in order to influence policy.

## More about Oceanography

EVENTS seems unkindly to have taken the gloss off the report on oceanography by the U.S. National Academy of Sciences (*Oceanography 1966, Achievements and Opportunities*, National Academy of Sciences, \$5.00). Although the responsible committee under Dr. Milton Schaeffer of the Scripps Institute of Oceanography has been at work since 1956—it issued its first report in 1959—its most recent document was on the way to the printers when the White House announced its plan to create two new public bodies for fostering the development of oceanography—the Commission on Marine Science, Engineering and Resources and the National Council on Marine Resources and Engineering Development. That step was part of a spate of activity about oceanography which included a report on the subject by the Science Advisory Committee recom-

mending, among other things, a doubling of expenditure on oceanographic research (see *Nature*, 211, 446; 1966). Although the committee says that the existence of the two new bodies will help to implement the policies it now commends, what it has to say would undoubtedly have been more influential a year ago.

Although the report is principally concerned with an attempt to describe the ways in which oceanographic research can be with advantage increased, it will be read with most attention for what it has to say about the principles on which decisions should be made for investing new funds. The committee seems to be dissatisfied with existing machinery for allocating resources, and writes of the way in which proposals for research are sometimes subjected to "reviews of previous reviews by the same reviewing bodies". It pleads for a distinction between "discipline oriented activities" and "mission oriented activities".

U.S. FEDERAL EXPENDITURE ON OCEANOGRAPHY  
(millions of dollars)

	Department of Defense	National Science Foundation	Total (all agencies)
1958	9.1	1.5	21.3
1961	31.6	7.9	62.1
1964	54.6	19.6	123.1
1967	114.9	28.5	217.5

The first category of research projects is said to include those whose object is to "increase understanding of ocean science as a field of knowledge", and for which the National Science Foundation should be the principal source of funds. (The committee recognizes that agencies such as the Office of Naval Research have played an influential part in supporting basic research in oceanography.) Among the "mission-oriented" projects the committee distinguishes some which are expected to have "near-term payoffs" and those which promise more distant results. It is afraid that long-term projects will be neglected for the sake of quick returns, and asks grant-giving bodies to keep to some more or less constant ratio of activity. In the same spirit, the committee exhorts the independent grant-giving agencies—the National Science Foundation and the Smithsonian Institute—to recognize that practically oriented research should be founded on a sufficient basis of "discipline oriented research". Further restraints on the pattern of spending are imposed by remembering, for example, that a 600 ton research vessel will require that \$1.5 million a year should be spent in the shore facilities to which the oceanographers return after their spells at sea.

## Government Social Survey

THE British Government Social Survey is to become an independent body responsible to the Treasury on April 1, 1967. Since it was set up in 1941, the survey has been part of the Central Office of Information, originally the Ministry of Information. In 1965 the report of the Committee on Social Studies under Lord Heyworth recommended that the survey should be separated from the COI because of the unavoidable weaknesses of administration. The committee considered that the services which the survey could provide were often inadequately understood by government departments, and that studies being carried out for different departments could in many cases be combined. There was also no provision in the organization of the survey for

advance planning to meet the needs of future government policy.

It is hoped that the reorganization will make it possible for the survey to take a more active part in the training of research workers in the social sciences. With an annual budget of about £400,000, the survey carries out a third of all government research in the social sciences. It is to have a new controller, Mr. T. D. Kingdom, formerly an under secretary at the Ministry of Social Security, who will report to an interdepartmental committee composed of people from the main user departments and from the Treasury.

## Some Cheer from Florence

THE Botanical Institute of Florence seems to have survived the floods of November 1966 with less damage than was at first expected. The director of the institute, Dr. Eleanora Francini Corti, says that the institute and the garden were flooded, the ground floor to a depth of 70 cm. Nevertheless the great herbaria, which are the most important collections at the institute and which were kept on the first and second floors, were quite undamaged. The underground archives were less well protected, but it seems that the inks of the oldest manuscripts have survived their submersion without damage. The newer volumes survived less well, partly because of the quality of the paper from which they were made. Stocks of the botanical journals published from Florence have been seriously damaged. As a whole, however, it seems that the flood waters which invaded the institute were not contaminated with the petroleum products which proved to be extremely damaging elsewhere in the city.

## Science Spending in New Zealand

THE New Zealand government has been doubling its expenditure on science every seven or eight years for the last three decades according to figures compiled by the New Zealand National Research Advisory Council and now published by the New Zealand Department of Scientific and Industrial Research, Wellington (*Scientific Research in New Zealand—Government Expenditure and Manpower 1926–1966*, 5s.). Even so, the total expenditure in New Zealand on scientific research by the government amounted to just under £6 million in 1966. The Department of Scientific and Industrial Research is the largest consumer of funds (£1.7 million), and agriculture takes more than a fifth of the total—a proportion which has been declining steadily over the years since the war, when more than a third of all public expenditure on research was spent on agriculture. These figures are reflected in the pattern of employment. Public service in New Zealand employs just under 1,000 people on research, with 562 of them in the Department of Scientific and Industrial Research, 255 in agriculture, and mere handfuls elsewhere except in forestry.

## Calouste Gulbenkian Foundation

DURING 1966 the Calouste Gulbenkian Foundation gave away £4,635,000 in the form of grants, £532,432 of it in Britain and the British Commonwealth. In the Commonwealth the interest of the Foundation is mainly in the arts, and about half the money spent

comes under this heading, but elsewhere the emphasis is different. In Portugal £635,225 went on science and medicine, £584,270 on education, and only £163,597 on the arts. Armenian communities throughout the world (£552,975), social and cultural projects in South America (£820,738) and gifts to charities in the Middle East and elsewhere made up the rest of the budget.

In Britain the foundation supports some fascinating projects. The University of Bristol was given a grant to study the social and psychological consequences of development and rehousing in Bristol, and group homes for former psychiatric patients and for unsupported mothers and their children have been supported in Colchester and Nottingham. The University of Kent at Canterbury was given £35,500 towards the cost of a theatre and arts centre at the university, and the universities of Warwick and Wales (Bangor) similar sums for similar projects. At Cambridge grants have been given to continue work on the publication of a new complete edition of the Linear B tablets from Knossos, and to study the demography and social structures of 18th century Britain. The University of York has perhaps the most unusual project of all: £7,850 to study the internal government of British universities.

### More for Research Associations?

At least one of the co-operative industrial research associations will be supported more generously by the Ministry of Technology in the year ahead. It is understood that the ministry has offered the Welding Research Association substantially improved terms of grants, and that the ministry is willing to contribute to the marginal income of the association no less than 150 per cent of what can be raised by means of subscriptions from industrial companies. The standard form of grant entails that the government should match the industrial contribution pound for pound. No doubt the ministry will be quick to inform other research associations that the welding research association is in a special position dictated by the character of its work as a provider of help and advice to a great many industries whose needs cannot always be specified in advance. But there is bound to be a feeling among the research associations that what is good for welding would also be good for others. The way in which the government is encouraging mergers between industrial companies is in any case a good reason for looking again at the financing of the research associations.

### In Charge of Static

A NEW group has been set up by the Institute of Physics and the Physical Society to study static electrification. The new group will encourage study in the generation, dissipation and convection of electric charge on, in and between solids, liquids and gases, and will include both desirable and undesirable electric charges. It is suggested that the group might consider theories of contact electrification, electrostatic charge generation and dissipation, the electrification of liquids during pipeline flow, electrostatic precipitation and coating, and the hazard from static electricity in the textile, chemical, petroleum and photographic industries.

The first chairman of the provisional committee will be Dr. P. S. H. Henry of the Shirley Institute,

and the secretary will be Dr. N. Gibson of Imperial Chemical Industries Dyestuffs Division. There has so far been very little fundamental work on static electrification, and although Dr. Henry believes that the industrial side is the most important (static interferes with production, and can cause fires) he is also hoping to stimulate some fundamental work. Already one three-day conference has been arranged, to be held in London from May 8 to 10, and next year it is likely that regular half-day meetings will be arranged. Although the terms of reference of the group include a variety of disciplines, the problems will apparently be common to all.

### Uproar at Millom

SPRAY steelmaking, a new process developed by the British Iron and Steel Research Association (*Nature*, 212, 331; 1966) seems so far to have created more problems than it has solved. The process, which offers the nearest approach yet to continuous steelmaking at a capital cost said to be less than a third of that of conventional equipment, was developed at the Cumberland works of Millom Hematite Ore and Iron Company. The company, which has run the process so far only on a relatively small scale, is anxious to go ahead with an ambitious plan which would give it a steel capacity of 500,000 tons per year, and continuous casting equipment to make best use of the new process. The plans, which necessarily include gas cleaning equipment, would cost £1 million, and the Iron and Steel Board, which has to approve all expenditure in the industry of more than £100,000, deferred its decision. This was taken as a refusal, for the board had said that it was reluctant to approve the creation of small scale capacity when the trend is towards large scale operation. This week the board gave grudging approval for Millom's plans, adding that it would have prohibited the move if it had had the power to do so.

The deferment of Millom's application had caused uproar in the north-west, aggravated by the success of Lancashire Steel Manufacturing Co., Ltd., in going ahead with a spray steelmaking plant capable of making 50 tons of steel per hour. This is producing steel only three months after building started—remarkable in an industry where new processes characteristically take a long time to catch on. Millom's case was given emotional impact because the town relies almost entirely on the ironworks for employment and without it would be hard pressed to survive. Ironically, since Millom is in a development area, now that it has permission to go ahead the government will make a substantial contribution to the cost of the plant, possibly as much as £450,000.

Despite the bitter arguments, the future for spray steel looks promising. Apart from Lancashire Steel, there are hints that another British company—possibly United Steel—is interested. A Canadian company, Dominion Foundries and Steel, Ltd., has already taken out a licence from BISRA to develop its own spray unit. The company intends to do theoretical research on the process before building a pilot plant. Criticisms in the United States and Canada include the possibility that the reaction chemistry could play havoc with furnace refractory linings (BISRA says that as the reactions do not take place in

contact with the refractory walls, life of the linings should be increased and not diminished) and whether the process is genuinely continuous. If the process has to be stopped to change the composition of the steel produced, much of its advantage would be lost. There is, too, the problem that the process has so far produced only plain carbon steel, and has not been used for alloy steels.

## Animal Sonar

THE international symposium on animal sonar held at Frascati, Italy, under the auspices of the North Atlantic Treaty Organization, the United States Office of Naval Research, and the United States Air Force, brought together for the first time workers in this and related fields from member countries of the North Atlantic Treaty Organization. A feature of the symposium was the opportunity for an exchange of views between biologists, engineers and physicists, and it was so organized that the participants were able to study the main contributions in advance.

It rapidly became clear from the discussions that, although life scientists working on animal sonar had been in contact with sonar engineers over a long period, there had been insufficient exchange of information. Biologists tend to know too little about the advanced physics and systems engineering involved in the work of sonar engineers, while the engineers often lack the biological knowledge to take advantage of the information available about animal sonar. These difficulties have in the past tended to decrease the usefulness of experiments with animals. Such experiments need considerable preparation: the animals must be trained to carry out simple tasks, and the training may take many months. Unless the relevant physical processes are taken into account when the experiment is being planned, the results will be far less useful than they might otherwise have been.

Indeed, despite the extravagant claims of some early workers, animal sonar seems to obey the same laws as do artificial systems. The animal differs from the machine, not in its ability to transcend the laws of physics, but in its ability to adapt, and in the greater storage capacity of its neural system. The animal is also a single integrated system and takes its own decisions. The closest approximately to this aspect of animal sonar that can be achieved with a machine is to link a man to a machine so that he can take decisions. This is the approach used in the portable sonar outfits now issued to some blind persons, and the information obtained as a result of the use of such outfits has given a useful insight into the mode of action of animal sonar. Sonar engineers are probably trying to incorporate some of the animal adaptability into their machines, but they are reluctant to talk about this aspect of their work in public.

The symposium was less successful in its attempt to relate the processing of sonar signals in the animal brain to the systems used in engineering. This is scarcely surprising: neurophysiology is still far from the type of complex model used in systems engineering. Cross-fertilization of ideas will undoubtedly stimulate both sides even in this difficult corner of the field. The symposium seems to have broken the ice separating the disciplines concerned with sonar, and some beneficial effects of the thaw should be evident at the next gathering.

## Ribonuclease Structure—Some Implications

from a Correspondent in Molecular Biology

THE solution of the ribonuclease structure at a resolution of 2 Å by Harker and his collaborators, as reported in *Nature* last week (Kartha *et al.*, *Nature*, **213**, 862; 1967), is clearly an event of unique importance and interest. Of all enzymes, ribonuclease has been the most intensively studied, and its structure is probably that which has been awaited most impatiently by protein chemists, physical chemists and enzymologists alike. The schematic illustration of the chain outline shown in the article gives only a small portion of the information which is expected shortly to emerge, but some interesting conclusions may already be drawn, particularly in connexion with predictions based on chemical evidence.

The active site, as identified by Kartha *et al.*, lies in a deep cleft between two wings of the molecule. There is remarkably good agreement with chemical evidence, due notably to Moore, Stein and co-workers (who also determined the sequence of amino-acids in the molecule). Two histidine residues, his-119 and his-12, were found to be necessary for activity and sterically related; thus, for example, the alkylation of either by a suitable reagent was prevented by the alkylation of its partner. These residues are indeed seen to flank the active centre cleft. Another residue which was unambiguously shown by Hirs in 1962 to be required for activity is lys-41, which appears at the third surface of the cleft opposite the two histidines. There is also some evidence for the involvement of lys-7 and indeed it has been shown that lys-7 and lys-41 can be joined by a bifunctional cross-linking reagent. With a minimum of hindsight it can be seen that other more equivocal identifications of active residues in the literature now appear to be wrong. Suggested mechanisms, of which a number have been put forward, will have to await critical examination until electron density maps of the enzyme with a bound substrate analogue have been obtained.

It is interesting to note that the segment corresponding to the *S*-peptide of Richards stands away from the molecule, almost over the cleft. This peptide (residues 1–20) can be split off by hydrolysis of a single peptide link with subtilisin. It will recombine non-covalently with the remainder of the enzyme molecule (*S*-protein) to give an active product. It was shown by Anfinsen and co-workers that residues 16–20 are in fact largely dispensable for binding and activity, but that 14 and 15 are required for proper binding. It is gratifying that residues 16–20 in the model appear to form a loop barely in contact with any other part of the protein, whereas in the region of residues 14 and 15, non-covalent interactions are likely.

The  $\alpha$ -helix content of ribonuclease is low, 17 per cent being apparently an upper limit. This agrees well enough with optical rotatory dispersion measurements, which give values around 15 per cent. No  $\beta$ -conformation is present.

The appearance of the ribonuclease structure necessarily represents, among other more important consequences, a day of reckoning for those bold enough to have offered three-dimensional structures based only on indirect evidence. Such structures do not appear to have fared well. The most recent, by Hammes and Scheraga, has features in common with reality (apart

from the constraints provided by the disulphide bridges) only to the extent that it incorporates the correctly identified residues mentioned above juxtaposed in the active centre. For the rest, the non-covalent interactions deduced by Scheraga and co-workers are evidently almost wholly specious. Earlier models, by Saroff and by Scheraga, are as different from the above model as they are from the actual structure. At the present stage it therefore appears that the indirect approach to the construction of three-dimensional protein structures is of dubious value.

The appearance of more complete structural data will offer the possibility of interpreting the mechanism of nucleolytic hydrolysis, and will now be awaited with the greatest interest.

## Momentum Dependent Interactions

### from a Correspondent in High-energy Physics

IN a recent experiment at the Cornell 2 GeV synchrotron, K. Kerkelman *et al.* (*Physics Letters*, **24B**, 165; 1967) have measured the momentum dependence of the magnetic dipole transition between a proton and the positively charged  $N^*$  resonance at 1,230 MeV.

The inelastic cross-sections for the processes in which an electron striking a proton produce in the final state an electron together with either a proton and a neutral pion or a neutron and a positively charged pion show a marked enhancement when the effective mass of the final nucleon and pion system is near 1,200 MeV. This effect is usually interpreted as a first order electromagnetic process in which the scattered electron emits a virtual photon—one for which the effective mass is not zero—which in turn excites the proton into a state with mass 1,200 MeV. A final pion and nucleon are then produced by the (very fast) strong decay of this  $N^*$  resonance—or particle as it is frequently called. On this interpretation of the state as a particle with isotopic spin  $3/2$ , positive parity relative to the nucleon and spin  $3/2$ , the transition from the nucleon induced by the virtual photon can be shown by general covariance and angular momentum properties to be of only three possible types. These are a magnetic dipole ( $M1$ ), an electric quadrupole ( $E2$ ) and a Coulomb octupole ( $C2$ ) transition, each of which is measured in terms of a corresponding form factor depending only on the momentum transfer (the effective invariant mass of the virtual photon).

If the analysis in terms of a virtual photon is valid, then these form factors have already been measured for zero momentum transfer by experiments in which the  $N^*$  state is excited by a real photon (Gourdin, M., and Salin, P. H., *Nuovo Cimento*, **27**, 193; 1963). Thus it is known that the form factor describing the  $M1$  transition has a value at zero momentum transfer of  $3.00 \pm 0.01$  in units of nuclear Bohr magnetons and that the other two transitions are much smaller (less than 5 per cent). In the electro-production experiment the analysis is therefore based on the assumption that only the  $M1$  transition contributes—a fair assumption for this first experiment. This simplification makes it possible in principle to measure the  $M1$  transition form factor  $G_M^*(k^2)$  by the standard techniques of observing only the scattered electron, provided that the dominant contribution is known to be from the  $N^*$

and not from non-resonant background effects. The technical advance which has made this experiment possible is that events have been selected in which a final proton and neutral pion are detected in coincidence. Since the photoproduction results indicate that this final state (in contrast with the neutron-positive pion state) is predominantly resonant, the background is largely eliminated.

The results obtained over a range of momentum transfers up to 8 (GeV)<sup>2</sup> extrapolate neatly back to the value at zero momentum transfer measured in the photoproduction experiments, and thus confirm the validity of the model assumed. Furthermore, the momentum dependence is roughly in agreement with the predictions of both the dispersion theory and the relativistic symmetry schemes for the strong interactions which are presumed to be the source of this effect, although detailed comparison requires much improved experiments.

This experiment surely marks a new era of sophistication in the use of electron machines. The new synchrotrons in Germany (DESY) and England (NINA) are starting operation without any substantial energy advantage over earlier machines. Crude elastic electron scattering experiments can hardly justify extensive running time, and inelastic experiments, particularly of the coincidence and polarization types, must figure largely in their future programmes.

## Egon Bretscher Retires

THE Atomic Energy Research Establishment at Harwell, now 21 years old, is branching out into new fields with the setting up of centres for ceramics and materials testing. It remains, however, a place with powerful associations for physicists, and two of them have recently been writing of the retirement of Dr. Egon Bretscher, head of the Nuclear Physics Division since 1948, who had been associated with work on atomic energy since the early days of the war. After graduating as a chemist in 1927, Bretscher had moved to Cambridge, where he collaborated with Feather on the problem of uranium  $Z$  when, according to Feather, "progress depended essentially on the trustworthiness of Bretscher's chemical preparations: time has shown that they were impeccable". This was followed by neutron bombardment with particles from the one million volt accelerator, and towards the end of 1938 the group was making preparations to study the X-radiation of the supposed transuranic elements—those which, a few months later, were shown by Hahn and Strassman to be radioactive isotopes of elements with smaller atomic number.

A. P. French explains how Bretscher turned from the radiochemistry of uranium to the measurement of fission cross-sections for fast neutrons using neutrons from the D+D reaction. One of Bretscher's innovations at this point, in 1942, was to devise a way of extending the technique of measurement with a homogeneous ionization chamber so as to measure neutron flux in the presence of a strong gamma-ray background as with neutrons from the  $^{12}\text{C}+\text{D}$  reaction. This work was helped along by the possession in Cambridge of 2 g of fully deuterated paraffin wax, prepared a few years earlier at the heavy water plant in Norway. French says that this was used to line a chamber that was filled with deuterio-ethylene and used differ-





entially with an otherwise identical chamber lined with ordinary paraffin wax and filled with ordinary ethylene—a very clever scheme for automatically balancing out the ionization current due to gamma rays while leaving a calculable effect due to neutrons.

In 1944 Bretscher and many of his colleagues moved to Los Alamos, and French notes that even then thinking had moved beyond the fission bomb to the possibility of making a hydrogen bomb. But experimental information on the relevant reactions involving deuterium and tritium was sadly lacking. As French points out, “the  $D + D$  reaction had never been followed down to thermonuclear energies of the order of 10 keV, and the reaction of deuterium with tritium was mostly a big question mark, though there were hints and speculations of a prodigious cross-section”.

Bretscher directed the design of a low voltage accelerator to record protons from these reactions between like nuclei. Some time in 1945, “the world supply of pile-produced tritium gas—a whole cubic centimetre of it, many times more costly than plutonium, weight for weight—was delivered to us, and with this we were to make our measurements. Bretscher himself undertook the nerve-racking task of breaking the vial of tritium inside a gas-transfer system. Late one night, shortly afterwards, the crucial moment came. The gas supply to the ion source was switched from deuterium to tritium. Instead of occasional pulses on the oscilloscope, there appeared an absolute hail-storm. The implications were frightening and as soon as we had convinced ourselves that the result was genuine we went home in a mood that was a strange blend of excitement and awe.

“Over the next few months the measurements were refined and extended, with the help of a couple more consignments of tritium. Everything confirmed the first indications; the  $T + D$  reaction had a monstrously large yield (of the order of fifty times greater than for  $D + D$ ) at fission-bomb temperatures. The theoreticians seized on these results, and it seemed obvious at the time that the super-bomb would consist of a great mass of liquid deuterium set off by a fission bomb fuse and a tritium/deuterium detonator. But practical realization of such a design was certainly not going to happen soon, especially as the ending of the war brought about a general lessening of urgency.”

## Parliament in Britain

In a written answer in the House of Commons on March 1, the Minister of State for Education and Science, Mr. G. Roberts, stated that studentships were only awarded by the Science Research Council and the Social Science Research Council to nominees of university departments. In 1966, the Social Science Research Council received 429 nominations, and 43 were not offered awards. The Science Research Council received 2,567 nominations in 1964, and 60 were not offered awards; for 1965, the figures were 2,928 and 143; and for 1966, 3,096 and 321.

On March 2, also in a written answer, Mr. Roberts gave figures for the average expenditure per full-time student of the universities for the academic year 1964-65. The figures vary from £2,377 (Essex), £1,268 (London), £1,125 (East Anglia), £1,121 (Reading), £1,092 (Manchester Institute of Science and Technology) to £695 (St. Andrews), £690 (Durham), £612 (Strathclyde), £580 (Hull), £563 (Exeter), and £537 (St. David's College, Lampeter). The figures for Cambridge (£805) and Oxford (£781) are not directly comparable as they exclude all expenditure by the Colleges.

In giving an analysis of the origin by country of the 2,686 Commonwealth students in Britain who are supported by British public funds, the Parliamentary Secretary to the Ministry of Overseas Development, Mr. A. E. Oram, in a written answer in the House of Commons on March 2, stated that the average cost of a student from the beginning to the end of his course and allowing for passage costs, etc., was about £2,425. This did not include the hidden subsidy represented by the uneconomic fees charged by institutions of higher education.

In a written answer in the House of Commons on February 28 the Minister of Technology, Mr. A. W. Benn, gave a long summary of the action taken to implement the fourteen recommendations of the Fielden Committee on Engineering Design which reported in June 1963. A series of regional conferences on engineering design were arranged during 1964 by the Federation of British Industries and the Department of Scientific and Industrial Research. The Ministry of Technology works closely with the Council of Engineering Institutions in its efforts to raise the status of the engineering profession as a whole, and after 1973 the academic standard required for the professional qualifications of “Chartered Engineers” will be of degree standard. The Science Research Council has continued to award grants for studies of the design process. Ways of introducing elements of engineering design into certain National Certificate and Diploma courses in engineering are being considered by the Department of Education and Science, while the Engineering Industry Training Board has set up a Technologist Training Policy Committee to prepare a manual on training professional engineers in industry. The first of the institutes recommended by the report has been established at the National Engineering Laboratory at East Kilbride. In addition, he added that the Ministry of Technology and the Science Research Council had placed contracts to further the development of computer-aided design.

## University News: Aston in Birmingham

PROFESSOR D. F. LAWREN, at present professor of mathematics in the University of Canterbury, New Zealand, has been appointed to the chair of mathematical physics in the Department of Mathematics.

## Bradford

PROFESSOR A. RAHMAN, scientist in charge of the Survey and Planning of the Scientific Research Unit of the Council of Scientific and Industrial Research, India, and in charge of the International Scientific Collaboration Bureau, has been invited to a visiting chair in science planning and sociology of science for one year.

## Nottingham

PROFESSOR H. J. KING, at present professor of applied mineral sciences in the University of Leeds, has been appointed professor and head of the Department of Mining Engineering in succession to Professor F. B. Hinsley.

## Reading

PROFESSOR J. WRIGLEY has been appointed to the joint post of professor of curriculum research and development in the university, and director of studies and research adviser to the Schools Council. Dr. J. B. Adams and Mr. A. E. Milward have been co-opted to membership of the Council of the University. Mr. Milward has been chairman of the Friends of the University since 1956, and Dr. Adams is member for research of the U.K.A.E.A.

## Appointments

DR. T. M. SUGDEN has been appointed director of the Thornton Research Centre of "Shell" Research, Ltd., near Chester. Dr. Sugden was reader in physical chemistry at the University of Cambridge before joining Shell in 1964, and is best known for his work on combustion, particularly in the detection of traces of metals by flame photometry.

MR. P. H. STEPHENSON, formerly chief mechanical engineer of the Pye Group of Companies, has been appointed director of the Institute of Advanced Machine Tool and Control Technology at East Kilbride, near Glasgow. The institute is the first to be established following the recommendation of the Feilden Committee on engineering design. Mr. Stephenson has also been appointed a professor of the University of Strathclyde.

DR. B. G. F. WEITZ has been appointed director of the National Institute for Research in Dairying in succession to Sir Ronald Baskett.

## Announcements

THE James Clayton prize for 1966 has been awarded by the Institution of Mechanical Engineers to Dr. S. G. Hooker and Mr. A. A. Lombard for their contribution to the design and development of aero-engines leading to great advances in aerial transport, and particularly for their work in the field of vertical lift for take-off and landing.

THE International Journal of Cancer wishes to publish a collection of brief reports of the occurrence of Burkitt's lymphoma outside the African region, and the editor would be interested to receive reports from workers in this field. Further information can be obtained from Professor E. A. Saxén, Editor in Chief, International Journal of Cancer, 111 Department of Pathology, University of Helsinki, Helsinki 25.

THE Summer Courses of the International School of Physics "Enrico Fermi" are to be held this year at Varenna. The first course, which is during June 26–

July 15, is entitled "Nuclear Structure and Nuclear Reactions"; the second course, which is during July 17–29, is entitled "Selected Topics in Particle Physics"; and the third course, which is during July 31–August 19, is entitled "Quantum Optics". Further information concerning all three courses can be obtained from the Società Italiana di Fisica, Via Irnerio, 46—Bologna.

A THREE weeks course on the "Application of the Electron Microscope to Problems in Molecular Biology", sponsored by the European Molecular Biology Organization, will be given in the Institut de Biologie Moléculaire in Geneva during September 4–23. The course will stress specimen preparation and examination techniques and will be designed for participants at the post-doctoral or advanced Ph.D. student level with a good background in some branch of molecular biology. Applications should be sent before June 1, and further information can be obtained from either Lucien G. Caro, Biology Division, Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tennessee, or Edouard Kellenberger, Laboratoire de Biophysique, Institut de Biologie Moléculaire, Université de Genève, 24 quai de l'Ecole-de-Médecine, 1211 Genève, 4.

A SYMPOSIUM on the "Systematics of Cultivated Plants and Domesticated Animals", organized by the Systematics Association, will be held at the Dunfermline College of Physical Education in Edinburgh during April 12–13. Further information can be obtained from Dr. Patricia J. Watson, Scottish Plant Breeding Station, Pentlandsfield, Roslin, Midlothian.

ERRATUM. In the article entitled "Evidence for a New Mechanism of Respiratory Stimulation and Proton Ejection in Ehrlich Ascites Tumour Cells dependent on Potassium Ions" by Edwin E. Gordon, Kerstin Nordenbrand and Lars Ernster (*Nature*, 213, 82; 1967), the concentrations of the reagents specified in the caption to Fig. 1 were wrongly printed as in moles/l. They should, of course, have been in mmoles/l. In the legend to Table 1, where the composition of media for depleting cells of potassium ions were specified, the sodium concentration should be 1.78 mmoles/l. In the lithium medium, the concentration of magnesium should have been given as 1.5 mmoles/l. (not 1–5 mmoles/l.), and the other ingredients should have been 1.5 mmoles/l. of magnesium, 154 mmoles/l. of chloride, 1.5 mmoles/l. of sulphate, 12 mmoles/l. of phosphate. The choline medium had 154 mmoles/l. of choline, 1.5 mmoles/l. of magnesium, 12 mmoles/l. of *tris*, 166 mmoles/l. of chloride, and 1.5 mmoles/l. of sulphate.

The following corrections should also be made. Page 83, left-hand column, line 32, should read: There was release of inhibition of respiration and inhibition of extrusion of hydrogen ions induced by oligomycin on addition of valinomycin. Last sentence, left-hand column, p. 83, should read: Further addition of potassium ions to the cells depleted of this ion resulted in a significant effect on both parameters for the cells depleted in the media containing either sodium or lithium ions. The effect . . . The legend to Fig. 2 should read: Release of respiration and proton extrusion inhibited by oligomycin in cells depleted of potassium ions. Page 84, left-hand column, line 23, should read: The release of respiratory inhibition induced with oligomycin by potassium ions plus valinomycin was similar to the release effected by dinitrophenol, except that the release by dinitrophenol was not dependent on the presence of potassium ions. The authors are indebted to Dr. J. C. MacDonald of the Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada, for providing the valinomycin.

ERRATUM. In a note entitled "British Science Spending" in News and Views (*Nature*, 213, 749; 1967), the second of the four research councils listed should have read Natural Environment Research Council and not National Engineering Research Council.

# Education in East Germany

by

NIGEL HAWKES

*Jena, February*

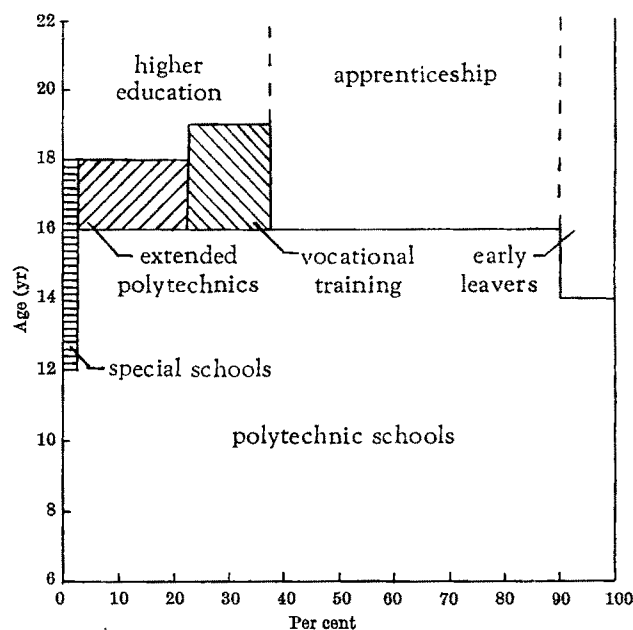
MR. McNAMARA thinks that Europe is educationally backward, and makes no bones about saying so (*Nature*, 213, 851 and 853; 1967). Certainly there are few European countries which can rival the United States in terms of the sheer quantity of education which it supports. There is, however, one country at least in Europe which claims to match America's commitment to education. Twenty per cent of its GNP is spent on education, and one-third of its students continue education beyond the age of 18. The education system has a distinctly technological bias—all of which would doubtless gladden Mr. McNamara's heart, if East Germany were not part of the communist bloc.

Since the war East Germans have shown a compulsive desire to eliminate all traces of the old Germany. West Berlin, like most cities, is a pleasing mixture of old and new, and has a traffic problem; East Berlin, on the other hand, is a city of sweeping boulevards flanked by ten-storey blocks of workers' flats built with wonderful thoroughness but a sorry sense of design. Education has also been completely reconstructed, in a way which illustrates that East Germans are pragmatists as well as socialists. What follows is based on a description of the East German system supplied by the director and assistant directors of the apprentice school at Jena, one of the largest in the country. While the figures are impossible to substantiate—if they are all true it is difficult to see where supplies of unskilled labour come from—there is no reason to doubt the general structure, although it may not be in general operation everywhere in the country.

The backbone of the structure is the system of "polytechnic" schools. Children enter them at the age of 6, and most stay until the age of 16. These schools give a general education, with a powerful push towards vocational training; in the last two or three years children spend one day in each week in practical training in factories, in a kind of inverted version of British "day release". The aim is for all children to stay at school until the age of 16, but it is admitted that at the moment about 10 per cent leave at the age of 14. Russian is taught from the age of 11, and English—as an option—from the age of 13.

At 16, the student has three choices. The commonest course is to enter an apprenticeship, which lasts two or three years, and leads to a nationally recognized certificate. Some 40 to 50 per cent of school leavers do this, East Germans claim. Training during apprenticeship is free, and a nominal allowance of 120 marks (about £10) a month is paid. When the apprenticeship is completed, an employment agreement is signed; most apprentices at the Jena apprentice school stay in Jena to work for Carl Zeiss "as a matter of course". The whole cost of the course, and the teachers, is paid by the state, and there are apparently more applicants than places.

Two other courses are open to the school leaver, both leading to higher education in universities and high schools. About 20 per cent of the 16 year olds go to extended polytechnics until the age of 18, when the



Apart from the special schools, the East German education structure shows no division until the age of 16, when transfer takes place. In Britain the Plowden report, devoting five pages to a discussion of the age of transfer, decided that 12 was the ideal age.

qualifying examination for university entrance—the "Abitur"—is taken. The other possibility is to take a combined "Abitur" and vocational training course, which takes three years to complete. At the end of the course the students are qualified either to enter university or high school, or to take up the vocation they have studied.

So far the system seems to conform with socialist principles. But there are other, newer schools which are by no means as egalitarian, and are clearly designed to cultivate an élite. These are the special schools, which only a tiny minority—about 2 per cent—of the population attend. The special schools cover subjects such as technology, sport, fine art, and music, and the children selected for them start at the age of 12 and continue until they are 18. The best pupils in the polytechnic schools are selected for special schools on the basis of reports from their teachers, and it is claimed that the method of selection is so good that only 2-3 per cent of those selected drop out before the age of 18. It is not possible for able pupils passed over at the age of 12 to enter the special schools at a later stage, so that the schools represent a complete separation of a small section of the population from the rest. The products of the special schools in technology are intended to be middle or top managers in industry, or to take up jobs as representatives overseas. In addition, although, of course, the East Germans are

not explicit about this, special schools offer very good opportunities of recruitment to the Communist Party, or at least of obtaining loyalty to the party line. This is particularly important for those who are allowed to travel overseas, either as sales or service engineers, or even as sportsmen.

Great stress is laid on group activities in all East German schools, and particularly in the special schools. The groups are organized by the teachers, assisted by youth groups such as the Youth Pioneers, and the aim is to promote "collective as well as individual achievement". To judge from the posters which are displayed by the youth groups in the apprentice school at Jena, political activities go at least as far as condemning American activity in Vietnam, and declaring support for the socialist state, if no further. The posters seem no more offensively propagandist than any that might be seen in Trafalgar Square, but their unanimity is depressing. The apprentices themselves—half are girls—seem very young by comparison with western 15 year olds, but a down to earth style of dress and absence of cosmetics have a lot to do with this.

Central control combined with a belief in education does provide one benefit which hard-pressed British teachers would welcome—East German teachers are paid more than their counterparts in industry. Their advantage is derived from an arrangement which provides for them to pay tax on only 70 per cent of their income. This fringe benefit, it is explained, is not a capitalist blunt instrument for persuading people to become teachers, but is intended to allow teachers to buy the books and literature they need. Whatever the reason, pupil-teacher ratios (not to be confused with the actual numbers in each class, which are slightly higher because not all teachers are working all the time) seem much the same as in Britain, 28:1 in the polytechnics, and 25:1 elsewhere. Teachers work a 45 hour week, and teach 24 lessons (each of 45 minutes) each week; the rest of the time is for preparation. This suggests that some of the

classes must be very large indeed, although the absence of separation into primary and secondary schools may allow the British primary school crush to be avoided.

To recruit people to important jobs in a country where salaries are rigidly controlled and do not respond to market forces obviously calls for efficient forward planning, and vocational guidance which falls just short of compulsion. Children decide on their professions at the age of 15, but vocational guidance begins with "toy construction sets" at the age of 11. Direct guidance begins at the age of 13, when "factory day release" begins. The East Germans admit that one advantage of the polytechnics is that "children become acquainted with production very early". They are taught to understand the country's needs, and apparently "conflict is rare". Changing from one form of training to another is also rare, and outright failure, the Germans believe, "belongs to the past". Whether this system works better than the unpredictable surges of the free economy is doubtful, but certainly it relies very heavily on accurate central planning, and risks being overtaken by events. There are, for instance, very few computers, and even fewer systems analysts, in East Germany.

East Germany suffers from a brain drain, though the flow has been staunch since the building of the Berlin Wall. It is a migration in two directions, as the very best students are often rewarded by being allowed to go to the Soviet Union to further their education. This the East Germans do not mind, for they regard it as a compliment to their educational system, in much the same way as Mr. Quintin Hogg applauds the migration of British scientists to the United States. There is, in addition, the drain to the West, from which, surprisingly, the East Germans wring some wry humour. Their favourite story, doubtless apocryphal, concerns Dr. Adenauer, the former West German Chancellor. Asked by a visitor where the West German universities were, he is alleged to have replied, "Our universities? Oh, they are all in the Eastern zone".

## Restoring Atmospherically Degraded Images

by

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How is it possible to extract the most information from optical images degraded by the passage of light through the atmosphere? This was the subject of the Woods Hole Summer Study on the Restoration of Atmospherically Degraded Images held at the Summer Studies Centre of the National Academy of Sciences between June 27 and July 22, 1966. The director of the study has singled out some of the principal themes and conclusions.

It was pointed out in Newton's *Opticks* (Book I, Part I, Prop. VIII, Prob. II; 4th ed., 1730) that even if "the Theory of making Telescopes could at length be fully brought into Practice, yet there would be certain Bounds beyond which Telescopes could not perform. . . . Long Telescopes may cause Objects to appear brighter and larger than short ones can do, but they cannot be so formed as to take away that confusion of the Rays which arises from the Tremors of the Atmosphere." For example, the diffraction limit of a 48-in. telescope is 0.1 sec of arc, but "seeing" effects limit the actual resolution to several tenths of a second. A recent study sponsored by the United States National Academy of Sciences undertook to investigate how far such non-Newtonian devices as electronic computers and lasers may be able to circumvent

the effects of atmospheric turbulence on the formation of optical images.

The Summer Study did not consider techniques of image enhancement that depend only on some form of contrast amplification to make a photographic image more open to interpretation, for it was assumed from the start that one would be able to perform more sophisticated operations on a recorded pattern of light intensity with the object of undoing degradations and of restoring, as nearly as possible, the image that would have been recorded in the absence of degradations. Among the resources of modern technology which may contribute to this end are high speed digital computers with large memories, powerful laser sources of coherent radiation, and electro-optical image sensor techniques which are fast

enough to "freeze" image motion due to atmospheric turbulence.

The attention of the Summer Study was focused on a relatively restricted problem—that of the restoration of images of objects which subtend small angles and which are viewed against a dark uncluttered background and which are photographed from the ground looking up. This problem arises, of course, in photographic natural or man-made objects in space through a ground-based telescope. It is perhaps the simplest real problem in image restoration, because of the limited number of resolution elements in a typical image; but it has the merit of being both intrinsically important and also a stepping-stone to more difficult problems. At the Summer Study we did not consider such sources of image degradation as imperfect optical systems or motion due to tracking errors, even though it is well known that many of the restoration techniques that are useful for atmospheric turbulence can correct for these factors as well.

Three principal lines of investigation were pursued at Woods Hole. Briefly, these may be called passive image processing, wavefront-reconstruction imaging and pre-detection image processing.

In passive image processing, one attempts to answer the question, "Given a conventionally formed and atmospherically degraded image, what can be done to make its information content more intelligible?" One way of looking at the problem is as follows.

Because the effect of atmospheric turbulence is, in general, to reduce the amplitudes of the higher spatial frequency components of an image, the objective is to restore these components to their proper values. During very short exposures, the high spatial frequencies are not attenuated as much as during long exposures, but they are provided with instantaneous phase shifts which must be compensated for. The long-exposure transfer function of the atmosphere is fairly well known, but for short exposures it is an important problem to obtain either exact or statistical knowledge of the instantaneous transfer function, so as to correct for it. Another question which has not yet been adequately studied concerns the restoration of extended images, where the effects of turbulence are not invariant over the image—when the object does not lie in a single isoplanatic region.

The most impressive examples of passive image restoration which were shown at Woods Hole were due to J. L. Harris (Visibility Laboratory, Scripps Institution of Oceanography, University of California, San Diego). Harris has restored both long and short exposure images of simple objects such as the characters "S" and "5" which have been degraded by heat-induced turbulence in the laboratory, by using digital Fourier techniques on a Control Data Corporation 3600 digital computer (*J. Opt. Soc. America*, **56**, 569; 1966). A typical restoration may involve  $60 \times 60$  image points, although larger formats are possible. Work at the Visibility Laboratory is being continued actively. In particular, Harris is making an experimental study of the statistics of the optical transfer function of a turbulent medium.

Digital techniques are extremely flexible and convenient for image processing research, but the time required for the computations depends critically on the number of points to be processed. An alternative method of spatial frequency filtering, which can accommodate very rapid processing rates, is provided by an optical analogue system (Cutrona, L. J., *I.E.E.E. Spectrum*, **1**, 10, 101; 1964). The basic principle of an optical analogue system is that if a lens is placed a focal distance away from a transparency, the light at the focal distance away from the lens on its output side will be distributed according to the two-dimensional spectral analysis of the object. A spatial frequency filter can be provided by placing at the output focus a transparency whose transmittance varies in any desired manner, and the inverse transform of the filtered image may then be produced

by a second lens another focal distance away. Some preliminary examples of photographic images degraded by turbulence generated in the laboratory and restored by optical analogue filtering were shown by Dr. G. O. Reynolds (Technical Operations, Inc., Burlington, Massachusetts).

Against the operating speed and conceptual simplicity of an optical analogue processing system must be balanced a number of practical difficulties. Among these are diffraction noise due to dust and lens imperfections, the problem of achieving linearity in photographic response—in principle, digital processing can correct for nonlinearity, whereas optical processing cannot—and the problem of dynamic range.

It is apparent that in several ways digital and analogue image processing techniques are complementary to each other. One might therefore imagine an ultimate system which would combine the best features of both in an on-line operation.

An important limitation of passive image restoration techniques is that one does not usually know the optical transfer function of the randomly varying atmosphere at the instant the image is recorded. An active technique recently proposed by Dr. J. W. Goodman and his colleagues (Stanford Electronics Laboratories, Stanford University, California) uses a laser beam to illuminate the object and essentially to record the properties of the atmosphere at the same time. This technique, which is based on the principles of holography, is potentially immune to many of the atmospheric effects that degrade the quality of directly formed images.

The Goodman wavefront reconstruction technique records on a photographic transparency the interference pattern between laser light reflected from the desired object and a coherent reference source near the object. When the transparency is regarded as a hologram, illuminated by a collimated coherent beam and followed by a converging lens, the two first-order diffraction patterns on either side of the lens axis provide a pair of twin images of the original object. It can be shown that if the light from the object and the light from the coherent reference source undergo arbitrary but nearly identical phase shifts in a random transmission medium, the reconstructed images will to a first approximation be unaffected by the presence of the random medium. The closer the phase-perturbing region is to the receiving aperture, the less will be the effect on the reconstructed images. Nearby phase perturbations, on the other hand, are exactly those which degrade the quality of a conventionally formed image most severely.

Dr. Goodman and his colleagues have restored in the laboratory images degraded by a piece of shower glass in front of the receiving aperture (*App. Phys. Lett.*, **8**, 311; 1966). They have also shown that wavefront reconstruction imaging is possible when the coherent reference source is a specular point on the object itself. For a co-operative object, of course, the reference source is most easily provided by a corner reflector. Laser technology would at present appear to provide power levels and coherence lengths which are adequate for imaging low-altitude objects in space, and work is continuing at Stanford.

Although most of the attention at Woods Hole was directed toward the methods just discussed, two systems were considered which in some sense use electronic means to improve the quality of an image before it is recorded.

The first system, due to Dr. R. L. Gregory (Psychological Laboratory, University of Cambridge), involves a technique for selecting automatically those instants when the major features of an image are in their statistically correct positions, as determined from a previously taken long-exposure negative, and maximally sharp. It then builds up a single picture from a series of very short

(continued on page 969)



# The Problem of Quasars

by

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Jodrell Bank, Macclesfield, Cheshire

Quasars create more problems than they solve. How can such small star-like objects emit such large quantities of energy? Why do their spectra show such anomalously high red-shifts? How are they related to radiosources and visible stars?

THE discrete sources of cosmic radio emission were once known as "radio stars", by analogy with the discrete sources of light which are scattered similarly over the sky. Identifications of many radio sources with visible galaxies soon ended this terminology, and ended also any thought that the radio sources could be associated with star-like bodies. As more detailed maps became available of the wide extent of radio emission outside the visible parts of these radio galaxies, so it appeared more obvious that radio sources were large clouds of electrons with associated magnetic fields, very distant, and not at all like stars. Following this simple view, the cosmological interpretation of the number-intensity counts gave a clear rejection of the steady-state theory, and the first details of the development of radio galaxies through the early history of the universe.

Only one puzzling observation remained unaccounted for. The angular diameters of radio sources can be measured by the use of interferometers with sufficiently long baseline, and it is fairly easy to achieve this for most radio galaxies. Many of the radio sources in the 3C Cambridge catalogue were found in this way to have angular diameters of the order of 1 minute of arc. In 1960 an attempt at Jodrell Bank to resolve the smallest angular diameters by extending the measurement to a baseline of 32,000 wavelengths was surprisingly unsuccessful; some radio sources were apparently less than 3 arc sec across. It was known that three of these radio sources were coincident with three bright blue star-like objects, but it was not until accurate positions were available

from a combination of measurements at Cambridge and Owens Valley, California, that the coincidence was taken seriously. Sandage then measured the spectrum of one of them, 3C 48, and found unusual and unidentified broad emission lines.

Then came, in 1963, the identification of 3C 273 with another "star", even brighter, with a position determined to 1 arc sec by the observation of a lunar occultation at Parkes, Australia. This "star" had a wispy cloud extending 19 arc secs from it, and a part of the radio source was located in the wisp.

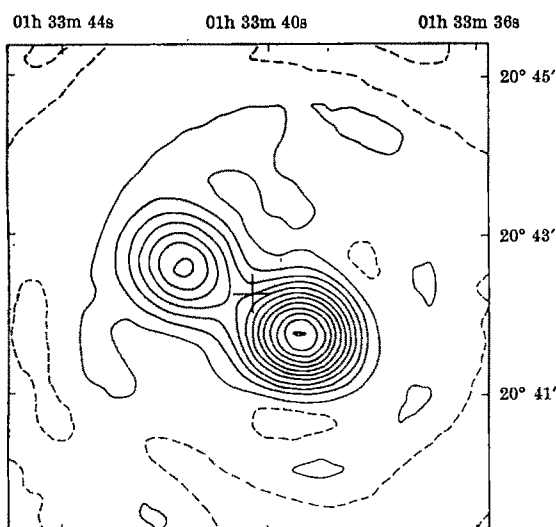


Fig. 1. Shape of the radiosource 3C 47 determined by the one-mile interferometer at the Mullard Radio Astronomy Observatory, Cambridge, at a wavelength of 21 cm. The optical quasi-stellar object is marked by a cross. The radio source consists of two components 62 sec of arc apart set nearly symmetrically either side of the optical object with flux densities in the ratio of 1.8 : 1. The optical object has a red-shift of  $\Delta\lambda/\lambda = 0.452$ .

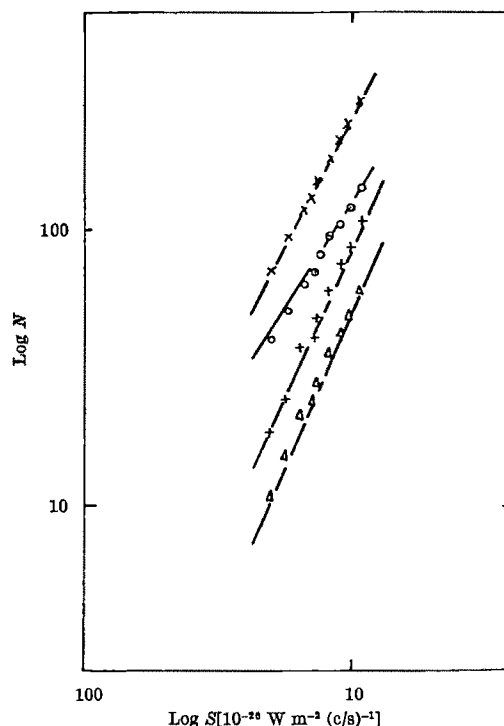


Fig. 2. The variation with apparent intensity of the numbers of celestial objects of a particular type can indicate their distribution throughout the Universe. The slope of  $\log N$  ( $N$ =number) against  $\log S$  ( $S$ =flux density) for radio sources away from the galactic equator, as measured at Cambridge, is  $-1.85$ , which is inconsistent with any model of equally luminous and uniformly distributed objects. In 1966 Véron (*Nature*, 211, 724) classified the extragalactic radio sources into radio galaxies and possible radio galaxies, quasars and possible quasars, and radio sources for which there is no visible star (empty fields) and radio sources the identification of which with a visible object is uncertain. The slopes of  $\log N$  against  $\log S$  for the different classes vary, as shown in the figure. The slope of the graph for the quasars is highest ( $-2.2$ ).  $\times$ , All extragalactic radio sources in the Revised 3C Catalogue;  $\circ$ , radio galaxies;  $\Delta$ , quasars;  $+$ , the quasars, the empty fields and the non-identified sources

The optical spectra of the three stars had shown unidentifiable emission lines and an exceptionally bright blue continuum. In 3C 273 the emission lines were identified by Schmidt as the Balmer lines from hydrogen,

shifted to the red by a factor 1.158 ( $z=0.158$ ). The spectra of the other three were re-examined and clearly fitted the same pattern, but with different values of  $z$ . About 100 of these quasi-stellar objects, the quasars, have now been identified, and it seems likely that a third of the 10,000 catalogued radio sources may turn out to be quasars. There are probably many more visible, but radio-quiet, quasi-stellar objects. Observational evidence is mounting rapidly but we still do not know for certain what they are, where they are, how they were made, or how long they last.

If the red-shift is indeed a Doppler shift conforming to Hubble's Law, the quasars are the most distant objects known. Several values of the red-shift parameter  $z$  greater than 2 are already known, while radio galaxies have values only up to 0.46. The emitted power is then calculable from the distance and the observed intensity over the radio spectrum; it amounts to  $2 \times 10^{47}$  ergs sec<sup>-1</sup> for 3C 273. A life-time of  $10^5$  years is plausibly assumed, and the total energy emitted is reckoned to exceed  $10^{50}$  ergs. This energy could be obtained by converting into helium a mass of hydrogen equal to  $10^9$  solar masses. There are only about  $10^{11}$  stars in a whole galaxy and even if we involve a whole galaxy it is difficult to find a sufficiently efficient physical process of energy conversion. So much energy can only have come from gravitational potential energy, or from a nuclear process, or a combination of both. But the explanation would be easier if the energy were less, which means either a shorter life or a smaller distance. Both are possible. There has been more discussion about distance, so we examine this first.

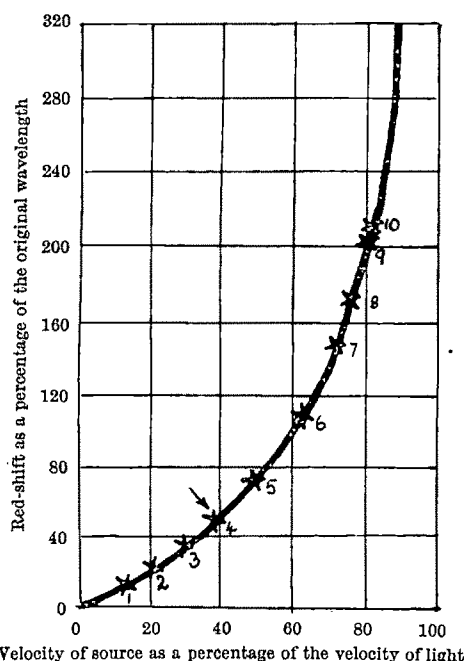


Fig. 3. A plot of the red-shift as a percentage of the original wavelength against the velocity of the source as a percentage of light (after J. L. Greenstein). The top of the curve is uncertain because as yet the large scale geometry of the Universe is unknown. The arrow marks the point of the most distant radiogalaxy and above this all points are for quasars. (1) 3C 273; (2) most distant (measured) ordinary galaxy; (3) 3C 48; (4) most distant radiogalaxy; (5) 3C 254; (6) 3C 287; (7) 3C 298; (8) 3C 454; (9) 3C 9; (10) 1116 + 12.

There are three disturbing aspects of the observed red-shifts which suggest that Hubble's Law may not be directly applicable to quasars. First, there is a strange distribution of red-shifts among the quasars. The high value of  $z=2.02$  appeared among the first ten to be measured; with 100 the largest value is still only 2.12, and there is a relative paucity of  $z$  values between 1 and

2. Second, the absorption lines now being found in the optical spectra do not have the same  $z$  values as do the emission lines. The third difficulty concerns the expected absorption of light on the short wavelength side of the red-shifted Lyman alpha line of hydrogen. This absorption is an extremely sensitive test of the presence of neutral hydrogen between the source and the observer. No absorption is seen and it is necessary either to assume that there is virtually no hydrogen or that the red-shift does not correspond to the expansion of the universe. Both represent distasteful complications of cosmology.

Could part of the red-shift be gravitational? The difficulty here is to form the sharp optical emission lines in regions where both the gravitational potential and its gradient are low enough, and where the potential is the same for all emission lines. This cannot occur for a single massive body, as was shown by Greenstein and Schmidt<sup>1</sup>. It could only occur if the light were emitted from inside a massive body; Hoyle and Burbidge<sup>2</sup> suggest that the optical lines come from a concentration of gas at the centre of a tight cluster of separate massive bodies. The absorption lines would then occur in cooler gas farther from the centre.

Gravitational red-shift is seen to be a very open question, but it does not follow that the quasars can be very close to us. They are distributed reasonably isotropically around us, and the only heavenly bodies of which the same can be said are the distant extragalactic nebulae. If they are local, and the red-shift is a Doppler effect, then they are receding from a point near us where the energy contained within all of them was once concentrated. No other such points are known in the universe because we do not see the very bright blue-shifted quasars which would emerge from them. At the very least, the distance of most quasars must exceed 10 megaparsecs, well beyond the local group of galaxies. The best interpretation at present is to ascribe most of the red-shift to Doppler effect, but allow a considerable contribution from gravity. The quasars are then very massive, containing  $10^9$ – $10^{11}$  solar masses, concentrated in large stars or protostars, and with dimensions of only a few parsecs. They contain the mass of a whole galaxy compressed to a size equal to the distance between the Sun and the nearest star.

The radio emission from some quasars changes appreciably in a few weeks, and the light emission may change within a day. Clearly some very large events are occurring in a very small space, as they do in a supernova explosion. There could, for example, be  $10^7$  or more large stars exploding or colliding, several times per year, keeping the quasar shining for  $10^5$  or  $10^6$  years. This is long enough to allow some material ejected from 3C 273 to have reached an elongation of  $19''$  arc.

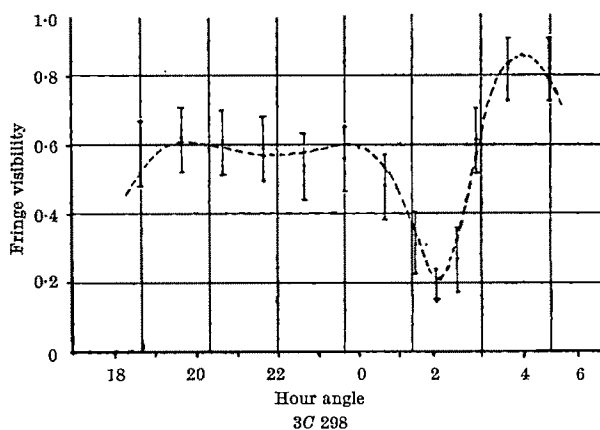


Fig. 4. The varying visibility of the radio interferometer fringes obtained from quasar 3C 298 as observed through 12 h of Earth rotation. The deep minimum indicates that the source is double. These fringes were obtained at a frequency of 408 Mc/s on a baseline of 180,000 wavelengths.

On this view the lifetime to be used in the calculation of energy could be shorter than  $10^5$  years but not so much shorter that the problem is significantly changed. We are in any event faced with the same problems for radio galaxies, the distances and lifetimes of which are much better known. Catastrophic releases of energy must occur; the extra problem of the quasar is the extreme concentration of the energy.

The arguments so far, which are due to many authors, notably Hoyle and Fowler<sup>3</sup>, and McCrea<sup>4</sup>, are converging on the idea of a distant, massive, condensed nebula. Some serious difficulties remain.

(1) The radio galaxy 3C 47 has an optical quasar at its centre. If quasars are related to radio galaxies, why are radio galaxies commonly found to be bright elliptical galaxies in clusters, while quasars are not associated with clusters?

(2) Why are quasars often double radio sources? There is even one pair of unidentified quasar-like sources, 3C 343 and 3C 343.1, which are 28 min of arc apart, which is 17,000 times their angular size. These two are identical in many of their detailed radio characteristics.

(3) How can any mechanism concentrate so much of the total available energy into electrons and magnetic field?

These problems are unanswered. The surprising thing is that such strange and inaccessible objects as quasars can have been the subject of such rapid advances recently, both in observations and in theory. The excitement is surely not yet over.

Further reading will shortly be available in books by G. R. and E. M. Burbidge (*Quasi-Stellar Objects*, Freeman, London) and by F. D. Kahn and H. P. Palmer (*Quasars*, Manchester University Press). The Physical Society and the Institute of Physics, together with the Royal Astronomical Society, are arranging for a conference on "The Physics of Quasars" to be held in Manchester during September 5-7, 1967.

<sup>1</sup> Greenstein, J. L., and Schmidt, M., *Astrophys. J.*, **140**, 1 (1964).

<sup>2</sup> Hoyle, F., and Burbidge, G. R., *Nature*, **213**, 373 (1967).

<sup>3</sup> Hoyle, F., and Fowler, W. A., *Nature*, **187**, 533 (1963).

<sup>4</sup> McCrea, W. H., *Nature*, **213**, 239 (1967).

(continued from page 966)

exposures taken at such instants of best seeing. The original purpose was to get improved lunar and planetary pictures, where the object to be photographed is available for periods of the order of an hour. Laboratory tests have been made (*Nature*, **203**, 274; 1964), and experimental lunar photography is under way. Some thought has been given to the problems of photographing fast-moving artificial satellites.

The other scheme is an electronic image stabilization system developed by Itek Corporation (Lexington, Massachusetts). Optical feedback and magnetic deflexion coils are used to keep the image of a small object, or the centroid of a larger image, fixed on the retina of an electronic image tube in spite of actual motion of the object, the propagating medium or the image tube mount. A prototype system has been tested extensively, and shows good frequency response up to 2,000 c/s. It appears reasonable that the system would work satisfactorily on a large telescope.

The general conclusion of the study was that image processing has advanced to the stage at which it is ready to make the step from experimental procedure to useful operational tool; in particular, it was recommended that Harris's digital processing techniques should be applied to real telescopic images as soon as possible, and that work should be pushed in the other areas to increase understanding and to develop more effective techniques and equipment.

## BOOK REVIEWS

### NEWTON'S PROGRESS

#### The Background to Newton's *Principia*

A Study of Newton's Dynamical Researches in the Years 1664-84. By John Herivel. Pp. xv+337+5 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 70s. net.

THIS work is a detailed study of Newton's dynamical researches during what was the most fruitful period of his life. In 1664 Newton came under the tutelage of Isaac Barrow; the following year he was compelled by the Great Plague to seek shelter in his native Woolsthorpe, and it was during his sojourn there that were laid the foundations of his future greatness. "All this," he wrote later, "was in the two plague years 1665 and 1666, for in those days I was in the prime of my age for inventions, and minded mathematics and philosophy more than at any time since."

It is to this period that can be traced the beginnings of those researches which were to prove of immeasurable value to science. Was the force which drew the apple to the ground the same as that which retained the Moon in her orbit? "In the same year" (1666), he wrote, "I began to think of gravity extending to the Orb of the Moon . . . having thereby compared the force requisite to keep the Moon in her Orb with the force of gravity at the surface of the Earth, and found them to agree pretty nearly." Thus began those investigations which led to the enunciation of the principle of Universal Gravitation.

On the cover of this book it is stated that Mr. Herivel's work is "devoted exclusively to Newton's dynamical thought leading up to the composition of the *Principia*". On this account, its appearance is all the more welcome. Only a fraction of Newton's vast output was made public during his lifetime; much of it has been hidden away for close on three centuries. A detailed study of the steps which led to the production of this monumental work is therefore long overdue, and we are grateful to Mr. Herivel for the painstaking way in which he has analysed Newton's original documents and presented them in a way which compels the reader's attention.

The book is divided into two parts. Part I is devoted to an extended commentary on the manuscripts which form the substance of Part II. In the early pages of Part I the main line of development of Newton's dynamical thought is traced. Apart from the contributions of Galileo, mechanics had made little headway during the early decades of the seventeenth century. Newton inherited much from Galileo, and Mr. Herivel rightly assesses the value of this inheritance. But he is far too kind to Descartes, whose notions on fundamental ideas were vague in the extreme. Motion, according to Descartes, was nothing more than the transport of a body from the vicinity of those in contact with it to the vicinity of others (*Prin. Phil.*, II, 25). It is thus contrary, not to a motion in the opposite direction, but to rest. To say that a body in motion possesses a certain "force" to resist any change in its state is not at all helpful if we are not told how this force is measured. Mr. Herivel tells us (on page 53) that "it seems probable that Newton took the idea of using momentum (and its conservation) in collision processes directly from Descartes". But did not Descartes base his conclusions on divine immutability? God always preserves in the Universe the same amount of motion as He created in it. Mr. Herivel is clearly aware of the weakness of Descartes's exposition when he tells us (on page 52) that "the laws of collision given by Descartes in Part II of the *Principia* were notoriously full of error".

He might have added that the simplest of experiments would suffice to disprove them, as Clerselier clearly pointed out. It is therefore no injustice to Descartes to declare, as did Whewell in the *History of the Inductive Sciences*, that "if we were to compare Descartes with Galileo, we might say that of the mechanical truths which were easily obtainable at the beginning of the seventeenth century, Galileo took hold of as many, and Descartes as few as was well possible for a man of genius".

Chapter 6, on order of composition and dating of manuscripts, is particularly valuable inasmuch as we learn from it the extent to which Newton was treading on virgin soil, and, here again, Mr. Herivel has placed us heavily in his debt. Newton, like so many of his contemporaries, was often careless as to dates, and in giving a precise chronology of the steps leading to the publication of the *Principia*, Mr. Herivel has rendered a very useful service.

One of the most valuable features of this work is the comprehensive index which has been supplied, and this the reader will find most helpful. If, and when, a second edition of this work is planned, Mr. Herivel might consider the provision, in an appendix, of brief biographical notes on some of the lesser known persons mentioned in its pages. Not every reader is familiar with the contributions of such men as Borelli, Bradwardine, Gassendi or Paget, to name but a few.

*The Background to Newton's Principia* is a work which should have a place on the bookshelves of all who are interested in the history of science during the illustrious seventeenth century.

J. F. SCOTT

## LECTURES ON MOLECULAR SPECTROSCOPY

### Molecular Spectroscopy—VIII

(Invited Lectures presented at the VIIIth European Congress on Molecular Spectroscopy, held in Copenhagen, Denmark, August 14–20, 1965.) (*Pure and Applied Chemistry*, Vol. 11, Nos. 3–4, 1965.) Pp. vi + 261–582. (London: Butterworth and Co. (Publishers), Ltd., 1966.) 75s.

THE organizers of the eighth European Congress on Molecular Spectroscopy at Copenhagen have been wise in restricting the publication to the invited papers presented at this meeting. They represent useful summaries or reviews of various topics, and any objections to the publication of conference proceedings in general do not apply to the present volume. The meeting in Copenhagen was a very large meeting with about 800 participants and many parallel sessions were necessary. Consequently, it was not possible for an individual participant to attend all the invited lectures, and it is very useful to have all (or almost all) the invited lectures collected together in one volume. The meeting in Copenhagen may have been the last meeting to cover the whole of molecular spectroscopy. Correspondingly, the present volume deals with a large variety of topics, several of them extremely well presented.

Many of those who attended the meeting will remember the beautiful opening lecture of Professor Daudel on the role of wave mechanics in the interpretation of molecular spectra. It is a fine summary which clearly brings out the constant dialogue between theory and experiment in the development of both the detailed theory of small molecules and the semi-empirical theory of large molecules. There are several other invited theoretical lectures, in which I. M. Mills considers the effect of Coriolis interactions on rotation-vibration spectra and is able to present examples which show the possibility of obtaining information about the potential functions from the effects of Coriolis interactions; H. Preuss deals with the potential functions of polyatomic systems; G. J. Hoijtink deals

with triplet-triplet spectra of alternant hydrocarbon molecules; J. T. Hougen gives a thorough discussion of the vibrations in molecules with nearly free internal rotations; and I. Fischer-Hjalmars presents a theoretical study of systems with heteroatoms.

In addition to these theoretical papers, there are a number of excellent papers on experimental subjects. Many will remember the very fine lecture by Mössbauer, who showed how the effect bearing his name can be applied to problems of electronic shielding in the rare earths. This paper deals, in other words, with the spectroscopy of very small wavelengths, that is, gamma radiation. There is very little discussion of the vacuum ultra-violet region or of spectra of diatomic molecules, at least in the invited papers, but the visible and near ultra-violet spectra of polyatomic molecules are well represented. Th. Förster deals with the polarization of the photoluminescence in solutions. M. Kasha deals with the exciton model and its use in the interpretation of the spectra of aromatic molecules, and H. Kuhn reports on very interesting experiments in which simple organized systems of molecules are built up and their absorption and fluorescence are investigated as a preparation for an understanding of biological structures.

A very stimulating paper is presented by B. P. Stoicheff on molecular spectroscopy with optical masers. Apart from giving a very useful summary of the present status of this field, he reports his recent discovery of the inverse Raman effect.

In line with the historical development of the European molecular spectroscopy meetings, a good deal of emphasis remains with vibrational spectroscopy. (The ninth meeting in Madrid next September is to be devoted entirely to this subject.) D. Hadzi reports on the infra-red spectra of strongly hydrogen-bonded systems, while L. A. Woodward discusses the Raman spectra of aqueous solutions, and D. O. Hummel describes the infra-red spectra of macromolecules. One of the most interesting developments reported at this meeting is the first success in obtaining infra-red spectra of free radicals produced by flash photolysis. This work is described by G. C. Pimentel in his invited lecture and at the meeting it was also discussed by R. M. Hexter in one of the contributed papers (not included in this volume).

Microwave spectroscopy is represented by the lectures of two of the pioneers in this field. W. Gordy reports on the extension of microwave spectroscopy into the sub-millimetre region, while J. Sheridan discusses some recent results and future trends in microwave spectroscopy.

Only one of the invited papers, that of R. A. Hoffman, deals with nuclear magnetic resonance spectra; in particular, with the analysis of high resolution spectra of this type. Finally, there are two important papers on spectroscopic technique. H. A. Gebbie describes the important developments in the field of interference spectroscopy to which he himself has made such fine contributions, and P. L. Richards describes the latest developments in infra-red detectors.

All in all, this volume represents a very useful summary by competent authors of some of the most important topics in spectroscopy. The book is well printed, and, in particular, the half-tone reproductions are well done. The lectures are published in the original language of the particular authors although the actual presentation of most of the lectures was in English. G. HERZBERG

## BROMINE HANDBOOK

### Bromine and Its Compounds

Edited by Z. E. Jolles. Pp. xxvii + 940. (London: Ernest Benn, Ltd., 1966.) 210s. net.

THIS book deals with almost every conceivable aspect of the chemistry of bromine and its compounds. There

are chapters by the editor, Dr. Jolles, and by twenty-three other authors from a large number of industries and universities. The book is divided into seven sections: the manufacture and physical and chemical properties of elemental bromine; inorganic bromine compounds; organic bromine compounds—this deals mainly with methods of bromination and related reactions, for example, addition, substitution and elimination; radioactive bromine and bromine in radiation chemistry; biological aspects of bromine and its compounds; industrial applications; and analytical chemistry of bromine and its compounds (including applications of most types of spectroscopy).

Such a multitude of topics are covered that an exhaustive appraisal would require a reviewer of exceptionally catholic tastes, but it is clear that the editor and his co-authors have produced an important reference work, and that they have largely succeeded in one of their objectives which was to summarize existing knowledge of bromine and its compounds. They also hope to stimulate the use of bromine and its compounds as an alternative to chlorine and its compounds, particularly in industrial processes, and the book will be invaluable to anyone considering this.

The cost and contents are such that the book will be bought by few individuals although all libraries should consider it an essential acquisition. This raises two points. At present the book stands alone: its value would be enhanced if it were accompanied by companion volumes on the other halogens. The direct orientation towards bromine chemistry also conflicts to some extent with the comparative approach which is so useful in dealing with the properties of the halogens.

Finally, a good point—the book is relatively free from errors—and a criticism—the references given to the thermodynamic properties of bromine are obsolete.

E. WHITTLE

## TASMANIAN JOURNAL

### Friendly Mission

The Tasmanian Journals and Papers of George Augustus Robinson, 1829–1834. Edited by N. J. B. Plomley. Pp. xiii + 1074 + 17 plates. (Hobart: Tasmanian Historical Research Association, 1966.) A.126s.

ALTHOUGH the aboriginal tribes of Tasmania were in contact with Europeans for some decades before they finally became extinct (approximately from 1770 to 1870), there is nevertheless very little worthwhile information about them. Before the publication of the book under review, much of the information was provided by the records of French explorers during the late eighteenth and early nineteenth centuries. The Robinson papers, missing for many years, therefore provide a large additional source of information on these people.

George Augustus Robinson began his work among the aboriginals in very troubled times, and was successively conciliator of the Tasmanian tribes, commandant at the Flinders Island aboriginal settlement, and chief protector of the aborigines at Port Phillip. During this time he kept various records of his work with these people, most important being his field journals. The book is mainly concerned with the period 1829–1834, giving a day by day account of his experiences with the tribes—and in the case of some of them, his information is all that we have.

The mass of writing left by Robinson was by no means orderly, and the editor has clearly been to great pains to sort and cross-check names, localities and other information. Additional hazards for him were poor writing and early nineteenth century punctuation (or lack of it). Plomley is to be congratulated on his careful and scholarly editing, and in lacing the journals together so well with the many additional notes.

The book is of value not only as a source of information about the physical and social anthropology of the indigenous Tasmanian, but also as a remarkable chapter in colonial history. To quote Plomley's apt comment about Robinson: "Usually humanity is a principle rather than a course of action. To find someone living up to his belief that all men are born equal but suffer from inequality of opportunity was and is extraordinary". Alas, all the good intentions of Robinson and certain other administrators only hastened final extinction.

Increased mortality resulting from early conflict with European settlers, and a decrease in births following the enslavement of numerous aboriginal women by the sealers, was followed by increasing mortality through European-introduced diseases.

In addition to the journals, there are valuable extra sections and appendixes compiled by the editor. These review the prehistoric background, biological affinities and variation in the Tasmanians; also the distribution and names of the numerous small tribes. The appendixes also provide a geographical check-list of aboriginal and alternative European place names, a review of the natural history of the country, and a useful summary.

D. R. BROTHWELL

## MENTAL SET

### The Psychology of Set

By D. N. Uznadze. (The International Behavioral Science Series.) Pp. xvii + 251. (New York: Consultants Bureau, 1966.) \$15.

THIS book appears in the International Behavioral Science Series, for which Dr. Joseph Wortis is the general editor. Other translations from the Russian in the same series are A. R. Luria's *Higher Cortical Functions in Man* (1966) and B. V. Andreev's *Sleep Therapy in the Neuroses* (1960).

Dmitrii Nikolaevich Uznadze (1886–1950) was for many years director of the Institute of Psychology of the Georgian Academy of Sciences in Tbilisi, where visitors today are hospitably received by Professor Prangishvili, one of his close collaborators and a former colleague. For many western readers, the first acquaintance with Uznadze's work probably came in the 1920s through the pages of the German journal, *Psychologische Forschung*, which published articles deriving from the Gestalt school. Since then odd papers have appeared in proceedings of conferences and in a few western journals, but the present work is the first systematic presentation of the approach of Uznadze and the Georgian school to be available generally in the West. It comprises two monographs, *Experimental Basis of the Psychology of Set*, originally written in Russian, but first published in the Georgian language in 1949, and *Basic Principles of the Theory of Set*, also written in Russian, which has not hitherto been published.

The influence of a "mental set" has long been known. A common example is the way in which one "sees" or "hears" the approach of the expected visitor only to find that other stimuli have been biased or distorted by the pre-existing "set" of thought. The book deals with methods of establishing sets experimentally in visual, auditory and kinesthetic modalities. A set established for one hand can "irradiate" to the other, spontaneously, and so too, particularly with young children, sets may transfer from one sensory modality to another. Individuals differ appreciably in the readiness with which sets can be formed, retained and generalized to other situations. Qualitative stages in the extinction of a set, such as the "plastic", "coarse" or "inert" forms, are distinguished. Sets may be established in animals such as rats, chickens and chimpanzees, a field of work where a keen debate could develop between those who would prefer to use the language of the "set" theorists and others who would



favour the terminology of learning theory and reinforcement. Talented actors can acquire a set described to them and maintain it much more precisely than others and some interesting possibilities of distinguishing the course of sets peculiar to schizophrenics and epileptics are outlined.

The methods used and the findings established in the many experiments described are usually not presented in great detail, but frequently in the form of conclusions. This has the merit of ensuring a wider coverage of topics within a shorter space; but would tend to limit precise repetition of the experiments. Nevertheless, psychologists who are interested in the experimental study of the thought processes, a very basic field of study, are presented with a valuable sketch of a considerable body of work already done and many possibilities for future experiments.

F. V. SMITH

## BASIS OF INTELLIGENCE

**Genetic and Environmental Factors in Human Ability**  
Edited by J. E. Meade and A. S. Parkes. (A Symposium held by the Eugenics Society in September–October 1965.) Pp. xi+242. (Edinburgh and London: Oliver and Boyd, Ltd., 1966.) 57s. 6d.

THE editors and the Eugenics Society are to be congratulated on an outstanding symposium in which a distinguished group of authors present a series of lucid and concise papers on the nature–nurture problem in human intellectual performance. The book deserves to be read by every student of psychology, genetics and the social sciences and by all who are concerned with education, social medicine and social welfare either as teachers, practitioners, administrators or policy makers.

After a brief introduction by Sir Robert Platt, the book is divided into four sections. In the first section, on intelligence tests, P. E. Vernon outlines the problem, B. B. Bernstein and D. Young report new work on the effects of relationships between mother and child, Hilde Himmelweit summarizes her work on social influences in school and Douglas Pidgeon discusses the effects of intelligence tests on British education.

In the second section Jean Floud discusses the problem of selection for university places; Stephen Wiseman summarizes evidence on innate and environmental factors in educational attainment; James Drever tells a cautionary tale to ask how far abilities are influenced by parental ambitions; and Liam Hudson describes some of his research relating test results to achievement at university and after. The section is briefly summed up by Lord Robbins.

The third section deals with causes of sub-normality and includes a lively contribution by James Walker on the effects of perinatal accidents and their relation to social factors. It also contains a discussion by Albert Kuschlick of the interaction between physical, social and mental factors, an account by C. E. Dent of genetically caused biochemical abnormalities affecting mental function, and a summary by Valerie Cowie of associations found between chromosomal abnormalities and mental defect. The section ends with a brief statement by Brian Kerman.

The papers in the last section are on the relationships between intelligence and fertility. B. Benjamin summarizes census data from several countries; C. O. Carter indicates that the fertility of the more intellectually able sections of the population is tending to rise; R. M. C. Huntley outlines elegant research comparing genetic influences on intelligence, on certain physical measures and on social adjustment; and D. S. Falconer considers the consequences of selection pressure on genetic factors in intelligence. J. A. Fraser Roberts makes a short concluding statement.

The contributions from psychology, genetics, education and medicine complement each other in a way which

realizes the aim of a true symposium: where one discipline is equivocal another is definite, where one is emphatic another may qualify. The only really disappointing papers are those by Douglas Pidgeon and Jean Floud, whose essentially political statements on education appear thin beside the scientific contributions. The time seems to be coming when education should be taken out of party politics, for a more rational approach is now evident.

A. T. WELFORD

## NERVOUS MECHANISMS

### Nerve as a Tissue

Edited by Kaare Rodahl and Bela Issekutz, jun. (Proceedings of a Conference held at the Lankenau Hospital, Philadelphia.) Pp. x+470. (New York and London: Hoeber Medical Division, Harper and Row, Inc., 1966.) \$18.50; 148s.

THE Lankenau Hospital research conference in November 1964 provided a unique opportunity for the discussion of a wide range of topics related to nerve and synaptic mechanisms. An impressive list of contributors assured the success of the conference and has resulted in a volume of undoubted value. The book is divided into four major sections: morphology, biochemistry, physiology and pharmacology, and the pathophysiology of nervous tissue. Within these sections the chapters present a coherent, sometimes controversial, and always stimulating, account of nervous mechanisms. Seventeen topics are covered in the book but, unfortunately, comment must now be limited to only a brief mention of a few of these.

The first chapter describes and illustrates the organization of the cell membrane, and E. de Robertis describes the biochemistry of subcellular fractions and provides evidence for the compartmentalization of 5-hydroxytryptamine and catecholamines.

The biochemistry section begins with a chapter by D. Nachmansohn in which he delivers his customary attack on the generally accepted views of neuromuscular transmission and nerve conduction. The arguments he uses lean, more heavily than usual, on the existence of rather inscrutable permeability barriers and on the cursory dismissal of well established experimental results. In a subsequent chapter these arguments are concisely and effectively dealt with by G. B. Koelle. It is hard that Nachmansohn should tell us that there is "no alternative" to his views, especially when misleading interpretations of experimental facts are made which have to be pointed out in the illuminating discussion which follows.

A balanced analysis by W. Nastuk and A. Gissen of the effect of depolarizing drugs at the muscle endplate leads to a reasoned explanation of the puzzling nature of "dual block" in terms of chemodesensitization.

Evidence for the identity of centrally transmitting chemicals is reviewed by D. Curtis, and he reasonably concludes that, although there is suggestive evidence indicating that several compounds may transmit, only acetylcholine at Renshaw cells can be considered, with any certainty, to be a transmitter. Progress in this topic has been rapid since the conference and further evidence has now accumulated to implicate acetylcholine and  $\gamma$ -aminobutyric acid as transmitters in the cerebral cortex.

The section on pathophysiology contains an intriguing chapter by A. Ward on the hyperexcitable neurones which are found in focal epilepsy. He describes the characteristics of these cells and the way in which they lose their dendritic spines. The abnormal quantal release of acetylcholine in myasthenia gravis is considered by D. Elmquist and, supported by experimental evidence, he suggests some possible defects in the transmitter mechanism that may occur in this disease. The volume ends, appropriately, with an appendix by Sir John Eccles on the strategy of neurophysiological research.

This book is exceptionally well produced and should appeal to all who have an interest in nervous mechanisms. The features one hopes for in a conference report of this kind are all present and include abstracts of chapters, author and subject indexes, full bibliographies and coverage of the discussions which followed each section of the conference.

J. F. MITCHELL

## MONOGRAPH ON NORADRENALINE

### The Uptake and Storage of Noradrenaline in Sympathetic Nerves

By Leslie L. Iversen. Pp. xiv + 253 + 8 plates. (London: Cambridge University Press, 1967.) 57s. 6d. net; \$11.

DURING the past decade there has been a remarkable increase in our knowledge concerning the neurotransmitter, noradrenaline. This development has been stimulated by the availability of specific and sensitive methods for the isolation and measurement of noradrenaline in tissues. These include the introduction of radioactive catecholamines of high specific activity; new histofluorescent techniques that make sympathetic nerves visible; and the sophisticated use of drugs that affect the adrenergic system. Thus, contributions have come from a variety of disciplines, including biochemistry, pharmacology, physiology and anatomy. In this monograph Dr. Iversen gives a lucid, informative account of our current knowledge of the formation, uptake, storage, release and metabolism of noradrenaline in peripheral sympathetic nerves, as well as in the central nervous system.

The first portion of the book describes the methodology for the chemical estimation of catecholamines, the pathways and enzymes involved in their biosynthesis and metabolism, the release of noradrenaline from nerves and its subsequent fate. A considerable portion of the remainder of the book deals with a newly discovered process which causes the rapid inactivation of the adrenergic neurotransmitter by uptake across the neuronal membranes and storage in vesicles within the sympathetic neurone. The uptake and release of noradrenaline in nerves and the action of adrenergic drugs are described in considerable detail. The author is very knowledgeable in this subject and he has made many of the significant contributions. Recent work on the exciting topic of the localization, storage and release of noradrenaline and other biogenic amines in the central nervous system and the effect of psychoactive drugs is described in the last chapter.

In this book considerable controversy is generated by conflicting theories, as is the case in most rapidly emerging fields of research. Dr. Iversen handles controversial questions critically but fairly, and places them in proper perspective. This monograph is concerned with an important problem in the biomedical sciences, and it should appeal to the general reader as well as the specialist.

JULIUS AXELROD

## PARASITIC PROTOZOA

### Malaria Parasites and Other Haemosporidia

By P. C. C. Garnham. Pp. xviii + 1114. (Oxford: Blackwell Scientific Publications, 1966.) 175s. net.

STUDENTS of the malaria parasites and other haemosporidia have depended on Wenyon's *Protozoology* as an indispensable guide for four decades. In the meantime, knowledge of these parasites has increased tremendously and a great need has developed for a modern book of comparable depth and quality. This book admirably fulfils the need with respect to the haemosporidia. It deals primarily with protozoology in both vertebrate and invertebrate hosts; clinical aspects, epidemiology, im-

munity and pathology of malaria are discussed when they have a direct bearing on the parasites.

Part I is a general review of the discovery of malaria parasites, their life cycle and morphology, classification and evaluation, and biochemistry. Part II, on the Plasmodiidae, starts with taxonomy, in which the mammalian parasites are divided into the sub-genera *Plasmodium*, *Laverania* and *Vinckeia*. It continues with detailed descriptions and illustrations of each mammalian species. The avian sub-genera *Haemamoeba*, *Giovannolaia*, *Novyella* and *Huffia* are reviewed next, and the species of avian parasites are described. This part of the book is completed by a review of the reptilian sub-genera *Sauramoeba*, *Carinamoeba* and *Ophidiella* along with descriptions of the species of parasites known in lizards and snakes.

Part III, on the Haemoproteidae, includes *Hepaticystis*, *Haemoproteus*, etc., and Part IV discusses and describes species in the family Leucocytozoidae. Malaria parasites of doubtful status are reviewed in Part V. The final part of the book is a quite detailed description consisting of sixty-five pages of technical procedures for use in the identification of malaria parasites and studies of their life cycles.

The morphology and staining reactions of the parasites are presented in numerous coloured plates of excellent quality. Extensive reference to the literature is made throughout, which results in a useful but understandably incomplete bibliography on each subject. Taxonomy is traditionally controversial and some authorities may not concur fully with the author's decisions.

The volume is recommended as a reference book for malariologists, protozoologists and others concerned with the laboratory diagnosis of malaria, as well as a text-book for students of parasitology.

PAUL E. THOMPSON

## PLANT MALADIES

### Annual Review of Phytopathology

Vol. 4. Edited by James G. Horsfall in Association with Kenneth F. Baker. Pp. vii + 423. (Palo Alto, California: Annual Reviews, Inc., 1966.) \$8.50.

THIS volume, which follows the format of its predecessors, contains sixteen articles which between them cover a wide field of phytopathology. Dixon Lloyd Bailey, in an introductory chapter assessing the present position of plant pathology, detects an increasing trend among pathologists to escape from the rigid boundaries of established disciplines, and he welcomes the development of an awareness and willingness to apply relevant discoveries in pure science. Few would disagree with him. E. C. Large reviews disease measurement, particularly in Britain. Grogan and Campbell contribute an article on fungi as vectors and hosts of viruses. In addition to the now established relationship of lettuce big vein and tobacco stunt viruses with *Oidium brassicae*, they suggest that tobacco necrosis may also prove to have a connexion with this fungus. The evidence for the possible association of cereal viruses with *Polymyxa graminis* is reviewed. The present volume also contains articles on chemical soil treatments by S. Wilhelm; virus transmission in woody plants by R. W. Fulton; air pollution in plant pathology by J. T. Middleton; pectic enzymes in tissue degradation by D. F. Bateman and R. L. Millan, and sections on host-pathogen interactions. These include epidemiology, by J. E. Crosse and K. F. Baker, and S. H. Smith, the action of toxicants—a paper on synergism among fungicides by Anna Scadavi—and chemical control—amino-acids and plant diseases by O. M. van Andel. The action of oil in disease control is discussed by L. Calpouzos, who presents a critical evaluation of experimental results, both published and unpublished, on the value of oil sprays in the control of plant

diseases, in particular banana leaf spot, *Mycosphaerella musicola* Leach. Effective oils apparently achieve their results in a number of ways and their effects appear to be on the disease itself rather than directly on the pathogen. For this reason the author prefers "antidisease agents" to "fungicides" for use in describing them.

There are author and subject indexes for the present volume and cumulative indexes of authors and titles for Volumes 1 to 4. The present volume has maintained the standard set by its predecessors and the series remains essential reading for plant pathologists. J. H. WESTERN

## CHEMISTRY AND PHYSICS OF LIPIDS

### Chemistry and Physics of Lipids

Edited by G. H. de Haas and five others. (Volume 1, No. 1; 1966/67.) Pp. 89. (Amsterdam: North-Holland Publishing Company, 1966.) n.p.

THE new journal, *Chemistry and Physics of Lipids*, first appeared in November 1966. The declared editorial policy of this new journal is to publish papers in the field of molecular biology, with particular emphasis on the chemical and physical aspects of lipids. At present there is already a journal exclusively devoted to lipid research and it is by no means bursting at the seams. Moreover, *Biochimica Biophysica Acta* offers a whole section for papers on the biochemistry and biophysics of lipids. One wonders what prompted the editorial board and publishers of *Chemistry and Physics of Lipids* to launch a new journal in this field. They may have felt that a journal should be exclusively devoted to the biophysics of lipids, but surely this is carrying super-specialization too far.

It is not easy to applaud the purposes of a journal the editorial board of which sees the chemistry and physics of lipids as a cloistered enclave insulated from the hurly-burly of general biological research. If this process of super-specialization proceeds much further, the time will come when a scientist will only be able to communicate with himself, because nobody else will understand his jargon.

It would be churlish not to wish success to this new journal. Nevertheless, if it should fail to thrive for lack of real support, it is to be hoped that the publishers will consider whether or not to continue its publication. There are already too many specialized journals that are barely viable: to add to their number serves no useful purpose.

C. W. M. ADAMS

## THE DRUG SCENE

### Extra Pharmacopoeia (Martindale)

Incorporating Squire's Companion. Edited by R. G. Todd. Twenty-fifth edition. Pp. xxviii + 1804. (London: The Pharmaceutical Press, 1967.) 150s.

THE twenty-fifth edition of Martindale is obviously destined to become a *vade mecum* for the increasingly harassed druggist and the equally tormented prescriber. The drug scene, as the popular press keeps reminding us, is getting out of hand. No one is more aware of this than the editor of a comprehensive pharmacopoeia—the editor of this one, Mr. R. G. Todd, writes in his introduction of the "formidability" of the task that faced him after the publication of the twenty-fourth edition in 1958. Since that time more than 1,200 drugs have been admitted to the book, and the number of new proprietary products developed over the same period is 2,400. The task of compiling a directory to all these compounds would daunt the bravest, but Mr. Todd has put together a comprehensive and even readable guide.

The first part of the book consists of monographs on the chief drugs of clinical and toxicological importance, the

drugs being grouped together according to their properties where possible. The articles on individual drugs are accompanied by quotations from articles in the medical and pharmaceutical press. The second part is a list of supplementary drugs of lesser importance. There is also a list of proprietary substances usually supplied "over the counter" on request, a list of manufacturers and a classification of drugs by the disease for which they are normally used. The index is unusually comprehensive and the book should be on the bookshelf of everyone who needs a guide to modern drugs.

JOHN SPENCER

## OBITUARIES

### Professor H. Munro Fox

HAROLD MUNRO FOX, who died on January 29 at the age of 77, was a zoologist of great distinction. His scientific output began with a series of ten papers, mostly on fertilization, hybridization and inheritance in sea urchins, published between 1911 and 1915. After an interlude as an officer in the Army Service Corps, London Mounted Brigade, he resumed his researches in the early twenties with a group of papers on various subjects: lunar periodicity; migration of invertebrates through the Suez Canal; animal orientation; reactions of flagellates to low oxygen tension and their use in detecting the respiratory surfaces of small invertebrates, and the chemical nature of chlorocruorin. Soon he settled down to a "main line" of work on invertebrate respiration. He was professor of zoology in the University of Birmingham during 1927–1941 and at Bedford College during 1941–1954, and finally professor emeritus and honorary research associate at Queen Mary College. For the whole of this time a steady stream of important papers flowed from Fox and his pupils, on such subjects as the nature and functions of the respiratory pigments haemoglobin and chlorocruorin; the relation of metabolic rate to environmental temperature and oxygen tension; the regulation of respiratory movements; and invertebrate blood circulations. At first, these papers were mostly concerned with worms, but later he produced a series of important works on the respiration physiology and haemoglobin of crustacea, especially *Daphnia*. This led him on to investigate the taxonomy and general biology of freshwater crustacea, with which his latest papers are concerned.

Fox was elected to the Royal Society in 1937, and was Fullerian professor of physiology at the Royal Institution from 1953 to 1956. He was awarded a gold medal by the Linnean Society of London in 1959, and the Darwin Medal of the Royal Society in 1966.

The whole of Fox's work is characterized by great clarity and precision, shown both in his experimental methods and in the style of his writing. His interest in the life of the animals on which he worked was unflagging. He was an indefatigable field naturalist, and honorary president of the London Natural History Society. His friends will remember the spotlessly clear marine aquaria in his London flat, where the most delicate animals thrived and bore witness to his devotion. He was also a great European, spending his working holidays at laboratories abroad, especially in France and Italy. He served as president of the International Union of Biological Sciences from 1950 to 1953.

His enthusiasm and precision made Fox a first-class teacher, and also—together with his international outlook—fitted him for his forty years in the editorial chair of *Biological Reviews*, a position which he occupied from the origin of that journal in 1926 until his death. He was indeed an outstanding scientific editor, and in building up *Biological Reviews* he made perhaps the greatest of all his contributions to the advancement of biology.

G. P. WELLS

# Pressure History of some Iron Meteorites

by  
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Comparisons with artificially shocked standards show that many iron meteorites have been shocked at pressures of at least 130 kilobars. These shocks probably originated from preterrestrial collisions, rather than from impact with Earth.

DURING the past few years, metallographic investigations have shown that shock pressures in excess of 130 kbars resulted in a number of microstructural changes in Grant and Canyon Diablo iron meteorites<sup>1-3</sup>. More recently, we have observed<sup>4</sup> that single crystals of meteoritic kamacite ( $\alpha$  Fe), cohenite ( $\text{Fe}_3\text{C}$ ) and schreibersite ( $\text{Fe}_3\text{P}$ ) are transformed, during artificial shock, into aggregates showing preferred orientation on X-ray diffraction investigation. Preferred orientation was also observed in grains of the same minerals from naturally shocked Canyon Diablo samples<sup>4</sup>.

Two previous reports<sup>5,6</sup> have mentioned that kamacite from eight additional iron meteorites showed unusual X-ray diffraction features ("asterism" or diffuse Laue spots). We thought that these abnormalities might indicate that the kamacite had been severely shocked, so we obtained a number of specimens of four of these (Table 1) for investigation by metallography and X-ray diffraction. In addition we investigated a number of samples of the Magura meteorite as it has been reported to contain diamonds<sup>7</sup> which may have been formed by shock<sup>8</sup>. Finally, we wished to make use of these observations in estimating the percentage of iron meteorites which have been severely shocked at some time during their history.

The metallographic and X-ray diffraction techniques used and the shock standards have been described elsewhere<sup>2,3</sup>. Although the microscopic shock-induced changes have been previously described in detail<sup>3</sup>, we shall review them briefly. Fig. 1 illustrates the appearance of Odessa samples shocked to 200 (a), 400 (b) and 600 kbars (c). The principal feature in Fig. 1a is the dense, fine-grained appearance of the  $\epsilon$ -iron structure. The localized nature of this feature in meteorites shocked to 200 kbars probably arises from differences in the relative orientation of the kamacite grains<sup>3,8</sup>. The 600 kbar transformation structure, which consists of a clearly resolvable background and Neumann bands with well developed transverse hatching (Fig. 1c), is readily distinguishable from that at 200 kbars<sup>3</sup>. X-ray diffraction patterns of kamacite, cohenite and schreibersite from such samples show pronounced preferred orientation, even if the regions from which the specimens were taken show no microscopically observable changes<sup>4</sup>.

The kamacite exposed in the polished surfaces of the meteorites Narrabura and Spearman uniformly shows a clearly resolvable  $\epsilon$ -iron transformation structure and "feathered" Neumann bands (Fig. 2a). The structure in

Narrabura has also been observed by Buchwald and Axon<sup>10</sup>. In Narrabura the kamacite grains in the very coarse plessite ( $\alpha$ - $\gamma$  iron mixture) even show this structure. The kamacite of both exhibits pronounced preferred orientation on X-ray examination. The phosphide grains of both meteorites show extensive fracturing and, in Spearman, corrosion (probably caused by terrestrial weathering). Based on comparison with shock standards, these meteorites apparently have been shocked to 400-600 kbars.

As in Narrabura and Spearman the  $\epsilon$ -iron transformation structure in Coopertown is present throughout the kamacite. It (Fig. 2b) closely resembles the 600 kbars standard, however, indicating that this meteorite was shocked to a somewhat higher pressure than were the first two. The plessite grains are somewhat less dense than in Spearman and there is localized kamacite recrystallization between the "teeth" of the comb-like plessite grains. The schreibersite grains are corroded although the rhabdites show sharp borders. The kamacite grains show preferred orientation.

All the specimens of Magura which we investigated showed patches of the  $\epsilon$ -iron transformation structure (Fig. 2c) previously observed<sup>10</sup> as well as regions of apparently unaltered kamacite. In the body of the transformation regions, the structure resembles the 200 kbars variety, while at the grain boundaries the structure was similar to that seen in Odessa samples shocked to 400 kbars. Some schreibersite and cohenite grains showed fracturing but no corrosion was apparent. Kamacite and cohenite grains showed preferred orientation on X-ray examination. We conclude that these specimens have been shocked in the pressure region of 130-400 kbars.

In all four specimens of Elbogen investigated the kamacite appeared to be completely recrystallized (Fig. 3a). The plessite grains were relatively clear and both taenite and plessite grains showed "thorny" edges, indicating nickel diffusion. While most phosphide inclusions were somewhat corroded, it could still be observed that the phosphide was dissolving into the kamacite matrix. In one specimen we observed a relict shear region which traversed kamacite, taenite and plessite but which had been subsequently recrystallized.

All the observations<sup>2,9</sup> suggest that every Elbogen specimen investigated has been reheated (either by shock or annealing) to  $700^\circ \pm 50^\circ \text{C}$  for a period of up to a few days. X-ray diffraction photographs of a number of Elbogen kamacite grains indicated randomly oriented polycrystalline aggregates. Such features, however, are apparent in both annealed and shock-recrystallized kamacite<sup>4</sup>. Schreibersite grains yielded typical single-crystal diffraction patterns (Fig. 3b) similar to those of unshocked meteorites (Fig. 3c), both unheated and annealed. Diffraction patterns of schreibersite from an Odessa specimen artificially shocked to 800 kbars (the pressure required to recrystallize kamacite) showed preferred orientation, however (Fig. 3d). It appears therefore that Elbogen kamacite was recrystallized by annealing rather than by shock-associated high temperatures.

Table 1

Meteorite (recovered mass in kg)	Source (specimen number)	Date found
Coopertown (17)	CNHM (Me 1126) Yale (P 231)	(fell) 1860
Elbogen (107)	BMNH (1922, 162: 90219) Yale (M 220: P 7)	1860 (1400)
Magura (1,500)	BMNH (33025: 19101 c) CNHM (Me 888)	1840
Narrabura (32)	Yale (M 288: M 307: P 18a) CNHM (Me 1142)	1855
Spearman (10)	CNHM (Me 2218)	1934

BMNH—British Museum (Natural History). CNHM—Chicago Natural History Museum. Yale—Bosch and Peabody Collections.

Of the meteorites which we have investigated, four seem to have been shocked to pressures of 130–600 kbars at some time during their history. It is impossible to say whether the shock(s) occurred preterrestrially or during impact with the Earth. The small masses of the recovered meteorites (Table 1) and their lack of association with terrestrial explosion craters would suggest, however, that the former alternative is the more likely. For these four cases, then, it seems that the abnormal diffraction features are strongly indicative of a severe shock.



Fig. 1. Microstructure of Odessa meteorites shocked artificially to (a) 200, (b) 400 and (c) 600 kbars. Note the localized nature of the  $\epsilon$ -iron transformation structure at 200 kbars. The textural differences permit estimation of peak shock pressures in naturally shocked iron meteorites. Scale bars are 0.1 mm.



Fig. 2. Photomicrographs showing microstructure of Narrabura (a), Coopertown (b) and Magura (c) iron meteorites. Apparently Magura has been shocked to 130–400 kbars; Narrabura (and Spearman) to 400–600 kbars; and Coopertown to 600 kbars.

Our observations on Elbogen would suggest that its recrystallization was caused by annealing at low pressures rather than by shock. This conclusion does not, however, explain the observation<sup>6</sup> of Elbogen's kamacite having preferred orientation covering  $40^{\circ}$ – $50^{\circ}$  of arc. We have observed that such arc segments can be obtained by incompletely etching a randomly oriented polycrystalline kamacite aggregate<sup>4</sup>. It would therefore seem either that Leonhardt's Elbogen sample was improperly prepared or that true shock alterations were not distributed uniformly throughout the meteoroid. Until it can be demonstrated



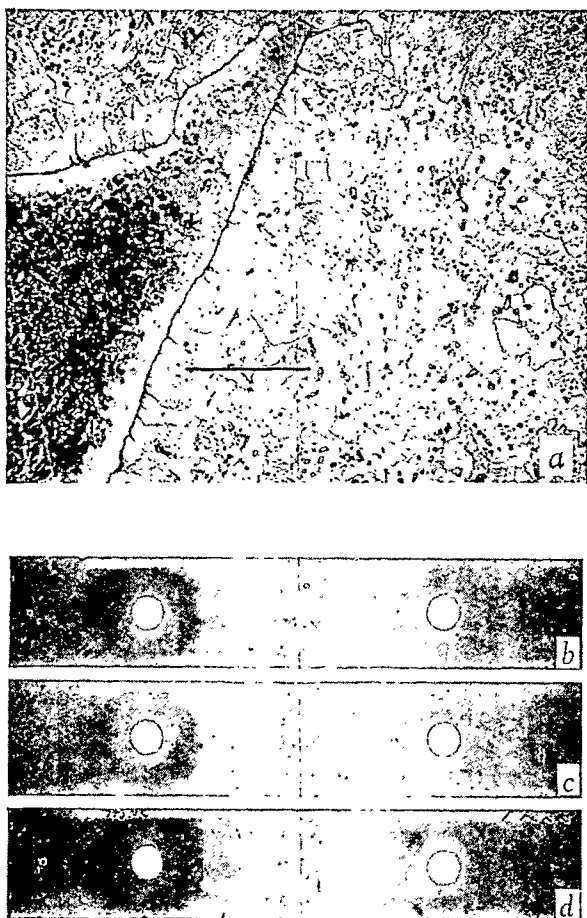


Fig. 3. Microstructure of the Elbogen meteorite (a) showing thermal alteration effects. X-ray diffraction photographs (manganese-filtered iron  $K\alpha$  radiation) of unrotated single grains of schreibersite ( $\text{Fe}_3\text{P}$ ) from: (b) Elbogen, (c) unshocked Odessa specimen, and (d) Odessa sample artificially shocked to 800 kbars. The diffraction photographs indicate that Elbogen specimens have not been shocked to high pressures at any time during their history.

that specimens of Elbogen have been shocked we feel justified in considering it to be an unshocked, thermally altered meteorite.

Our observations and those of Leonhardt<sup>5</sup> and Short and Andersen<sup>6</sup> on sixteen randomly chosen specimens suggests that shock effects in iron meteorites are not rare. If we eliminate Elbogen from consideration, eight of the fifteen

meteorites have been shocked to pressures in excess of 130 kbars. Although the number of meteorites investigated is small, it is large enough to indicate that, at the 95 per cent confidence level, at least 7 per cent of the known iron meteorites (nearly 600) should have been severely shocked at some time during their history. One additional significant point should be raised in this connexion. After completion of our study, it was brought to our attention that the  $\epsilon$ -iron transformation structures in Magura and Narrabura had been reported previously<sup>10</sup>. Furthermore, Buchwald<sup>10</sup> has observed it also in two other meteorites, Treysa and Bella Roca. It is important to note that most of the small shocked meteorites reported here and elsewhere<sup>1,5,6,10</sup> belong to the Ga-Ge group III as we will discuss in a forthcoming publication.

We conclude by considering the occurrence of diamond<sup>7</sup> in the Magura meteorite. In a previous paper<sup>2</sup> we reported that one Magura specimen showed evidence for shock-induced recrystallization of kamacite, while two others did not. A re-examination of one of these two and examination of five other Magura specimens showed that the  $\epsilon$ -iron transformation structure was invariably present, indicating a minimum shock pressure of 130 kbars. Recent work by De Carli has shown that diamond can be formed from graphite by shock pressures as low as 100 kbars. Unfortunately all the specimens of Magura which we investigated were devoid of troilite ( $\text{FeS}$ )-graphite nodules, so that no diamonds could be expected. In view of Magura's shock history, however, it seems quite possible that some specimens may indeed contain diamonds. Because many meteorites seem to have been shocked, a search for diamond among these might be expected to yield positive results.

This research was supported by grants from the U.S. National Aeronautics and Space Administration and the Advanced Research Projects Agency. We thank Dr. M. H. Hey, Dr. E. Olsen and Professor K. K. Turekian for the loan of the meteorite specimens.

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## Variations in the Flux Density of Some Quasi-stellar Sources

by  
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Further data on the variations in the 1.96 cm flux density of the quasars 3C 273, 3C 279, 3C 446 and 3C 454-3 are reported. The data support the theory that the variation is produced by repeated injection into magnetic field of clouds of relativistic electrons, which then expand, but some difficulties remain.

DURING 1966, periodic observations of the variable quasi-stellar sources 3C 273, 3C 279, 3C 446 and 3C 454-3 have been made at a frequency of 15.3 Gc/s (1.96 cm) in order to determine accurately the nature of their time variations. Previous observations of 3C 273 and 3C 279 at 2 cm and at longer wavelengths had shown that significant

variations occur with time scales of a few months or less<sup>1</sup> (Allen, R., and Dent, W. A., communicated at the Union Radio-Scientifique Internationale Spring Meeting, Washington, D.C., April 18-21, 1966, and Pauliny-Toth, I. I. K., and Kellermann, K. I., communicated at the 121st meeting of the American Astronomical Society, Hampton, Va., March 28-31, 1966.) It has been suggested<sup>1</sup>, on the basis of the wavelength dependence of the variations and

\* Operated by Associated Universities, Inc., under contract with the National Science Foundation.

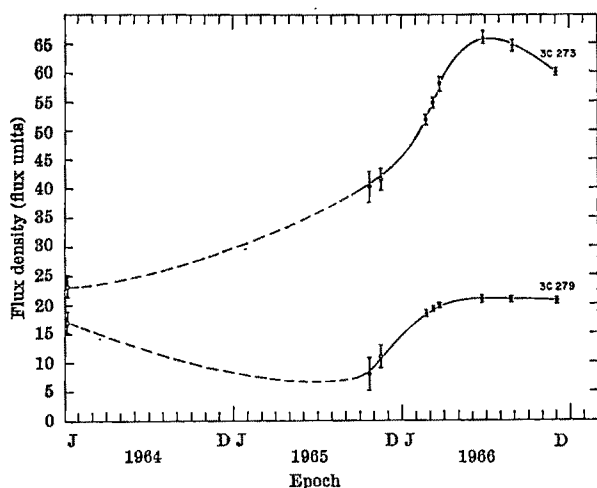


Fig. 1. The flux density of 3C 273 and 3C 279. The observations of the U.S. National Radio Astronomy Observatory are shown as solid circles and the error bars represent the relative accuracy of measurements made at different epochs. The measurements of Dent at 1.8 cm are shown as open circles and the error bars for these represent the absolute accuracy.

the form of the radio spectra, that these variations are caused by repeated injection into a magnetic field of a dense cloud of relativistic electrons. This cloud is initially optically thick to synchrotron radiation at centimetre or even millimetre wavelengths. Expansion of the cloud causes it to become optically thin at successively longer wavelengths. In the region where the cloud is optically thick, the flux density increases with time, while it decreases at frequencies where the cloud is optically thin.

In this communication we present the results of observations made in June, August–September, and December 1966, and compare them with earlier measurements made in October 1965 and February–March 1966. All of the observations were made with the 140-ft. radio telescope at the National Radio Astronomy Observatory. During the first observing period, the radiometer consisted of a crystal mixer which gave a system noise temperature of about 500° K and a bandwidth of 10 Mc/s. In all of the following periods, this was replaced by a tuned radio frequency tunnel diode radiometer having a system noise temperature of 1,200° K and a bandwidth of 2 Gc/s. In order to reduce the effect of atmospheric fluctuations, the radiometer was switched between the main beam, directed along the electrical axis of the paraboloid and an offset reference beam 6.5 minutes of arc (about 3 half-power beamwidths) away<sup>2</sup>.

In each observing period, a number of other sources of small angular diameter were observed as calibrators. Two or more of the stronger sources were also observed over a large range of hour angles in order to determine the variation of the aperture efficiency of the telescope and of the atmospheric extinction as a function of the antenna orientation. To within an accuracy of 5–10 per cent the aperture efficiency was found to be a function of the zenith angle alone up to a zenith angle of about 50°. At this point the antenna temperature of a point source falls to about half of its value at the zenith, partly as a result of the decrease in aperture efficiency and partly because of the increased atmospheric extinction\*.

Nearly all the observations were made within 30 min of meridian transit and the known change of the telescope gain with hour angle was used to correct the measurements to the meridian. Each observation consisted of a measurement of the ratio of the antenna temperature to a calibration signal from a gas discharge tube. For the stronger sources, the antenna beam was first positioned roughly on the source, the feed horn was then focused for maximum

antenna temperature, and the antenna beam was positioned on the source with an accuracy better than 6 sec of arc (0.05 of the half-power beamwidth). A measurement of the antenna temperature of the source relative to the calibration signal was then made by pointing the beam first towards the source and then towards a reference point about 1 min away in right ascension. For weaker sources, on which the antenna could not be positioned accurately, a series of on-off measurements was carried out not only at the expected position of the source but also at each of four positions about 1 min of arc away in right ascension or declination. A two-dimensional gaussian distribution curve was then fitted to the five measurements to determine the true antenna temperature of the source. Each observation was made in two orthogonal polarizations, so that the total flux density, including any linearly polarized component, could be determined.

The sensitivity and stability of the tunnel diode radiometer were such that the uncertainty caused by the receiver noise in a single on-off measurement was a few tenths of a flux unit (1 flux unit =  $10^{-26}$  W/m<sup>2</sup>/(c/s)). For the weaker sources, this uncertainty was reduced to about one tenth of a flux unit or less by repeating the on-off measurements. With the exception of the observations in October 1965, the day-to-day scatter in the relative antenna temperatures during a single observing period was of the order of 2–3 per cent for the stronger sources 3C 273, 3C 279, 3C 454.3, Virgo A and Jupiter. Observations made at different periods were related by expressing all the results as ratios to Virgo A. Measurements of the planets Jupiter and Saturn, the planetary nebula NGC 7027 and the non-thermal source 3C 123 showed that the flux density of Virgo A remained constant within 3 per cent over the period February 1966 to December 1966, and that it was the same to within 10 per cent in October 1965.

In October 1965, the accuracy of the measurements was determined mainly by the receiver noise and amounted to 2–3 flux units. In 1966, for any one of the sources 3C 273, 3C 279, and 3C 454.3, the accuracy with which the relative flux density at different times could be determined was not limited by the receiver noise, but by other effects, such as variations of the gain of the antenna, of the atmospheric extinction or of the calibration signal, or by uncertainties in the pointing of the telescope and in the focusing of the feed horn. All these amounted to an uncertainty of about 2 per cent. For 3C 446, the accuracy was about 0.1 flux units and was limited by the receiver noise. The relative flux densities of sources at different declinations measured during the same period are less certain because of the uncertainty of about 5 per cent in the variation of the gain of the antenna with its orientation. The scale of flux density is based on

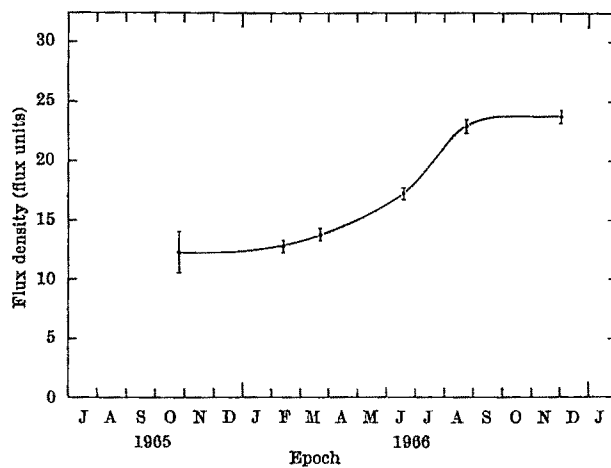


Fig. 2. The flux density of 3C 454.3. The error bars represent the relative accuracy of measurements made at different epochs.

\* Measurements made on more extended sources such as Cas A and Cyg A, which have angular sizes comparable with the beamwidth, indicate that the beam efficiency is much less dependent on the zenith angle than the aperture efficiency, as determined from the measurements of "point" sources.

observations of Virgo A, Hydra A and 3C 161, the flux densities of which at 2 cm were estimated to be 28, 4.6 and 2.6 flux units respectively from an extrapolation of their spectra at lower frequencies. It is thought that this scale is accurate to within 15 per cent.

The measured flux densities for the variable sources are given in Table 1 together with the epochs of the observations. The values for 3C 273 and 3C 279 are plotted in Fig. 1 together with those of Dent at 1.8 cm (communicated by Dent, W. A., Conference on Observational Aspects of Cosmology, Miami, Florida, December 15-17, 1965). The flux density of 3C 279, which showed a rapid increase at the beginning of 1966, has remained almost constant since April 1966, while the flux density of 3C 273 reached a broad maximum in June 1966, and has now begun to decrease. The flux density of 3C 454-3, shown in Fig. 2, began to increase in February 1966 and approximately doubled by September 1966. The peak was apparently reached sometime in October or November 1966. Previous observations at 20 cm (ref. 1) had suggested that this source is variable. The flux density of the source 3C 446 has increased by nearly 50 per cent during 1966 as is shown in Fig. 3. (*Note added in proof.* Measurements made during January 12-15, 1967, give the following flux densities:

3C 273	3C 279	3C 446	3C 454-3
58.9	23.7	4.63	28.1

There is thus evidence for renewed activity in 3C 279 and 3C 454-3.)

Table 1. OBSERVED FLUX DENSITIES\* FOR VARIABLE SOURCES

Epoch/Source	3C 273	3C 279	3C 446	3C 454-3
Oct. 1-5, 1965	41	8		12.3
Feb. 19-28, 1966	51.8	18.3		12.8
Mar. 3-15, 1966	54.9	19.3	3.29†	
Mar. 16-24, 1966	58.3	20.0		13.8
June 24-26, 1966	66.5	21.0	3.89	17.2
Aug. 21-30, 1966	64.9	21.2	4.12	22.9
Dec. 1-4, 1966	60.0	20.4	4.76	23.8

\* In flux units. For any one of the sources 3C 273, 3C 279 and 454-3, the random errors in the flux densities are estimated to be 2 to 3 flux units for the first period and 2 to 3 per cent thereafter, while for 3C 446 this error is 0.1 flux units. The absolute scale of flux densities is thought to be accurate to 15 per cent.

† Measured on March 11.

For a uniform, optically thick source expanding at a constant rate, the flux density increases approximately as  $t^3$  and the wavelength at which the optical depth is unity varies as  $t^2$  (ref. 1). The flux density at any frequency will be observed to increase when an appreciable fraction of the total flux is contributed by the expanding, optically thick component. The flux density will continue to increase until the expanding component becomes optically thin, at which time it will begin to decrease. Van der Laan<sup>3</sup> has given a more detailed mathematical description of this model and has shown that if the

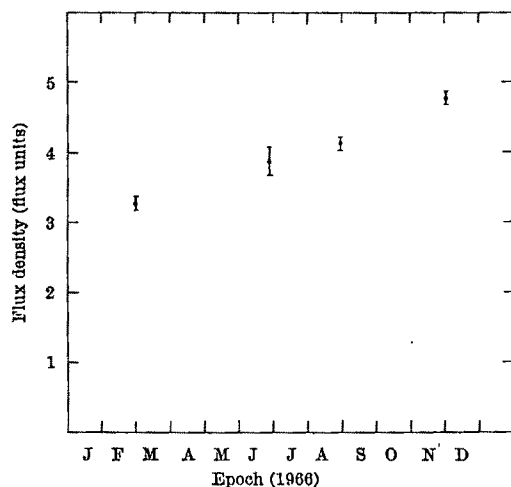


Fig. 3. The flux density of 3C 446. The error bars represent the relative accuracy of measurements made at different epochs.

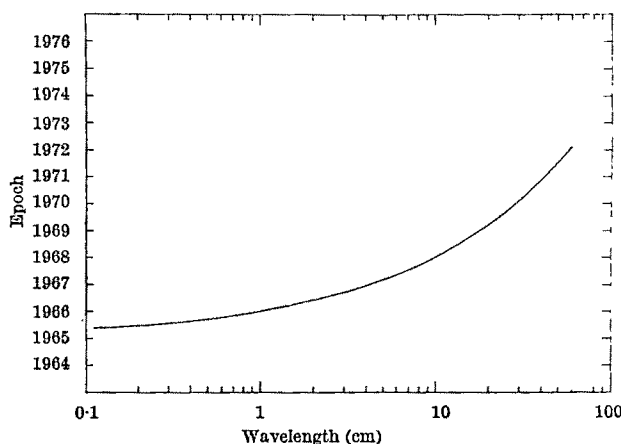


Fig. 4. The epoch at which the flux density of 3C 273 is expected to reach its maximum at different wavelengths, according to the simple model of a uniformly expanding source.

maximum flux density reached at frequency  $f_1$  is  $S_1$  then the maximum at frequency  $f_2$  is

$$S_2 = S_1 (f_2/f_1)^{5-7\alpha/5-4\alpha} \quad (1)$$

where  $\alpha$  is the radio spectral index in the range of frequencies where the source is optically thin. Thus, a detailed measurement of the variation at one frequency can in principle be used to predict the variations at any lower frequency.

The actual situation, however, is more complex because of the simultaneous presence of several components at different states of evolution. Although at any one frequency the spectrum may be dominated by a single strong component of increasing flux, there may remain an older and weaker component with a decreasing flux density. This makes the determination of the flux density of the main variable component and its rate of change more difficult. In 3C 273, for example, at least three components can be recognized<sup>1</sup>. From the form of the radio spectrum and from the rate of increase of the flux density at 2 cm in the spring of 1966, we estimate a characteristic age for the strong 2 cm component of about 1.3 yr in April 1966, when the flux reached a maximum at 2 cm. The epoch at which the flux density will reach a maximum at longer wavelengths has been estimated from the characteristic age and the relation

$$t(f_2) = t(f_1) \left( \frac{f_2}{f_1} \right)^{1/2} \quad (2)$$

and is shown in Fig. 4. A similar age is found for 3C 279 in the early spring of 1966 when the maximum flux was reached at 2 cm. The corresponding maxima at 3.75 and 6 cm are expected in late 1966 and early 1967 respectively.

The simple model predicts that the flux will begin to decrease after the maximum is reached at a rate which depends on the radio spectral index<sup>1,3</sup>. Inspection of Fig. 1 shows that this is clearly not the case for 3C 279 where the flux density has remained at a high level for six months. A possible explanation is that the density of relativistic particles increases towards the centre of the source, so that the inner region is still optically thick at a time when the outer parts are becoming optically thin. Such a gradient in the density of particles could occur if their production took place over a period of time, rather than in a short burst at the beginning of the expansion.

For 3C 454-3, the characteristic age derived from the rate of increase of the flux density at 2 cm is 1.2 yr for the epoch August 1966 when the maximum was reached at this wavelength. The most recent observations indicate that this component started to become optically thin at 2 cm shortly after the middle of 1966. In May 1966, when this component was still optically thick at 2 cm, its flux density at that wavelength was about 13 flux

units and at longer wavelengths its contribution should have been

$$S_{\lambda} \sim 13 \left( \frac{2}{\lambda_{\text{cm}}} \right)^{2.5} \quad (3)$$

This corresponds to an increase of about 2.7 flux units at a wavelength of 3.75 cm and of about 1 flux unit at 6 cm for the variable component—a change in flux density that should be easily detectable. The maxima at 3.75 and 6 cm are expected to occur in early and late 1967 respectively, and the corresponding maximum flux densities from equation (1) should be about 6.5 and 4 flux units (for  $\alpha = -0.2$ ).

Although the number of sources discussed here and the time span over which the observations have been made are limited, a few comments can be made about the general nature of the variations. First, the qualitative form of the variations differs somewhat from source to source. Second, the time scales for variations at 2 cm are considerably less than 1 yr. Third, if the sources are at the cosmological distances indicated by their red-shifts, the absolute monochromatic power radiated at 2 cm has increased by  $10^{27}$  W (c/s) for 3C 273 to  $10^{28}$  W (c/s) for 3C 454.3. The latter value is based on a red-shift of 0.87 obtained by Lynds (private communication). The change in the total radiated power is uncertain because of the lack of spectral data at shorter wavelengths but it is certainly greater than  $10^{45}$  ergs/sec for 3C 454.3, and not less than  $10^{44}$  ergs/sec for 3C 273 and 3C 279. This is equivalent to the creation of a radio source as intense as the most powerful radio galaxies such as Cygnus A or 3C 295 in a time interval of 6 months. Similarly, the energy requirements involve the production, in a time of a few months and in a volume of space smaller than 1 pc,

of relativistic particles with a total energy comparable with that found in the strong radio galaxies. Rees<sup>4</sup> has suggested, however, that the actual time scales may be longer than the observed values if the source is expanding with a velocity near that of light.

It might be expected that such a violent event would be observable at optical wavelengths and that the "light flashes" observed in the quasistellar sources may represent this explosion. At least in the case of 3C 273 no change greater than 0.07 magnitudes has been reported during the past two years<sup>5</sup>, although, of course, no optical observations are available for mid-1965 when the event possibly occurred, because the source was near the Sun at that time. In the case of 3C 279 (ref. 6) and 3C 454.3 (ref. 5), significant variations have occurred sometime between 1950 and 1966. In 3C 446 changes of as much as three magnitudes have been observed during the past year<sup>7</sup> and it will be of interest to see if the 2 cm flux increases more rapidly during the next year. On the other hand, there are several sources such as 3C 48 and 3C 196 which are known to be variable at optical wavelengths<sup>8</sup> but for which no radio variations have yet been detected. Clearly, more or less simultaneous observations are needed at radio and at optical wavelengths to determine if the intensity variations at the two wavelengths are at all correlated, or if they are caused by different mechanisms.

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## Altithermal Timberline Advance in Western United States

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A combination of radiocarbon dating and counting of annual rings of the remains of trees has been used to establish the time of retreat of forests from sub-alpine mountains in California and Nevada. The information obtained can serve as a palaeoclimatic indicator.

THE position of the mid-latitude alpine timberline may be a sensitive climatic indicator. Evidence from widely separated localities in California and Nevada shows that a rise and later recession of timberline have occurred during the past several thousand years, in agreement with other indications of a postglacial "thermal maximum". Dead trees and large fallen remnants occur on mountain slopes above the highest living "timber-sized" trees in the White Mountains, California, and in the Snake Range, Nevada (Fig. 1). The dominant sub-alpine tree in the White Mountains is the bristlecone pine (*Pinus aristata* Eng.), which is accompanied by limber pine (*P. flexilis* James)<sup>1</sup>. Engelmann spruce (*Picea engelmannii* Parry) is the typical timberline tree in the Snake Range, but bristlecone and limber pine are locally abundant. In both localities, the remnants appear to be those of bristlecone pines. Exposed wood of trees of this species can persist for several thousand years<sup>2</sup>, and thus can provide direct evidence of the former distribution of trees.

The discontinuity of the sub-alpine forest means that a climatic timberline exists only locally in the White Mountains. It is clearly defined where the upper limit of a wooded area coincides with topographic contours.

Extensive unwooded tracts below timberline correspond to areas of unfavourable sandstone, shale or granite<sup>3</sup>. Timberline is highest on the dolomite that makes up the east slope of Sheep Mountain (Fig. 1). It is lower on sandstone areas such as Campito Mountain and County Line Hill. Despite the differences in absolute altitude of timberline on different rock-types, the highest remnants have been found at similar vertical distances above timberline on both dolomite and shale. In each case tree remnants are most abundant near the present timberline and include numerous dead, but still erect, trees. Only the greatly reduced and deeply weathered remains of fallen trees occur at the higher altitudes at each locality.

Fire or other catastrophic agencies cannot be responsible for the occurrence of tree remnants through comparable altitudinal ranges above timberline on several isolated peaks. The low density of trees and the sparsity of litter and flammable ground-cover preclude widespread burning of the sub-alpine forest near timberline. Although a few of the remnants have been charred, local fires caused by lightning are probably responsible. A number of factors may have contributed to the death of individual trees above timberline, but only a climatic change seems

to explain adequately the absence of living trees on these once-wooded summits.

The present position of a dead tree or large remnant marks the lowest possible altitude of timberline during the lifetime of the tree. Dating of these remains, therefore, establishes the minimum timberline altitude in the past. Radiocarbon age determinations were made of samples from the wood of four tree remnants in the White Mountains, and from three in the Snake Range (Table 1). In addition, the number of years of growth represented by the wood of the entire specimen was determined by counting annual rings exposed in a supplementary transverse section or increment core. Because the chronological position of the radiocarbon sample relative to the ring-count is known, the approximate dates of the earliest—and latest—formed growth increments present in the specimen can be determined.

Table 1. AGE AND ALTITUDE DATA FOR TREE REMNANTS ABOVE MODERN TIMBERLINE

Specimen	Altitude (m)	Age data ring count (yr before present)					
Map No.	Field designation	Above sea level	Above timberline	Approx.	From		Radiocarbon date
				date of estab-lishment	Inner ring	Outer ring	
White Mountains, California							
1	202	3,480	120	3,100	2,663	789	840 ± 80 (UCLA-1070F)
2	195	3,490	130	3,200	3,193	2,367	2,540 ± 80 (UCLA-1070B)
3	382	3,510	150	4,300	4,214	3,501	3,565 ± 80 (UCLA-1070G)
4	231	3,360	120	5,800	4,507	3,784	4,000 ± 80 (UCLA-1070A)
Snake Range, Nevada							
—	362	3,540	120	2,488 ± 2,488	2,003	2,350 ± 80 (UCLA-1070C)	
—	364	3,540	120	3,049 ± 3,049	2,028	2,240 ± 80 (UCLA-1070D)	
—	363	3,540	120	4,017 ± 4,017	2,627	3,930 ± 80 (LJ-1336)	

Weathering and decay have destroyed the early formed wood in most of the specimens. The date of establishment of the tree, therefore, can only be estimated. The time-span represented by the missing inner wood is obtained by combining the probable amount of radial growth missing with the probable average growth rate during the period. The original location of the stem

axis is estimated by projection of intact radial branches or the radii of curvature of concentric growth-rings. The average growth rate indicated by the innermost few inches of remaining wood is inferred to equal that in the missing portion. The resulting figure is added to the age of the innermost remaining ring to give the estimated date of establishment. This is listed in Table 1 for those specimens in which evidence of the original size and form of the tree is sufficiently well preserved.

Ranking of tree remnants in the White Mountains according to position shows that the time that has elapsed since death increases progressively with increasing altitude of the site (Table 1). Except that the two highest remnants are also the oldest so far discovered at each locality (2 and 4 on map, Fig. 1) there is no consistent pattern of ages with respect to altitude above local timberline. The times of death of two specimens (1 and 2 on map) representing the highest dead, but still erect, trees on Campito Mountain differ by 1,500 yr. The younger of these is 120 m above timberline, but died only 750 yr ago. The fact that both trees were established at about the same time, at similar altitudes, suggests that certain trees can survive in conditions that contribute to the death of less favourably situated specimens, and that preclude the establishment of seedlings. Thus, where long lived trees such as bristlecone pine are involved, changes in the position of timberline defined by the highest living trees may lag several hundred years behind changing environmental conditions. The time of establishment of individual trees seems to be of greater significance than the time of their death as an indicator of past environmental conditions. The four dated remnants from the White Mountains were established at altitudes 120–150 m above present timberline before about 3,000 yr ago. We conclude that the onset of adverse conditions has occurred since that time.

The existence of a similar "fossil timberline" in eastern Nevada, about 400 km north-east of the White Mountains, suggests that a net retreat of the upper forest limits in the past few thousand years is a phenomenon of at least regional extent. On the south slope of Mount Washington (altitude 3,460 m), near the centre of the Snake Range, Nevada, bristlecone pine forms a timberline at an altitude of 3,420 m. Although scattered clumps of Engelmann spruce and bristlecone pine krummholz reach nearly to the summit, no large, erect trees are now living above timberline. Large wood fragments, however, are abundant on the slope up to 3,540 m. One of these (364) listed in Table 1 is more than 2 m long. A total of 1,030 annual rings was counted along the measured 67 cm radius. Because the prostrate stems of the dwarfed krummholz forms reach only a few centimetres in diameter, this is clearly the weathered remnant of a large tree similar to those now living at much lower altitudes. No date of establishment was estimated for any of the three dated specimens, because their original form cannot be confidently inferred. They were all, however, established about 2,500 yr ago, and all died within the same period, between 2,000 and 2,700 yr ago.

Alpine timberline may fluctuate in response to long term temperature trends. In north temperate latitudes, the position of the timberline seems closely associated with the altitude at which the near July temperature is about 50° F. (refs. 4 and 5). Climatic cooling would lower this apparent altitudinal limit. Thus interpreted, evidence of timberline retreat within the past several thousand years can be compared with other palaeoclimatic indicators. An extended period of higher than present temperatures, variously termed the Climatic Optimum, Hypsithermal, or Altithermal, has been widely recognized. It followed the cold period of the Late Wisconsin glacial maximum, when montane life-zones were presumably depressed far below present altitudes. Antevs<sup>6</sup> originally considered the Altithermal to have lasted from about 7,000 to 4,000 yr ago in the western United States, and

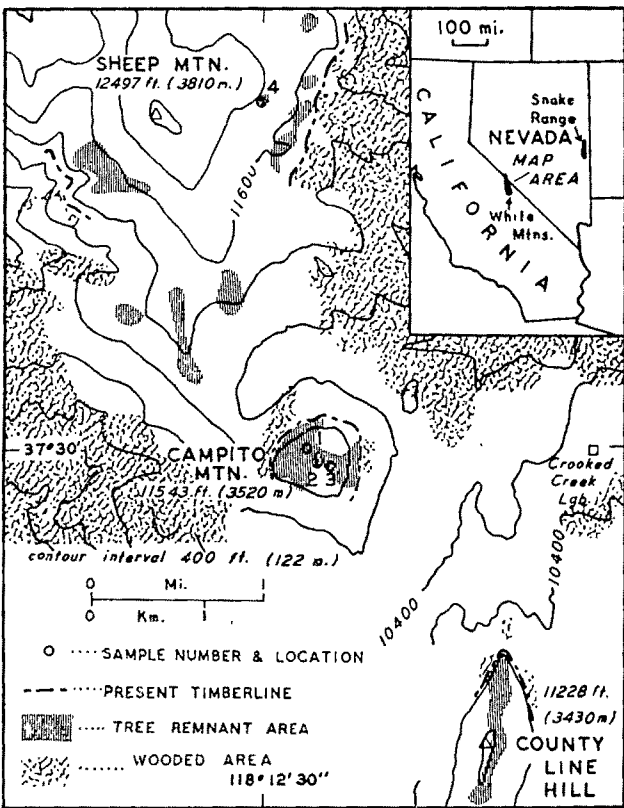


Fig. 1. Generalized topographic map of southern White Mountains, California, showing distribution of tree remnants above modern timberline.



to have ended with the reappearance or readvance of alpine glaciers of the "little ice age" of Matthes<sup>7</sup>. More recent work has shown that this widespread glacial readvance occurred between 2,600 and 2,800 yr ago at many localities in western North America<sup>8</sup>. At about the same time, our evidence shows that reproduction of bristlecone pine ceased near what was then the upper forest margin. Timberline retreated erratically with the death of individual trees that had been previously established. We conclude that the shrinkage or disappearance of alpine glaciers in Altithermal time was accompanied by an upward advance of sub-alpine forests to altitudes at least 150 m above the present timberline, and that timberline retreat began at the time of renewed glacial activity of the "little ice age", in response to climatic cooling. Although its retreat to present levels may have been punctuated by long periods of comparative stability,

or even by significant readvances, alpine timberline in the western United States rose to maximum post glacial height in Altithermal time.

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## Some Biological Properties of Dimethyl Sulphoxide

by

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Dimethyl sulphoxide decreases the viability of HeLa cells actively metabolizing in tissue culture. It inhibits attenuated virus strains more strongly than virulent strains, but seems to have little effect on the rate of virus adsorption.

THE penetration of a variety of topical pharmaceuticals is dramatically improved when they are used in conjunction with dimethyl sulphoxide (DMSO) (ref. 1). As a vehicle, DMSO could conceivably enhance the penetration of viruses *in vivo* as well as *in vitro* and possibly allow the crossing of species barriers. We have found that DMSO administered with the virus did not alter the number of passages required to adapt an egg-passed influenza virus strain to C57BL6J mice. In addition, intranasal inoculation of 3–5 per cent DMSO had no readily apparent adverse effects on the animals. These observations, coupled with the ability of dimethyl sulphoxide to protect cells during freezing<sup>2</sup>, suggested that further information about the biological properties of DMSO was necessary. This investigation therefore consists of a series of observations dealing with the influence of DMSO before and during viral penetration and synthesis.

The clone of Gey HeLa cells used in these studies was obtained from the University of Michigan, where it was given the name HCAAT. These cells were propagated as suspension cultures in a medium free from antibiotics consisting of 2X Eagle's vitamins, amino-acids and glutamine, 10 per cent calf serum, 15 per cent tryptose phosphate, 2 per cent sodium citrate, 0.12 per cent methylcellulose, suspended in Hanks balanced salt solution (BSS). For plaque assay of poliovirus, 4 ml. of cells at a concentration of  $7.5 \times 10^5$  cells/ml. were suspended in the medium without methylcellulose and sodium citrate and introduced into 2 oz. tablet bottles. The cells were incubated for 24 h at 34° C, and the monolayers thus formed were washed twice with Hanks BSS. These cells were then infected with the appropriate dilutions of poliovirus, incubated for 30 min at 34° C with gentle bottle agitation, and overlaid with 3 ml. of 1 per cent

agar consisting of monolayer growth medium (less calf serum and phenol red) containing 100 U of penicillin and 100 µg of streptomycin per ml. In certain experiments various concentrations of DMSO were incorporated either into the suspension culture medium or the agar overlay. After incubation at 34° C for 72 h the plaque bottles were further overlaid with 3 ml. of 1 per cent agar in Hanks BSS and 1 : 20,000 neutral red; the plaque counts were performed 12 h later.

In those experiments where cell viability was studied, the erythrocin B dye exclusion technique<sup>3</sup> was used. Cloning efficiency could not be measured because of the relatively poor attachment characteristics of the HCAAT cell line. The cells were sized using an electronic cell counter and cell size analyser.

Nucleoside incorporation was analysed by a method previously described by Bollum<sup>4</sup> and modified for tissue culture studies by Regan and Chu<sup>5</sup>. This was undertaken in an effort to determine the influence of different concentrations of DMSO on cellular nucleic acid metabolism. At various intervals after exposure to DMSO, 1 ml. quantities of suspension-culture HCAAT cells were pipetted into 5-ml. plastic test tubes. 0.1 ml. of fresh suspension culture medium containing 2–5 µc. of tritiated thymidine (approximately 2 c./mmole) or 2–5 µc. tritiated uridine (approximately 0.825 c./mmole) was added. The tubes were mixed well, incubated for 30 min at 37° C, and then placed in an ice bath. The cells were then lysed in an oscillator and 100 µl. of the sample was applied to a disk of filter paper 2.3 cm in diameter. After adsorption for 1 min the disk was submerged in 5 per cent trichloroacetic acid at 4° C. When the experiment was completed, all the disks were washed three times for 10 min in cold 5 per cent trichloroacetic acid, three times for 10 min in 95 per cent ethanol at room temperature, and twice rapidly in ether. The disks were dried com-

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Table 1. PERCENTAGE VIABILITY, ESTIMATED BY ERYTHROCIN B, OF HELA CELLS GROWN IN SUSPENSION CULTURE AND EXPOSED TO SEVERAL CONCENTRATIONS OF DMSO FOR VARYING INTERVALS OF TIME

Time (h)	DMSO (%)				
	0	1	2	3	4
0	96	96	96	96	96
4	—	—	—	96	—
8	—	—	—	90	—
12	96	93	89	85	73
16	—	—	—	86	—
21	94	95	86	80	55
33	95	94	74	49	20
48	93	95	47	46	10
70	97	95	50	5	3

pletely after each submersion. They were then placed in glass scintillation vials containing 5 ml. of 2,5-diphenyl-oxazole and 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene in toluene, and counted in a liquid-scintillation spectrometer.

The toxic effects of various concentrations of DMSO on HeLa cell suspension cultures, using the morphological criteria of cell viability and cell size, are indicated in Table 1 and Fig. 1, respectively. Cell viability decreases rapidly after exposure of the cell population to concentrations of DMSO greater than 1 per cent for periods greater than 12 h. This decrease in cell viability is further shown by an increase in the number of smaller cells (indicated by the shift of the cell population to the left in Fig. 1). The rapidity of this sequence of events depends on the concentration of DMSO to which the cell population is exposed.

Changes in cellular metabolism due to exposure to several low concentrations of DMSO are illustrated in Figs. 2 and 3. The rate of nucleoside incorporation (uridine and thymidine) is rapidly altered as the cell population is exposed to DMSO incorporated in the growth medium. Uridine and thymidine incorporation are proportionately affected by increasing concentrations of DMSO. There is a lag in the incorporation of uridine at the 1, 2 and 3 per cent DMSO concentrations. This delay in incorporation is partially overcome at 24 h. With 4 per cent DMSO this lack of incorporation is irreversible. There is an apparent depression of thymidine uptake after the first 24 h of exposure to DMSO; this depression is proportional to the concentration of DMSO. Decreased incorporation at the 1 per cent level is overcome during the next 12 h.

The influence of several concentrations of DMSO on the competence of HeLa cells in the synthesis of polioviruses was considered another criterion of cellular integrity. Depending on the concentration of DMSO incorporated into the agar overlay of plaque bottles after viral adsorption for 30 min, it was possible to differentiate

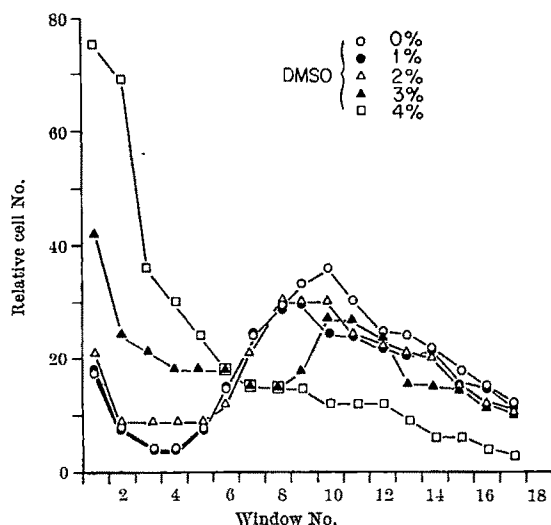


Fig. 1. Cell size distribution of HeLa cells exposed for 24 h to 0-4 per cent DMSO (each window is equal to a cell volume of  $240 \mu^3$ , and cell volume is therefore equal to  $240 \mu^3 \times$  window number).

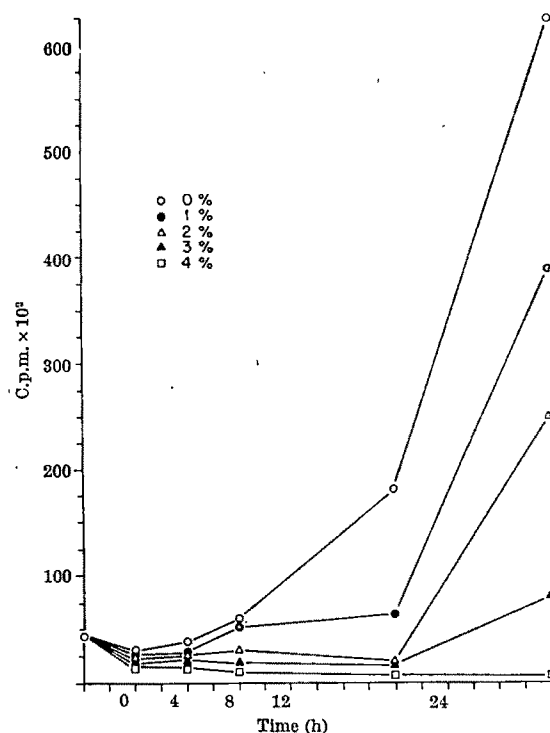


Fig. 2. Incorporation of tritiated uridine into HeLa cells treated with DMSO and propagated in suspension culture.

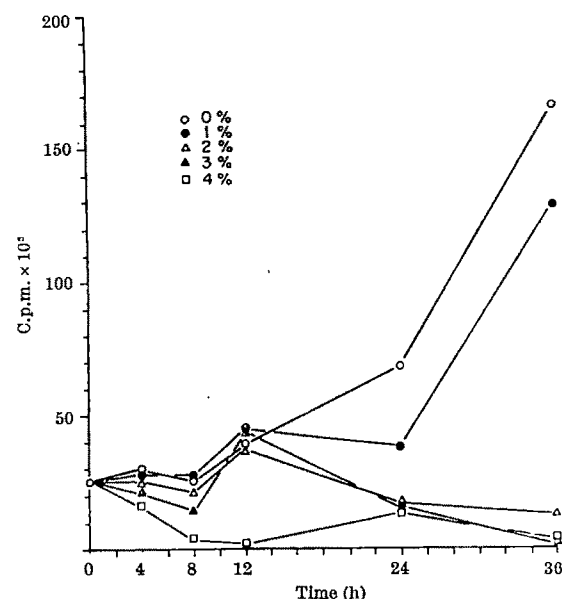


Fig. 3. Incorporation of tritiated thymidine in HeLa cells treated with DMSO and propagated in suspension culture.

between the attenuated and virulent strains of poliovirus (Fig. 4). In all cases, the attenuated strains were more adversely affected than the virulent strains, with the Sabin Type I strain (PLS) and the Mahoney strain (PLM), respectively, being the most competent in replication. To determine whether these results were due to direct inactivation of the virus by DMSO, we incubated the Mahoney and Sabin I strains for 24 h at  $4^\circ \text{C}$  in the presence of concentrations of DMSO ranging from 1 to 100 per cent. At concentrations of DMSO greater than 65 per cent no virus could be detected (Table 2). The observations in Fig. 4, therefore, cannot be due to viral inactivation but are probably due to effects of DMSO on the cell. Fig. 5 shows one such indication of a cytotoxic effect—the effect of DMSO on doubling time of exposed cells.

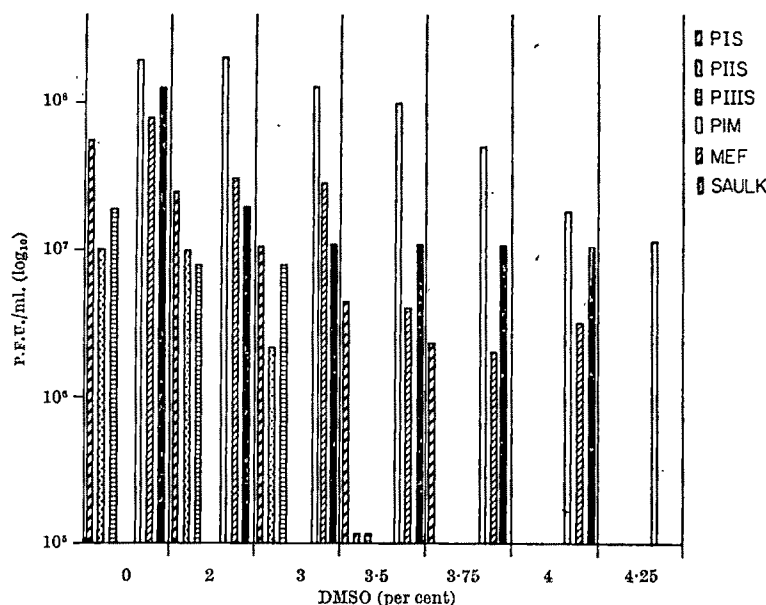


Fig. 4. Effect of various concentrations of DMSO on the selective P.F.U. inhibition of attenuated (PIS, PIIS, PIIS) and virulent (Mahoney (PIM), MEF, Salk) polioviruses.

To determine further the particular stage of viral propagation affected by DMSO, we studied the interim period between viral adsorption and viral release. We were unable to demonstrate a significant influence of 3 and 4 per cent DMSO during the first hour of virus adsorption (see Table 3).

Because none of these observations answered attenuated/virulent virus selectivity by DMSO, we believed these results could possibly be explained on the basis of elongation of the eclipse phase. As seen in Figs. 6 and 7, using a virus multiplicity of 20, virus release in the virulent strain begins 1 h before that in the attenuated strain; moreover, the peak virus yield is attained at least 1–2 h before that of the attenuated strain. A concentration of 3 per cent DMSO somewhat inhibits total virus yield in both strains, though appreciably more so in the attenuated strain. Similarly, DMSO has an appreciable sparing action with regard to loss of cell viability as determined by dye exclusion. Cells

exposed to virus alone lose their viability more rapidly than cells exposed to virus and DMSO.

We have been able to confirm a previous report<sup>6</sup> which indicated that, after 24 h association, concentrations of DMSO above 1.5 per cent are inhibitory to cell replication. The cytotoxic effects of 2, 3 and 4 per cent DMSO can be demonstrated after as little as 12 h using the criterion of vital stain exclusion. We are able to demonstrate that DMSO delays nucleoside incorporation; this can be observed as early as 12–24 h after the addition of 1 per cent DMSO to a cell population. The fact that, with the 1 per cent DMSO, both uridine incorporation and thymidine incorporation are merely delayed may indicate certain compensating or detoxifying factors present in the cell or its environment. The labelled nucleoside pool may be influenced by DMSO. This was not, however, determined in the present study.

The influence of DMSO on viral synthesis, another criterion of cell competence, sheds still more light on the possible mode of action of DMSO. The observation that DMSO seems to have a selective effect, depending on concentration, between the attenuated and virulent strains of poliovirus may be due to a longer eclipse phase in the attenuated strain, which causes it to reach optimal viral yield at least 1 h after the virulent strain, even when a similar viral multiplicity is used. In view of the nucleoside incorporation, a delay in viral synthesis places at a distinct disadvantage the virus with a longer eclipse phase. Once more, one must consider that plaque forming units cannot be found macroscopically until approximately 48 h after virus adsorption. During this interval, 2, 3 and 4 per cent DMSO has inactivated more than 50 per cent of the cell population. It should also be mentioned that although up to 4.25 per cent DMSO was incorporated into the agar overlay, the quantity of DMSO to which the cells were exposed at any one time was lower due to diffusion of DMSO through the agar. Therefore, by the time DMSO demonstrates its maximum

Table 2. PERCENTAGE OF DMSO REQUIRED TO INACTIVATE ATTENUATED AND VIRULENT POLIOVIRUS\*

DMSO (%)	Sabin Type I (P.F.U./ml. $\times 10^7$ )	Mahoney (P.F.U./ml. $\times 10^6$ )
0	3.0	1.1
1	3.5	0.9
5	3.2	1.6
10	1.0	0.9
20	2.5	1.7
50	2.7	1.0
65	0.03	0.02
75	0	0
85	0	0
100	0	0

\* DMSO diluted in Hanks BSS. In all instances undiluted virus was added to the appropriate DMSO concentration and incubated for 24 h at 4° C.

Table 3. RATE OF VIRUS ADSORPTION\*

Duration of virus adsorption (min)	Sabin Type I P.F.U./ml. $\times 10^6$			Mahoney P.F.U./ml. $\times 10^7$		
	0	3	4	0	3	4
1	3.2	1.9	2.0	1.1	1.2	1.5
3	3.6	2.4	4.3	3.5	3.3	2.7
5	3.6	4.2	3.4	3.5	1.5	5.0
7	6.4	3.6	4.3	6.4	3.1	4.9
10	8.1	2.9	5.0	6.4	3.6	2.5
13	9.0	7.6	7.7	6.3	3.9	5.0
15	9.4	4.6	7.0	6.8	3.4	5.5
20	11.0	17.0	10.0	8.8	4.5	7.5
30	17.0	14.0	14.0	9.4	7.5	11.0
60	12.0	10.0	13.0	9.2	13.0	15.0

\* Poliovirus was diluted in 0, 3, and 4 per cent DMSO–Hanks BSS. Virus was allowed to adsorb for various time intervals; bottles were washed twice with Hanks BSS and overlaid with nutrient agar.

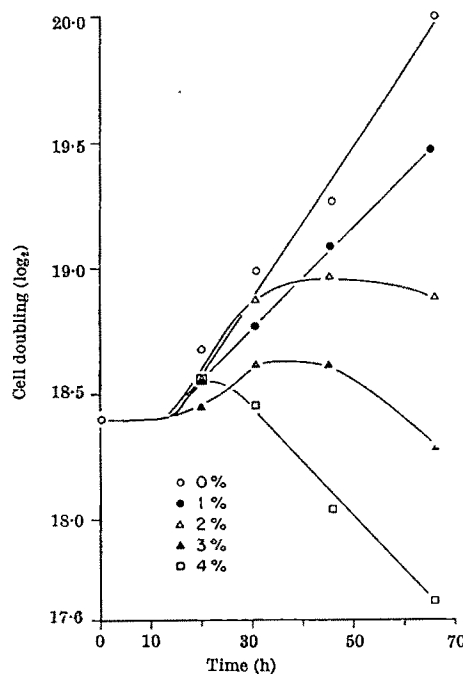


Fig. 5. Doubling time of HeLa cells grown in suspension culture media containing 0, 1, 2, 3, and 4 per cent DMSO.

inhibitory effects, the virulent virus has replicated to the point at which it has formed a visible plaque.

The optimum virus yield of DMSO-treated and untreated cells again indicates that DMSO in the virulent strain is inhibitory to about 50 per cent. The attenuated strain is inhibited by better than 95 per cent. We therefore propose that the selectivity of DMSO to the attenuated and virulent strains of poliovirus is associated with the influence of the compound on cell metabolism and is magnified by the difference in rate of virus synthesis. The sparing action of DMSO on cells infected with virus, as indicated by vital staining, can again be explained by the fact that virus infection and synthesis may bring about an alteration in cell permeability which in time may alter the capacity of the cells to exclude erythrocin B. In the presence of DMSO, virus synthesis is reduced, which eventually delays the cytotoxic consequences of viral infection. We have no evidence that DMSO increases

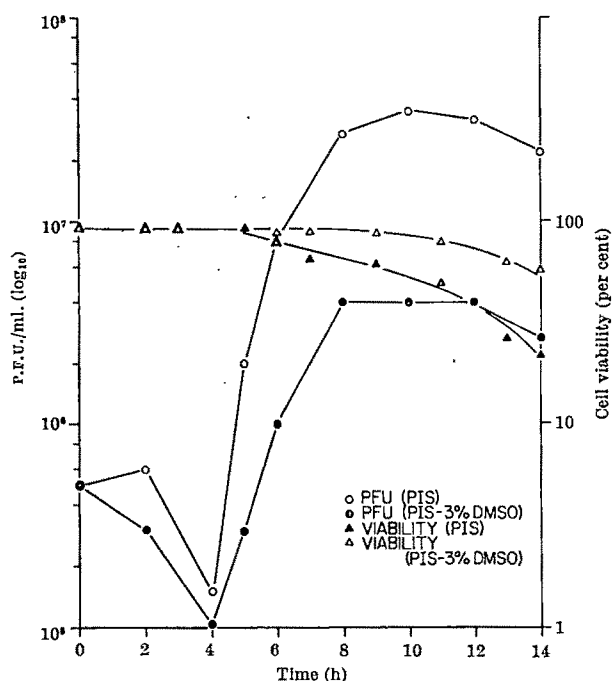


Fig. 6. Growth curve of Sabin Type I poliovirus in HeLa cells propagated in suspension culture. Cell viability estimated by erythrocin B dye exclusion.

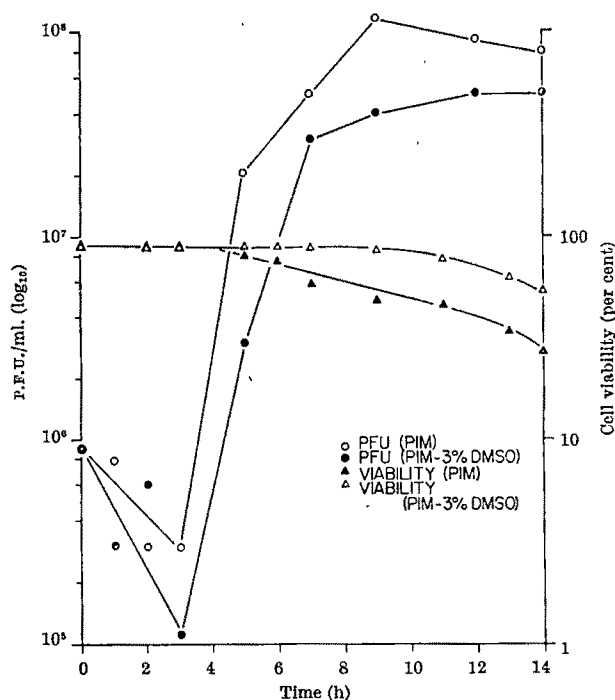


Fig. 7. Growth curve of Mahoney poliovirus in HeLa cells propagated in suspension culture. Cell viability estimated by erythrocin B dye exclusion.

the permeability of HeLa cells or the synthesis of poliovirus. Our results indicate that there is no difference in the rate of virus adsorption. Using low multiplicities of infection, we could find no increase in optimum virus yield. This again suggests that the sequence of events leading from virus adsorption to release and subsequent adsorption is not enhanced by DMSO. If anything, DMSO decreases optimum viral yield.

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## Glucose Binding by Homogenates of Intestinal Mucosa

by

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Glucose binds to soluble material in homogenates of intestinal mucosa; the degree of binding is dependent on pH and is increased by drugs which block glucose absorption.

In 1964 a new theory of the mechanism of intestinal absorption was proposed, a theory which was developed from the elucidation of the blocking actions of phloridzin and of cetrinide on the first and second receptors of intestinal sugar transport *in vivo*<sup>1,2</sup>. It was also shown that the mechanism of active transport involving the second receptor applies widely to nutrients other than

glucose and also to a number of species including man<sup>3,4</sup>. The results also indicated that the second receptor is situated on mobile intracellular proteins and that nutrients become associated with the protein on the luminal side of the cell and are released on the basal side. It was suggested that the dissociation constants between the nutrients and proteins are a function of the metabolic activities at the two opposite poles of the cell and the investigation of these proteins was thus the logical next

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step in the further elucidation of the mechanism of absorption.

As a preliminary to such an investigation it was decided to find out whether glucose binding to intestinal mucosal homogenates could be demonstrated *in vitro* by simple techniques. Brief descriptions of the methods and a summary of the results obtained are given in this article.

Male albino rats, deprived of food overnight, were killed by a single blow on the head and immediately bled. The small intestines (except the distal 5 in.) were crushed using a pestle and mortar and the mucosa expelled by digital pressure along the length of the intestine. The mucosal tissue was collected and homogenized by further crushing with a pestle and mortar, or in an electrically driven homogenizer, and the volume measured. Usually about 7–8 ml. of tissue were obtained from three rats. After dilution four times in freshly gassed Krebs bicarbonate solution, 0.124 ml. of 65 per cent glucose solution in Krebs solution was added to each tube to bring the concentration of glucose to 200 mg per cent. This allowed for a mean contribution of 5 mg per cent from the intestinal mucosa, that is, of 40 mg per cent in the original undiluted tissues, this being the mean of thirty estimations by a modification of the method of Haslewood and Strookman<sup>5</sup>. By contrast, the value obtained using animals allowed free access to food was 95 mg per cent. The pH of the homogenate, measured using a glass electrode, was then adjusted to the required value by adding 0.1 normal sodium hydroxide or hydrochloric acid. As the volume of fluid added for adjustment of pH was less than 0.05 ml., corrections in glucose concentrations were neglected. After standing at room temperature for an average of 1.5 h the homogenate suspension was spun down at 12,000 r.p.m. for 30 min so that some of the bound glucose might go down with the protein. The supernatant fluid was again diluted with its own volume of Krebs buffer and the glucose content estimated by the specific glucose-oxidase method<sup>6</sup> in an auto-analyser. The readings were compared with control glucose estimations in Krebs buffer at the same concentration, so that the difference between the mean control value and the mean of any set of experiments would represent a measure of the glucose uptake by the mucosal homogenate.

Preliminary experiments were carried out at the pH of the homogenate (6.8–7.2). The mean of glucose readings after centrifuging, for eleven experiments on mucosal homogenates without added drug, was  $75.18 \pm 3.91$  mg per cent as compared with a control value of  $99.45 \pm 1.26$  mg per cent in Krebs solution. Because this difference is significant ( $P < 0.001$ ) it was concluded that glucose had been bound by the mucosal homogenate.

Because there was evidence that cetrимide, phloridzin and sodium lauryl sulphate blocked intestinal absorption by interfering with the glucose receptors<sup>1,2</sup>, the effect of these drugs on glucose uptake by the mucosal homogenates was next investigated. The drugs were dissolved in the Krebs solution before being added to the homogenate, to give concentrations of 0.1 per cent and 1.0 per cent. The results shown in Table 1 indicate clearly that only phloridzin was significantly effective in reducing uptake at the lower concentration, while all three were effective at the higher concentration. On the other hand, phloridzin had no greater effect at 1 per cent than at 0.1 per cent.

One property of the cell, which may vary within the cytoplasm, is the pH, and it was thought possible that a difference of pH between the two poles of the cell might explain the association and dissociation between nutrients

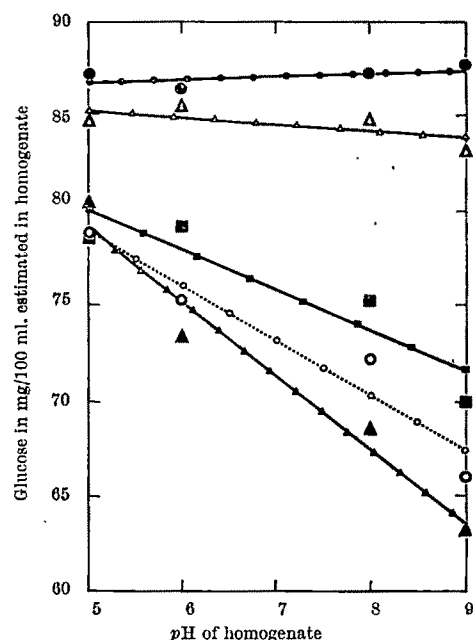


Fig. 1. Estimated concentration of glucose in the homogenate at various pH values in controls and in the presence of different concentrations of cetrимide. Initial glucose concentration was 104.3 mg per cent. ○, Control; ●, 0.1 per cent cetrимide; △, 0.25 per cent cetrимide; ■, 0.5 per cent cetrимide; ▲, 1.0 per cent cetrимide.

and proteins. For this reason the effect of pH on the uptake of glucose was investigated in some detail. In this set of experiments, glucose binding in the absence of the drugs was studied at pH 5.0, 6.0, 8.0 and 9.0. The results are shown in Fig. 1 and Table 2. The corresponding dose response curve showed a highly significant linear regression ( $P \ll 10^{-3}$ )—deviation from regression was only just equal to 0.05 and the equation for the curve is  $y = 93.27 - 2.89x$  (Table 3). Furthermore, the slope of the curve indicates much greater glucose uptake at higher pH. It is significant that the corresponding glucose control readings in twenty-eight experiments with which the homogenate glucose values have to be compared was  $104.35 \pm 0.64$  mg per cent. The differences between this value and those of the mucosal homogenate values at all pH levels was clearly significant. Parallel sets of experiments were carried out with 0.1, 0.25, 0.5 and 1.0 per cent cetrимide. The results are shown in Fig. 1 and Table 2, while a skeleton analysis of variance appears in Table 3. There is a continuous change in the slope of the dose-response curve from the lowest to the highest concentration. The difference in slope from that of the control is highly significant ( $P < 10^{-5}$ ) for the 0.5 per cent and 1.0 per cent but not significant for the two lower concentrations.

Table 2. MEAN GLUCOSE CONTENT OF HOMOGENATE WITH DIFFERENT CONCENTRATIONS OF CETRIMIDE AND AT DIFFERENT pH VALUES

Cetrимide (%)	pH 5	pH 6	pH 8	pH 9
0.1	81.88	74.84	70.08	64.8
0.25	79.00	78.90	75.81	71.80
0.5	84.37	85.17	84.37	82.93
1.0	84.97	83.71	85.13	84.65
0 (control)	79.28	75.16	72.40	65.92

After this work was concluded a number of auxiliary experiments were carried out to exclude ambiguities in the interpretation of results. In order to find whether the glucose was thrown down with the precipitate of protein, two tests were carried out in which the glucose estimation was performed on the whole homogenate without spinning. The means of four values at pH 5, 6, 8, 9 were 79.5, 72.0, 71.5 and 67.5 mg per cent respectively. Because this indicated that, contrary to the original concepts, the glucose was bound to soluble material in the

Table 1. GLUCOSE IN MG PER CENT ESTIMATED IN SUPERNATANT FLUID OF HOMOGENATE OF RAT INTESTINAL MUCOSA CONTAINING DIFFERENT CONCENTRATIONS OF DRUG

Mean reading of drug free controls was $75.18 \pm 3.9$ mg per cent		
	0.1%	1%
Cetrимide	80.13	87.22
Phloridzin	92.97	90.53
Sodium lauryl sulphate	78.73	86.59



homogenate, two experiments were carried out to test for uptake of glucose by the supernatant portion of the homogenate after spinning. The uptake was comparable since the reading gave a mean of about 73 mg per cent at pH 7.0. To exclude the possibility that the glucose was metabolized by surviving cells at room temperature, the glucose was estimated in a number of experiments immediately after addition. The readings obtained were 81, 74, 74, 71 mg per cent at the four pH values respectively, indicating that the bulk of the uptake occurred immediately. It was also found, in two experiments in which the glucose was estimated by the cyanide method<sup>7</sup>, that the readings did not differ from those of the control at any of the four pH levels. It became clear that the glucose which was present in the supernatant material all the time was split off by the more drastic cyanide method of estimation. In other words, the glucose-oxidase method was gentle enough to leave the binding intact, but even with this method this may only be relative and it is possible that the actual binding may be greater than is apparent from this method of estimation. In either case the question of conversion of glucose to other metabolites by surviving enzymes as a significant factor has been satisfactorily excluded.

Table 3. ANALYSIS OF VARIANCE, DOSE-RESPONSE LINES AND DIFFERENCE BETWEEN SLOPES WITH FIDUCIAL LIMITS FOR VARYING CONCENTRATIONS OF CETRIMIDE

	Control 0%	0.1%	Cetrimide 0.25%	0.5%	1.0%
Analysis of variance					
Source of variation					
Linear regression	$P \leq 0.001$	$P \leq 0.001$	$P < 0.005$	$P > 0.2$	$P \approx 0.2$
Deviation from regression	$P < 0.05$	$P > 0.2$	$P > 0.20$	$P \approx 0.1$	$P \approx 0.1$
Dose response line					
$x = \text{pH}$	$y = 93.27$	$y = 98.18$	$y = 90.41$	$y = 87.1$	$y = 85.97$
	$-2.9x$	$-3.8x$	$-2.1x$	$-0.38x$	$+0.125x$
Differences of slopes from control		$P > 0.2$	$P > 0.2$	$P < 0.001$	$P \leq 0.001$

To determine whether the extent of the binding was constant, experiments were carried out to measure the unbound glucose remaining after standing with homogenate for different periods of time. The homogenate, diluted as already mentioned, was mixed with glucose to give a final concentration of 100 mg per cent, and analysed after varying time intervals. The results of these experiments are shown in Fig. 2, and indicate that the binding

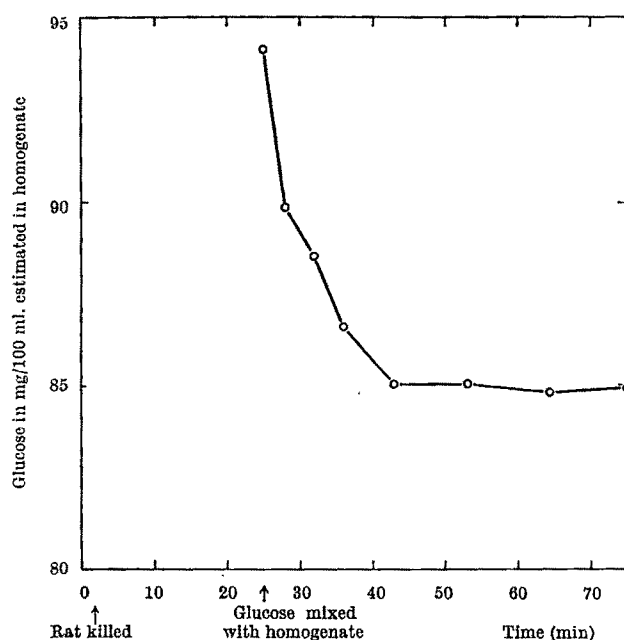


Fig. 2. Estimated concentration of glucose in the homogenate measured at intervals after the addition of the glucose.

reaction was complete after about 20 min. Indications had been given in previous work that the capacity for binding glucose increased with the length of time the homogenate had been stored after the death of the animal. After standing for varying intervals of time, mucosal homogenates were mixed with glucose. These samples were analysed after 1 h, to measure the total extent of binding. The results indicated that the extent of binding increased markedly at first, but became almost constant 3 h after the death of the animal. In all the previous experiments the mucosa had been tested approximately 5 h after death and the degree of binding possible was therefore considered to be constant at this point.

Further experiments indicated that the rate of this change was affected by temperature. Both the rate and extent of glucose binding were less when the homogenate was stored at 4° C than at room temperature, and conversely were greater when stored at 37° C. It was therefore necessary to ascertain whether or not glucose binding occurred in the homogenate at the time of death, or whether it resulted from denaturation of the constituents of the homogenate.

To facilitate rapid removal of the intestine, the rat was anaesthetized with pentobarbitone and the intestine washed out and cleared of fat before its death. It is known that pentobarbitone does not affect the binding or the analysis. The homogenate was then rapidly prepared and diluted, mixed with glucose and analysed after 3 min. The readings were repeated at intervals of 3 min. The results of nine such experiments are summarized in Fig. 3, which shows that within a few minutes of the death of the animal the homogenate is capable of binding glucose. The regression coefficient is  $0.197 \pm 0.035$ , giving the apparent intercept on the ordinate axis of  $90.78 \pm 0.70$ . The correlation-coefficient ( $r = 0.774$ ) indicates that  $P$  is less than  $10^{-3}$ . It therefore seems probable that glucose binding is an inherent property of the intestinal homogenate but that the capacity for binding increases after death.

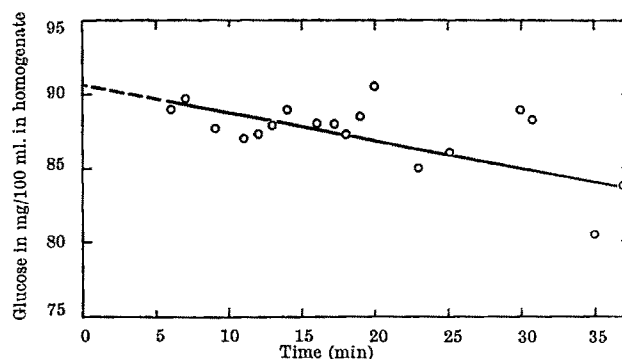


Fig. 3. Estimated concentration of glucose in the homogenate during the first 0.5 h after the rapid preparation of the homogenate.

Preliminary experiments have been carried out using homogenates obtained from sheep and pig intestines collected from a slaughterhouse. The results were qualitatively the same as those obtained with the rat. Preliminary experiments also indicate that glucose binding is increased after dialysis of the homogenate against distilled water, but the extent to which this is due to removal of electrolytes or to storage or denaturation of the protein requires further investigation.

We thank Dr. R. W. R. Baker for his assistance in the preparation of this paper.

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# Respiration and EEG Synchronization in the Frog

by

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The correlation between respiration and the rhythmic EEG waves seems to be a result of stimulation of the olfactory system by the inflow of air.

SINCE Adrian's original report of the induction of synchronous electroencephalograph (EEG) activity by olfactory stimulation in the hedgehog<sup>1</sup>, a similar phenomenon has been observed in other mammalian species<sup>2-5</sup>. In a series of acute experiments in the frog, Ottoson has made an analysis of the induced slow potential and rhythmic waves in the olfactory bulb and presented strong evidence that the latter is the result of postsynaptic discharge of secondary olfactory neurons in response to excitation of receptors in the olfactory mucosa<sup>6</sup>.

In the course of an investigation of EEG activity in relation to the behaviour of unanaesthetized, freely moving frogs, a striking correlation between EEG synchrony and respiratory rate was observed. It is the purpose of this paper to show that such synchrony is the result of olfactory stimulation by nasal air flow incidental to normal respiration and that it is responsible for much of the grossly visible variability of the surface EEG in the frog. Both EEG synchrony and respiratory rate are positively correlated with the amount of spontaneous and responsive movement of the frog, and so it is possible that they are both valid indices of "arousal" in this species.

Twelve bullfrogs (*Rana catesbeiana*) weighing between 100 and 200 g were investigated in two acute and ten chronic experiments. Bilateral section of the olfactory nerves was performed in two frogs by opening the canal in which they course from the nasal to the cranial cavity and cutting the nerves under direct vision. Brain stem section was accomplished in two frogs by enlarging the foramen magnum until the medulla, cerebellum, and optic tecta could be seen and the midbrain interrupted between the latter two structures. All chronic animals were implanted with electrodes and allowed to recover for 48 h before recording began. Acute experiments were performed under urethane, light ether or after high cervical spinal section and local anaesthesia with 2 per cent metacaine infiltration. Silver-silver chloride recording electrodes were placed on the olfactory bulb, cerebral hemisphere, and optic tectum under mineral oil and led to the electroencephalograph. Electrical stimulation of nervous tissue was effected through insulated stainless steel electrodes with a Grass SD-5 stimulator. Experimental stimulation of the olfactory mucosa consisted in both acute and chronic preparations of blowing puffs of room or odorized air from a tuberculin syringe through a polyethylene tube (inside diameter, 0.5 mm) inserted up to 3 mm within an external nare. Respiration was recorded as the movement artefact between alligator clip electrodes attached to the skin of the throat and flank.

Bilateral synchronization of the EEG followed each exchange of air across the nares. As shown in Fig. 1, the response followed the initiation of expiration by 0.25–0.50 sec, consisted of a more or less rhythmic fusiform train of waves, increasing in amplitude from 10 to 25  $\mu$ V, and decreasing in frequency from 12 to 8 cycles/sec early in the 2 sec of its duration. Background activity could be faster or slower but was always of lower amplitude and usually irregular or desynchronized. EEG activity

in general, and synchronous bursts in particular, were of greater amplitude between anterior and posterior than between left and right electrodes. In monopolar or bipolar left-right derivations, the activity was seen most prominently over the bulb and hemispheres, but was often also clearly present in the tectal leads; this relationship followed a trend in background activity from slow synchronized rhythms anteriorly to rapid and irregular ones posteriorly as has been previously reported<sup>7</sup>. The activity completely disappeared when the animal stopped breathing, as it did for long periods at rest, or when it submerged, and it could be prevented by blocking the nostrils mechanically with vaseline. On the other hand, when the respiratory rate was high, either spontaneously or in response to 5 per cent carbon dioxide, it became continuous.

Insufflation of room air, ether, propanol or butyrate, in varying concentrations and at varying rates, to either nostril produced a train of waves qualitatively similar to those following each spontaneous respiration (Fig. 2). The induced activity could be seen to interact with the spontaneous bursts on which it was superimposed but it had essentially the same form even if the background activity was flat when the animal was not breathing. The frequency was highest at the onset of a train and fell to a constant level as the amplitude peaked and began to wane. The amplitude and duration of the response were proportional to the strength and duration of air flow but no odour specificity could be demonstrated. Thus, rapid injection provoked a short high-frequency burst whereas prolonged injection was accompanied by a sustained low-frequency discharge. The waves were quite resistant to adaptation as indicated by their appearance constantly in the spontaneously breathing animal and their repeated evocation by artificial stimulation. Failure to obtain a response, or unsystematic variations in responsiveness, seemed to be related to movement of the tip of the tube within the nasal cavity where only the central area of the mucosa is excitable<sup>8</sup>. When the tube was pushed through

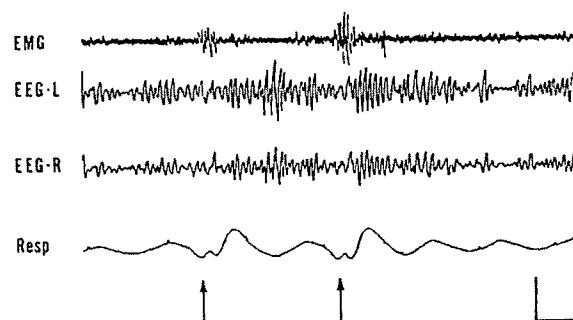


Fig. 1. Intensification of amplitude and frequency of rhythmic EEG activity after expiration. The EEG is recorded from both left and right antero-posterior derivations (middle two channels). Expiration, beginning at arrows, is reflected in a burst of fast potentials in the EMG (top channel) and by a brief deflexion in the pneumograph (bottom channel) caused by flank contraction which is followed by increased amplitude of the slow, regular oscillations that are a measure of inspiratory throat movements. Calibration 25  $\mu$ V, 1 sec.

the internal nare into the mouth there was no response, which showed that expansion of the nasopharynx and lungs *per se* was not an effective stimulus. Similarly, artificial external contraction of the abdominal wall did not induce rhythmic waves. Continuous and intermittent visual, auditory and cutaneous stimulation neither produced nor blocked the response. Thus passage of air across the olfactory mucosa would seem to be the necessary and sufficient ingredient of effective artificial and natural stimuli, and it is likely that the response depends on the same structures and mechanism in both cases. No odour specificity was apparent but tests were limited and not conclusive.

Bilateral olfactory deafferentation had no effects on respiration but completely eliminated spontaneously and artificially induced rhythmic EEG activity in chronic animals, which indicated that the response is absolutely dependent on a pathway between the mucosa and olfactory bulb in the first cranial nerves. As has been reported in the cat<sup>3</sup>, acute section of the brain stem and division of the forebrain between tectum and hemispheres and between hemispheres and bulb eliminated the activity behind the lesion but did not alter the response in the anterior structures. The response can be seen in the absence of neural connexions between the brain stem and forebrain, justifying the conclusion that it is not primarily dependent on centrifugal influences. Anaesthetic doses of 'Nembutal', ether and urethane usually eliminated respiration and always abolished the induced waves; the EEG was characterized by continuous low-voltage fast activity in these conditions. Local installation of 2 per cent metacaine and 1 per cent procaine to the nasal cavity suppressed but did not entirely eliminate the response. The medullary brain stem was stimulated with currents of 100–200 cycles/sec at 1–3 V at several points after preparation of the animal under light ether anaesthesia; the characteristic response was inhibition of respiration and desynchronization of the EEG. If the stimulation enhanced respiratory rate or amplitude, synchronous waves were seen. After section of the cord at C-1, EEG synchrony could no longer be produced by brain stem stimulation. At no time did stimulation of the brain stem *per se* provoke rhythmic EEG activity and it had no consistent effect on artificially induced rhythmicity.

Identity of the response seen in the chronic frog with that elicited in the olfactory bulb by Ottoson<sup>6</sup> using natural and electrical stimulation in acute animals can only be assumed, but the frequency, amplitude, waveform and certain stimulus-response relationships favour this assumption. Ottoson demonstrated that one component of the response, a slow potential, originated in the receptor and represented synaptic activity at the level of the bulb. This change was not seen in the experiments reported here, probably because of differences in recording technique. A second component, the rhythmic waves, was

sensitive to asphyxia and could be blocked by antidromic stimulation of secondary olfactory pathways suggesting that it originated in postsynaptic neuronal discharge. More direct evidence shows that the mitral cells of the bulb fire in synchrony with the induced waves in the rabbit<sup>8</sup>. Although the precise relationship between cellular events and the EEG is not yet known, it is generally assumed that EEG synchrony is a reflexion of simultaneous electrical changes in many neurons. In the frog it is possible that such synchrony is established by the excitation or inhibition of many interconnected cells of the bulb and transmitted through parallel chains of neurons through axodendritic connexions in the surface of the brain. Such connexions have been known to exist since the work of Herrick<sup>9</sup>, who also predicted, on anatomical grounds, the predominant influences of olfactory input on the activity of the hemispheres in amphibia.

Rhythmic waves have now been reported to arise in response to olfactory stimulation in the brains of humans<sup>5</sup>, monkeys<sup>4</sup>, dogs<sup>4</sup>, cats<sup>3</sup>, rabbits, and hedgehogs<sup>1</sup> among the mammals, and frogs<sup>6</sup> and toads<sup>10</sup> among the amphibia. No reports have been found of such activity in the intervening reptilian and avian classes. In an early investigation they were sought but not found in fish<sup>11</sup>.

The response would seem to be physiologically homologous in all species. After latencies of 0.2–0.4 sec, rhythmic bursts of 15–40 cycles/sec with amplitudes up to 1 mV have been recorded in the bulb, pyriform and prepyriform cortices, the olfactory striae, amygdala, and hypothalamus in various animals. The intensity and duration of the response vary with the intensity and duration of stimulation. It is always eliminated by deep anaesthesia, and local anaesthesia of the olfactory mucosa usually eliminates the response, but isolation of the rhinencephalic structures from the rest of the brain does not alter the response. Stimulation of the reticular formation has been found to enhance the amplitude of the response but not to produce it in the absence of respiration.

There is thus reason to believe that the induced waves recorded in mammals and those reported here in the frog may share the same physiological mechanism. An important difference between the mammalian and amphibian response is, however, indicated by the wide distribution of the evoked activity in the frog. This difference is most likely a reflexion of the exclusively "olfactory" constitution of the amphibian forebrain which consists of bulb, pallial hippocampus, pyriform, and dorsal areas, and on the kinds of connexions within this primitive neuropil. There is no true cortex, not to mention neocortex, hence homologues of the mammalian cerebral areas which show this response are all that exist in the forebrain of the frog. An important consequence of this difference is the correlation of EEG synchrony in the frog with behavioural states such as movement or "arousal" in which respiratory rates are high; by contrast, resting states of the animal are associated with a low-voltage, fast EEG<sup>12</sup>. This is an opposite relationship to that which holds between the neocortical EEG and mammalian behaviour.

I thank Dr. E. Henneman for advice and James Spelios for technical assistance. The work was supported by a grant from the U.S. Public Health Service.

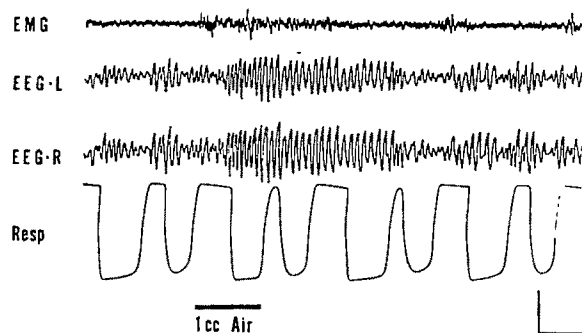


Fig. 2. Train of synchronous waves induced by injection of air into the nasal cavity. The channels are as in Fig. 1 and the time of the stimulus is approximately indicated by the solid black line under the tracings. Note that the background activity is synchronous in relation to a one-to-one throat-to-flank pattern of respiration and can be seen to interact with the evoked response. Calibration 25  $\mu$ V, 1 sec.

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# Senescence and the Fidelity of Protein Synthesis in *Drosophila*

by

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The possibility that ageing may be a consequence of the failure of cells to maintain accurate protein synthesis has prompted experiments in which the larvae of *Drosophila melanogaster* are treated with agents expected to induce errors in protein synthesis. Treatment of larvae with amino-acid analogues or with streptomycin does indeed decrease the life-span of adults.

ORGEL<sup>1</sup> has suggested that a failure to maintain accurate protein synthesis could cause cellular senescence. Although the genetic material provides the precise information required for the synthesis of proteins, it would be expected that occasional mistakes in the transcription and translation of this information would occur<sup>2</sup>. He argues that for the majority of enzymes the presence of a few defective molecules would result merely in a slight lowering of metabolic efficiency, but that this would not be so for that group of enzymes which are themselves necessary for transcription or translation (RNA-polymerase, aminoacyl-*t*-RNA synthetases and probably those involved in *t*-RNA synthesis). Here a defective molecule with a lowered specificity of action would cause further defective molecules to be produced. Orgel shows that although the initial frequency of errors in a cell may be low, in the absence of cell division and selection for the most rapidly growing cells there would be an exponential increase in the frequency of errors leading to an "error catastrophe" and the death of the cell. In differentiated organisms in which the rate of cell turnover is low, with increasing age it might be expected that more and more cells would be affected in this way. This could in the end lead to the death of the individual, although other causes of senescence may of course be operating at the same time.

Orgel points out that one way of testing the hypothesis would be to increase artificially the frequency of mistakes in protein synthesis by treating cells with a non-toxic amount of an amino-acid or RNA base analogue. A pulse treatment should initiate an incipient error catastrophe, the effects of which should be seen on the cell, or the organism, long after the analogue has been withdrawn. We have carried out this test with positive results with *Drosophila melanogaster*, which is particularly suitable for this type of experiment. The actively growing larvae will readily feed on supplements added to their medium. After metamorphosis into the adult there is little or no further cell division, apart from the germ cells, so there is no likelihood of selection of healthy cells to replace any dying from inaccurate protein synthesis. The life span of the adult fly is relatively short, and it is of course possible to use genetically homogeneous material. Amino-acid analogues were used rather than base analogues, because the latter could possibly get incorporated into DNA and cause somatic mutations. An effect on longevity by base analogue treatments could therefore be attributed to this, rather than to an effect on proteins; whereas it is scarcely likely that mutations could be induced by amino-acid analogues. We should point out that there is no direct evidence that amino-acid analogues do get incorporated into proteins in *Drosophila*, but from the results with other animal cells or micro-organisms it would be expected that they would do so<sup>3</sup>. Amino-acid analogues could lower the

specificity of enzymes simply by getting incorporated in place of the natural amino-acid, or alternatively, they could get incorporated into the position normally occupied by some other amino-acid, if the appropriate activating enzyme for that amino-acid sometimes used the analogue as substrate. We have also used streptomycin, because there is now good evidence that by acting on the ribosomes this substance can induce mistakes in protein synthesis in both resistant and sensitive cells of *Escherichia coli* as well as in the *in vitro* protein synthesizing system<sup>4</sup>. It has not been possible to obtain comparable results with the *in vitro* system from mammalian cells (Williamson, A., personal communication), although there is a report that streptomycin may induce misreading in the synthesis of antibodies<sup>5</sup>.

In the first experiments, *F*<sub>1</sub> larvae from a cross between Oregon (v), that is with vermilion eye marker, and a black body, purple eye stock both inbred for more than 125 generations were used. In later experiments the parental line Oregon (v) inbred was used. The mated females were allowed to lay for 24 h on standard molasses/oatmeal medium, so the larvae were only partly synchronized. Third instar larvae were used in all the experiments; they were washed and then transferred to small Petri dishes containing a semi-synthetic medium essentially similar to the one developed by Prof. J. Crow, University of Wisconsin (5.4 per cent sucrose; 3.2 per cent dried yeast; 0.2 per cent 'Nipagin M' (methyl-*p*-hydroxybenzoate); 0.75 per cent agar) and either supplemented with analogues or streptomycin, or unsupplemented. Usually about 200 larvae were used for each treatment. The treatment was terminated by removing the larvae to tubes containing the same unsupplemented medium for pupation. When the pupae emerged the sexes were separated, and the males were kept in batches of ten flies in containers consisting of a 3 in. × 1 in. specimen tube containing about 5 ml. of a yeast extract, peptone medium (1 per cent yeast extract; 2 per cent bacto-peptone; 5 per cent sucrose; 0.2 per cent 'Nipagin M'; 1.5 per cent agar) which was attached by a short piece of 1 in. rubber tubing to a 4 in. length of 1 in. glass tubing plugged at the end with cotton wool. (This arrangement made it possible to provide fresh medium and remove dead flies without etherizing or manipulating the flies.) The tubes were incubated in the dark at 27° C and the flies were scored daily. Females were not used because there is good reason to believe that they are less good material for this kind of experiment than males. It has been shown that treatments which interfere with or prevent egg production (as might any effect on protein synthesis) have the effect of increasing the life span in *Drosophila*<sup>6</sup>.

Five amino-acid analogues were used. These were (natural amino-acid in brackets): canavanine (arginine), ethionine (methionine), *p*-fluorophenylalanine and β-2-

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thienylalanine (phenylalanine) and 4-methyl tryptophan (tryptophan). The first four of these are known to become incorporated into the protein of micro-organisms or animal cells<sup>3</sup>. In initial treatments it was found that none of the analogues had an effect on normal larval development at concentrations of  $10^{-3}$  moles/l. and only canavanine was noticeably toxic at  $10^{-2}$  moles/l. In all the longevity experiments the analogues were included in the medium each at a concentration of  $10^{-3}$  or  $2 \times 10^{-3}$  moles/l. (or twice these values for DL mixtures); either all the analogues were used, or 4-methyl tryptophan was omitted. The higher concentration of analogues did in fact have the effect of slowing down development in the longer treatment, but there was no detectable effect on larval feeding, pupation or on the phenotype of the adults. The streptomycin was included in the medium at a concentration of 1 mg/ml. Even a level five times higher than this had no effect on the larvae.

In the untreated populations a survival curve characteristic of a population showing senescence was always obtained. The curve consists of a long shoulder with very few deaths followed by a steep fall in the number of viable flies. As expected, the longevity of the  $F_1$  was greater than that of the inbred line, although the shape of the curve was much the same. It would appear that the conditions used allow a normal life span followed by senescence. In several experiments, treatment with either concentration of analogues was for 4, 8 or 24 h, using either  $F_1$  or inbred larvae. The general shape of the survival curve was similar to the control, but there were usually more early scattered deaths and the steep part of the curve was some days earlier than in the control. (The presence or absence of 4-methyl tryptophan made no difference to the result; as yet, however, we have no information concerning the separate or combined activity of the other four analogues). The survival curves for two of the experiments are given in Fig. 1. On the basis of the  $t$  test, the mean life spans of the treated populations in these experiments, as well as those in the other figures, are significantly lower than the controls ( $P < 0.02$ ).

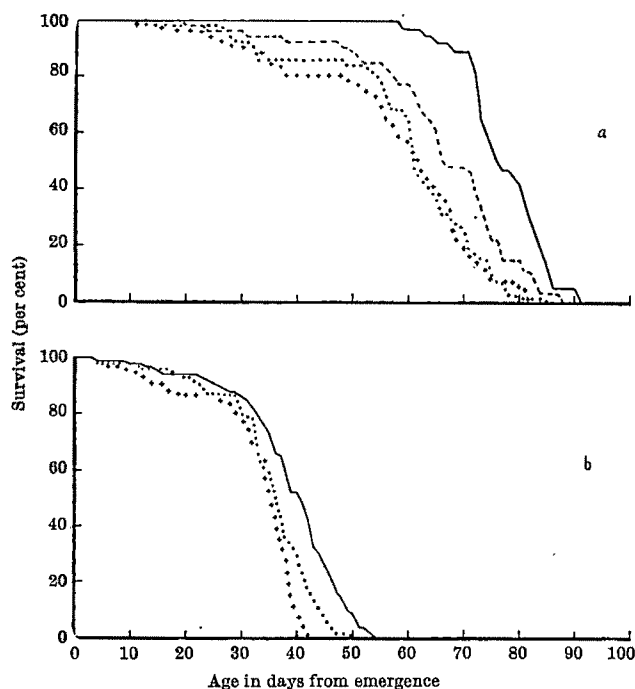


Fig. 1. *a*, Third instar  $F_1$  larvae (*b pr* inbred  $\times$  Oregon (*v*) inbred) treated for 4, 8 and 24 h with ethionine, *p*-fluorophenylalanine, canavanine,  $\beta$ -2-thienylalanine and 4-methyl tryptophan, each at  $10^{-3}$  moles/l. —, Control (38 flies); — — —, 4 h treatment (54 flies); . . . . . 8 h treatment (64 flies); + + + +, 24 h treatment (56 flies). *b*, Third instar Oregon (*v*) inbred larvae treated for 8 and 24 h with ethionine, *p*-fluorophenylalanine, canavanine and  $\beta$ -2-thienylalanine each at  $2 \times 10^{-3}$  moles/l. —, Control (123 flies); . . . . . 8 h treatment (82 flies); + + + +, 24 h treatment (66 flies).

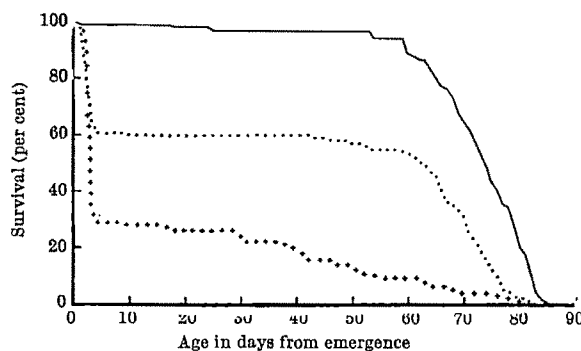


Fig. 2. Late third instar  $F_1$  larvae (*b pr* inbred  $\times$  Oregon (*v*) inbred) treated for 8 and 24 h with ethionine, *p*-fluorophenylalanine, canavanine,  $\beta$ -2-thienylalanine and 4-methyl tryptophan, each at  $2 \times 10^{-3}$  moles/l. —, Control (145 flies); . . . . . 8 h treatment (107 flies); + + + +, 24 h treatment (112 flies).

In one experiment we obtained a rather different result. In this the larvae were very near to pupation when they were put in the analogue mixture, and in fact some of them pupated during the treatment. The survival curves are shown in Fig. 2. It is evident that the treated population consists of two fractions: one which dies off 2–3 days after quite normal emergence, and the other which is much the same as those in the other experiments. The fraction of flies which died off quickly is greater (70 per cent) for the 24 h analogue treatment than for the 8 h treatment (40 per cent). The experiment suggests that there is a period immediately before the larvae pupate when incorporation of analogues has a very drastic effect on survival after metamorphosis. The life span of the non-sensitive fraction is affected in the same way as in all the other experiments.

We have found that treatment of larvae with streptomycin also causes premature death in the adult, but the extent of this effect varied from experiment to experiment. In general the  $F_1$  stock was more affected than the inbred one. An experiment showing an extreme effect and one showing an effect similar to that in most of the analogue experiments is shown in Fig. 3. In one experiment with the inbred line there was no detectable reduction in longevity.

It was essential to eliminate one possible trivial explanation of our results. Because all the media and tubes were kept sterile, apart from the medium on which the larvae were reared, it is possible that the analogues and streptomycin effectively sterilized the gut and the outer surface of the larvae, while the control larvae might remain infected. If a particular micro-organism enhanced the vigour of the adults, perhaps by producing an essential growth factor in the gut, then the sterile flies from treated larvae might be at a disadvantage and die earlier. We tested this by including some genetically marked flies (white eye and yellow body), reared in the usual way, with the flies from larvae treated with analogues or streptomycin. In this situation the experimental flies would be re-infected from the marked ones. It was found that the presence of these marked and naturally infected flies had no effect on the life span which was still materially reduced in comparison with flies from untreated larvae.

The finding that agents which might be expected to increase the normal frequency of errors in protein synthesis have the effect of shortening the life span does not of course prove that Orgel's hypothesis is correct, but it does confirm one of its main predictions. Other explanations are possibly applicable to our results. Clarke and Maynard Smith<sup>7</sup> have presented evidence that protein molecules in *Drosophila subobscura* have a long half-life, and it could be that if amino-acid analogues are present in the protein of young flies a substantial amount might remain in old flies. Assuming that this protein is defective in function, then these flies would have lessened vigour



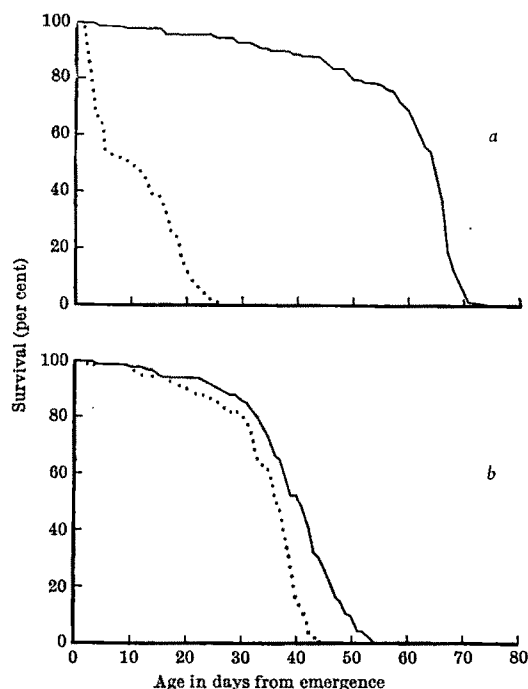


Fig. 3. *a*, Third instar *F<sub>1</sub>* larvae (*b pr* inbred × Oregon (*v*) inbred) treated for 24 h with 1 mg/ml. streptomycin sulphate. —, Control (108 flies); ·····, streptomycin treated (50 flies). *b*, Third instar Oregon (*v*) inbred larvae treated for 24 h with 1 mg/ml. streptomycin sulphate. —, Control (123 flies); ·····, streptomycin treated (107 flies).

and the normal ageing process might be accelerated. This could be easily tested by experiments with labelled analogues. Another possibility would be much more difficult to test. If death of the organism is due to a random accumulation of deaths in a given number of irreplaceable cells, and if the larval treatments were toxic enough to kill a proportion of these cells during metamorphosis or soon after, then the normal process of random death of the remaining cells would kill the animals pre-

maturely. The fact that streptomycin is active in accelerating senescence argues against both these possibilities. First, there is no evidence that streptomycin would be incorporated into biological molecules and it is therefore unlikely to persist in the adult for long periods. Second, streptomycin, unlike the analogues, seems to have no toxic effect on the whole larvae at very high concentration; it therefore seems unlikely that it would kill substantial numbers of cells at the much lower concentrations which were used in the longevity experiments. Another relevant point is the main finding of Clarke and Maynard Smith that the rate of turnover of protein in aged *Drosophila* was twice as high as in young adults, which result can be explained on the basis of Orgel's hypothesis. If some of the protein being synthesized in aged animals is defective, then it is quite likely that there would be control mechanisms which would compensate for the lowered enzyme activity by stimulating further synthesis, while much of the abnormal protein would be denatured and degraded.

In contrast to many other theories of ageing, Orgel's has the virtue that it clearly defines in biochemical terms a possible basis for cell senescence. For this reason it makes rather specific predictions which can be tested both at the biological and the chemical levels. So far, in preliminary experiments, the main biological prediction has been confirmed. As well as expanding the present investigations, it is now clearly necessary to test whether the biochemical basis of the induced senescence is also as predicted.

We thank Professor J. Maynard-Smith for valuable discussions on this subject.

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## Metabolism of Ribonucleic Acid during the Oestrous Cycle

by

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Variations in nuclear synthesis of RNA and turnover of RNA to the cytoplasm, as well as variation of the concentrations of RNA and protein in uterine cells during dioestrus and oestrus, appear to reflect changes in the rate of ribosome formation during the oestrous cycle.

It has been generally accepted that the cyclical nature of biological changes in the mammalian female urogenital system reflects responses to the variations in ovarian hormone titres. These titres are small during dioestrus or the luteal phase of the ovary and large during oestrus or the follicular ovarian phase<sup>1,2</sup>. Since the classical work of Long and Evans<sup>3</sup>, which described the oestrous cycle of the rat, this animal has proved to be a very convenient experimental organism for correlating fluctuations in endocrine activity with the responses of the target tissues, especially the interaction between oestrogen and the uterus.

Although the precise regulatory systems involved in the responses of uterine cells to fluctuations in the con-

centration of oestrogenic hormone remain unknown, work from many laboratories has now shown that oestrogen acts through the genetically controlled protein synthesizing machinery of the uterus<sup>4-7</sup>. In investigating the sequences of events during the early period of action of oestrogen on the uterus of the ovariectomized rat, we found that the acceleration of the rate of rapidly labelled nuclear RNA synthesis *in vivo* was the first response of the tissue to the hormone<sup>8</sup>. This response was also noticeable longer after hormone administration by a stimulation of DNA-dependent RNA polymerase activity in isolated nuclei, if the incubation was performed in the presence of magnesium ions only. There was only a slight and still later effect of hormone on the polymerase activity if the nuclei were activated with manganese ions and ammonium sulphate. It is significant that the RNA product of the

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polymerase assayed in the absence of the salt is of a ribosomal type, whereas it is more DNA-like in the presence of the salt<sup>9,10</sup>, and that most of the rapidly labelled nuclear RNA in the uterus, the synthesis of which is accelerated by oestrogen, is also predominantly ribosomal. The maximum acceleration of the rate of nuclear RNA synthesis *in vivo* was observed within 20 min of a single injection of oestrogen; this immediacy of effect prompted us to look at possible variations in nucleic acid metabolism of the uterus during the two most prominent phases of the oestrous cycle in the rat.

We wish to report our observations of changes in RNA metabolism that accompany those of size and activity of the uterus during dioestrus and oestrus in the normal rat. Our findings show that (a) the concentrations of DNA, RNA and protein in the whole tissue or its cytoplasmic fraction vary during the two phases; (b) there is no variation in the concentrations of nuclear RNA and protein; (c) the rates of nuclear RNA synthesis *in vivo* and the activity of RNA polymerase activated by magnesium ions in isolated nuclei were significantly greater at oestrus than at dioestrus; and (d) the activity of RNA polymerase assayed in the presence of manganese ions and ammonium sulphate remained constant during the two phases of the cycle.

Wistar rats weighing about 185 g and repeating their oestrous cycles at intervals of 4-8 days were used in all our work. The state of the oestrous cycle was determined routinely by microscopic examination of vaginal cell types<sup>11</sup>. Vaginal washes were collected, dried, fixed and stained with methylene blue and the proportion of vaginal epithelial cells of non-nucleated (squamous or cornified) and nucleated types and polymorphonuclear leucocytes was then determined. In this way, rats in dioestrus and oestrus, but not in pro-oestrus or metoestrus, states were selected. In some experiments, vaginal cell-type determinations were made twice daily for 1 week before selecting the animals, and in all experiments the uteri were divided into two groups according to their weight. As a rule, the larger uteri, that is, those weighing 500-800 mg, were obtained from rats in oestrus, and the smaller uteri of 95-190 mg from animals in dioestrus as determined by vaginal cell-type ratios. In all experiments, a final vaginal histological preparation was made at the time the animals were killed for removal of the uteri.

Nuclei were isolated from the uteri, within a few minutes of their removal, by a procedure described by us earlier<sup>8</sup>. According to this procedure, 30 per cent to 59 per cent of the DNA was recovered in the nuclear fraction. The rate of nuclear RNA synthesis *in vivo* was followed by measuring the incorporation of uridine labelled with tritium into nuclear RNA obtained at different time intervals after injection of the radioactive precursor<sup>8</sup>. The activity of DNA-dependent RNA polymerase was determined by measuring the incorporation of ATP labelled with carbon-14 into RNA during the incubation of nuclei with magnesium ions alone or with manganese ions and ammonium sulphate, as described elsewhere<sup>10</sup>. Chemical procedures for estimating DNA, RNA and protein have also been described previously<sup>8</sup>.

Fig. 1 shows schematically the very marked differences in the size of the uterus and in the vaginal cell types from animals in the oestrous or dioestrous phases of the cycle. These changes found in our animals agree well with the cyclical variations described in the rat by other workers<sup>1,11</sup>.

Accompanying the cyclical variations in uterine size and vaginal cell types were corresponding changes in total DNA, RNA and protein in each uterus, as shown in Fig. 2. Uterine concentrations of DNA during oestrus were 15 to 20 per cent higher than those in dioestrus, indicating an increase in uterine cell population during this phase of the oestrous cycle. The physiological significance of this variation in the number of uterine cells for the two phases of the uterine cycle here examined is unknown. RNA levels increased during oestrus from 100 per cent to 125 per

Table 1. RNA/DNA AND PROTEIN/DNA RATIOS FOR UTERINE NUCLEI AND WHOLE-TISSUE HOMOGENATES AT DIOESTRUS AND OESTRUS\*

Nuclei	Dioestrus		Oestrus	
	RNA/ DNA	Protein/ DNA	RNA/ DNA	Protein/ DNA
Experiment 1	0.26	3.44	3.26	3.82
2	0.22	3.16	3.22	3.33
3	0.20	3.19	3.23	3.88
Average	0.23	3.26	0.24	3.68
Whole tissue				
Experiment 1	0.53	4.16	0.71	27.1
2	0.41	5.64	0.62	25.1
3	0.43	4.78	0.79	21.7
Average	0.46	4.86	0.81	24.8

\* Each value is the result of analysis of uteri pooled from ten animals.

cent over the values obtained at the dioestrous state. That this rise is not entirely a result of increase in cell number, but rather an increase in cellular content of RNA, is demonstrated by RNA/DNA ratios in whole-tissue homogenates for dioestrus and oestrus (Table 1). The data of Fig. 2 and Table 1 also show that whereas total uterine RNA and protein/DNA at oestrus were respectively nearly double and triple that at dioestrus, the RNA and protein contents of nuclei from animals in the two states were essentially the same. The wet weight of the uterus varies even more than the content of the foregoing constituents, having increased during oestrus by 400 per cent to 500 per cent over the dioestrous state. At a qualitative level these findings parallel Drasher's investigation<sup>12</sup> of variation in nucleic acid content of mouse uteri during the oestrous cycle.

The rate of nuclear RNA synthesis *in vivo* during dioestrus and oestrus was estimated from the incorporation of tritiated uridine into RNA after short-term and long-term exposure of the animals to the radioisotope (Table 2). For a 10 min pulse period the uptake of radioactivity by the whole tissue was greater during oestrus than in dioestrus. The changes in the total amount of radioactivity recovered, however, were also accompanied by a change in the distribution of tritiated uridine between acid-soluble and acid-insoluble fractions. There was a

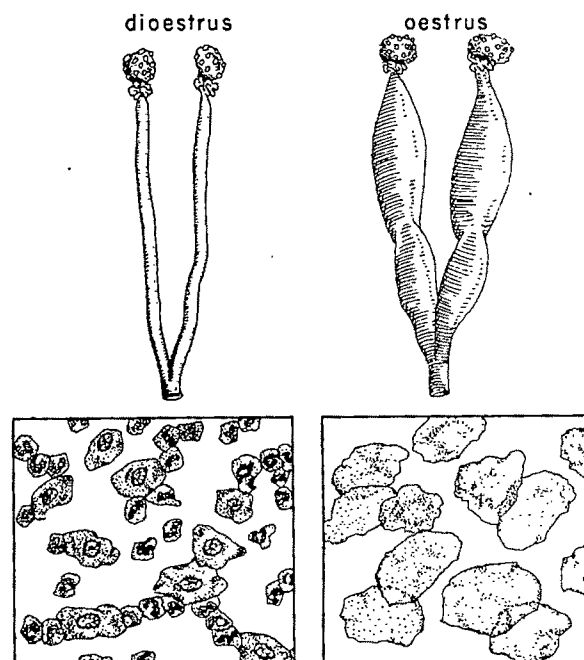


Fig. 1. Illustration of the dioestrous and oestrous phases of the oestrous cycle of the female rat. Uteri are shown with ovaries but without posterior portions of vaginas. Insets show types of vaginal epithelial cells histologically recognizable at the two stages of the oestrous cycle. At dioestrus, vaginal cell populations are of two types: relatively small, nucleated epithelial cells and polymorphonuclear leucocytes. At oestrus, the epithelial cells are larger and of a non-nuclear, squamous or cornified type, and the leucocytes are absent.

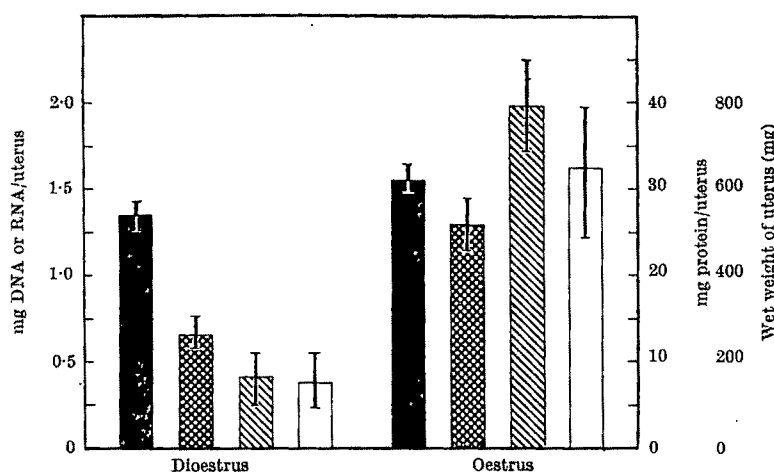


Fig. 2. Variations in the concentrations of DNA, RNA, and protein in the uterus at dioestrus and oestrus. Wet weights are based on blotted but unruptured uteri immediately after excising. Bars denote ranges for triplicate determination on organs pooled from ten animals. Black columns, DNA; cross-hatched columns, RNA; hatched columns, protein; and white columns, wet weight (mg).

drop in the fraction of acid-insoluble radioactivity at dioestrus and the differences between dioestrus and oestrous states in the total tissue uptake of radioactivity and the fraction of acid-soluble radioactivity became more marked with increasing time after administration of tritiated uridine. When RNA was extracted from the acid-insoluble material obtained at 10 min after labelling with tritiated uridine, nearly all of the acid-insoluble radioactivity was recovered as nuclear RNA, whereas labelling of cytoplasmic RNA was observed only with the longer isotope pulse periods of 2 and 4 h.

The specific activities of nuclear RNA at 10 min to 4 h after administration of tritiated uridine for uteri in both the dioestrus and oestrous states (Fig. 3) are compatible with a turnover of RNA from the nucleus to the cytoplasm. These values were higher in oestrus than dioestrus for all intervals of exposure to the isotope, the differences being most pronounced at the earliest intervals. These results are interesting in view of previous findings from investigations of the synthesis and accumulation of cytoplasmic RNA in the uterus<sup>13,14</sup>. A significant increase in newly labelled cytoplasmic ribosomes of the uterus of the ovariectomized rat was observed 4 h after administration of oestrogen, after an earlier stimulation of nuclear RNA synthesis.

When the activities of the two DNA-dependent RNA polymerase reactions in isolated nuclei were examined, only the activity of the magnesium ion-activated RNA polymerase reaction was enhanced during oestrus, being 60 per cent to 70 per cent greater than in the dioestrus condition (see Fig. 4A). No difference between dioestrus and oestrus could be discerned when the nuclei were incubated in the presence of manganese ions and 0.4 molar ammonium sulphate. That the variation in the activity of the magnesium ion-stimulated RNA poly-

merase reaction is specific<sup>15</sup> for the uterus can be judged from the absence of any variation in hepatic nuclear RNA polymerase reactions during dioestrus and oestrus (Fig. 4B). A similar tissue specificity was observed in RNA polymerase activity when the growth of the ovariectomized rat uterus was stimulated by the administration of exogenous oestrogen<sup>8</sup>. Whether other tissues of the rat urogenital system which respond to oestrogen also exhibit the differential variation in RNA synthesis remains to be determined.

Our results show that cyclical variation in uterine cell mass and population is most markedly obvious both in the high content of cytoplasmic RNA and protein and in the nuclear capacity to synthesize RNA at oestrus which then declines, to possibly the lowest levels of the cycle, at dioestrus. The association between the two phases of the oestrous cycle on one hand and the rate of nuclear RNA synthesis *in vivo* or *in vitro* and in cytoplasmic levels of RNA and protein on the other is the physiological counterpart of experimental variations in uterine activity controlled by ovariectomy and exogenous oestrogen. It is interesting to note that the RNA polymerase activity of nuclei from normal rat uteri assayed in the presence of manganese ions and ammonium sulphate shows no response to fluctuations in titres of endogenous oestrogen, whereas, as already noted, nuclei from uteri of

Table 2. UPTAKE OF TRITIATED-URIDINE AND ITS DISTRIBUTION BETWEEN CYTOPLASMIC RNA AND ACID-SOLUBLE FRACTION OF UTERI FROM RATS AT DIOESTRUS AND OESTRUS

Oestrous cycle stage	Time after tritiated uridine (min)	Uptake of tritiated uridine (c.p.m./mg uterus)	Acid-soluble fraction (percentage of total tritium)	Specific activity of cytoplasmic RNA (c.p.m./mg.)
Dioestrus	10	220	68	0
	120	335	79	300
	240	596	86	1,824
Oestrus	10	375	54	0
	120	624	62	2,148
	240	890	72	4,212

Rats were killed at the different time intervals indicated after the intraperitoneal injection of 100  $\mu$ c. of tritiated uridine (1,000 mc./mmole). Uteri from three animals in dioestrus or oestrus were pooled and processed as described elsewhere<sup>8</sup>. Cytoplasmic RNA was obtained from the supernatant fraction left after the sedimentation of nuclei from tissue homogenates.

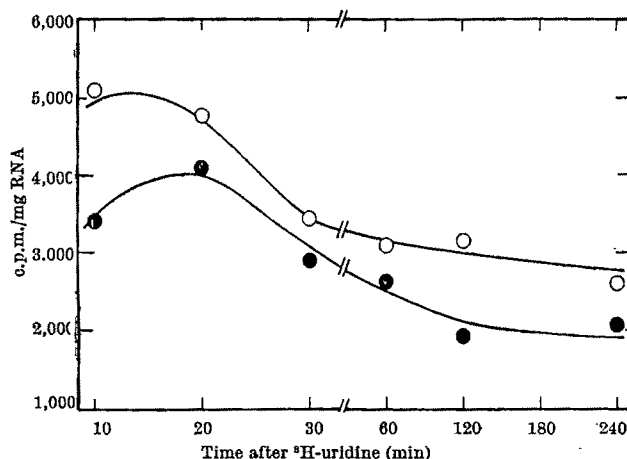


Fig. 3. Incorporation of tritiated uridine into nuclear RNA isolated from uteri of rats at dioestrus and oestrus. 100  $\mu$ c. of tritiated uridine was injected intraperitoneally at different times before killing. Uteri from three rats were pooled and nuclear RNA isolated as described elsewhere<sup>8</sup>.  $\circ$ , Oestrus;  $\bullet$ , dioestrus.

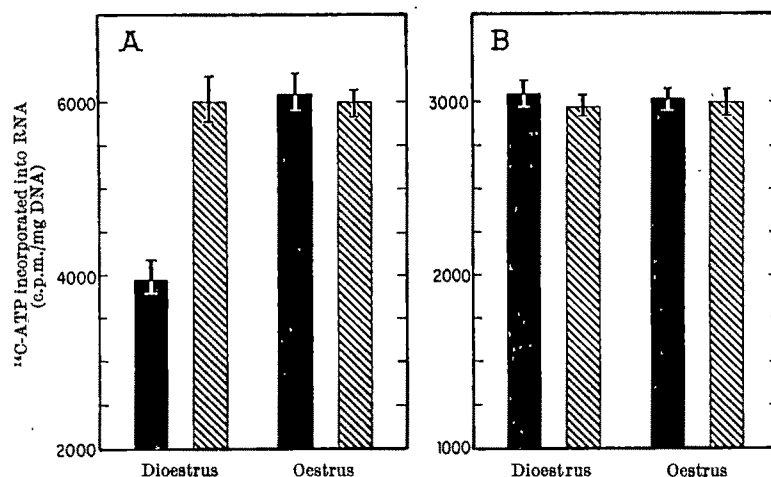


Fig. 4. Activities of the two DNA-dependent RNA polymerase reactions in nuclei isolated from the uterus (A) or the liver (B) of rats at dioestrus and oestrus. Nuclei were prepared from organs pooled from ten rats and the enzyme activities were quantitated from the incorporation of ATP labelled with carbon-14, according to methods described elsewhere<sup>10</sup>. Both the magnesium ion-activated and the manganese ion and ammonium sulphate-activated reactions were dependent on the presence of all four nucleoside triphosphates and inhibited by ribonuclease and actinomycin D. Bars denote variation ranges for three determinations for rat-uterus nuclei and two for rat-liver nuclei. Black columns, RNA polymerase activity assayed in the presence of magnesium ions; hatched columns, RNA polymerase activity assayed in the presence of manganese ions and 0.4 molar ammonium sulphate.

ovariectomized rats exhibit a slight but delayed response to administration of exogenous oestrogen when assayed for this polymerase reaction (ref. 8 and unpublished work of T. H. Hamilton, C. C. Widnell and J. R. Tata). The product of the RNA polymerase reaction activated by manganese ions and ammonium sulphate is a more DNA-like type of RNA and of that activated by magnesium ions at low ionic strength is of a ribosomal type<sup>8</sup>. Much of the rapidly labelled uterine RNA in oestrogen-stimulated or unstimulated organs<sup>3-7</sup>, in common with that of other mammalian tissue nuclei<sup>16</sup>, is also of the ribosomal type. It is, therefore, quite likely that the rate of ribosome formation may be the rate limiting step in the regulation of uterine size and growth. A similar effect on the rate of ribosome formation preceding an accelerated protein synthetic activity has been found to be a key feature of the action of other growth-promoting and developmental hormones in their target tissues<sup>17</sup>. A continuous generation of ribosomes or ribosomal precursors is thought to be essential for the transport of messenger RNA from the nucleus to the cytoplasm<sup>17-20</sup>, and it is quite possible that the variations in RNA metabolism we have observed largely reflect a modulation of this process of RNA transport in the uterus during cyclical fluctuations in titres of oestrogenic hormone.

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## Action of Drugs in the Central Nervous System

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Titration of a series of closely related  $\beta, \beta$ -dialkylglutarimide homologues with each other, and against a series of depressant drugs, provides evidence that stimulation and depression can be initiated at the same sites in the central nervous system.

THE mechanism by which a hypnotic drug produces sleep in the intact animal and an analeptic drug effects convulsions when given alone, or arousal from such sleep when given together with hypnotic has been the subject of much intense investigation. Many hypotheses have been proposed which attempt to explain these processes

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in terms of the physico-chemical properties of the drug and certain physiological or biochemical functions of the responsive neurones, but the basic mechanisms are still largely unknown<sup>1,2</sup>.

On the basis of quantitative titration and structure-action investigations with  $\beta, \beta$ -disubstituted glutarimide and related drugs, it has been proposed that certain

hypnotic and analeptic drugs produce their opposed effects by a physical mechanism at common sites in the central nervous system (CNS) of mice and higher animal species including man, and the action and interaction of these substances, and of related anticonvulsive and psychoactive drugs, have been described in terms of present day theories of drug action. Analeptics are thought to act as agonists, hypnotics as antagonists, and drugs which show dual stimulant and depressant central actions as partial agonists by affecting the liberation or action of a transmitter substance, or both, at excitatory synaptic sites which may form part of reticular neurones concerned in the maintenance of arousal (refs. 3-7 and our unpublished work). It would follow, then, that titration of CNS stimulant and depressant drugs which act at these common sites against a homologous series of  $\beta, \beta$ -disubstituted glutarimides which show agonist, partial agonist and antagonist action should give rise to a series of titration curves of predictable form. This was confirmed in titrating pentobarbitone sodium against each of four homologous glutarimides with agonist (methyl, ethyl), partial agonist (methyl, *n*-propyl), or antagonist (methyl, *n*-butyl; methyl, *n*-amyl) action<sup>6</sup>.

This article describes the titration of the same series of homologous glutarimides against two further depressant drugs, hydroxydione (21-hydroxypregnane-3,20-dione sodium succinate), which is a sterol of high hypnotic potency and structurally unrelated to pentobarbitone sodium, and trimethadione (3,3,5-trimethyl-2,4-diketo-oxazolidine), which has very low hypnotic potency and is only remotely related to this barbiturate in structure and provides further evidence that these two hypnotics also act at the same common central sites<sup>3</sup>. The glutarimide homologues have also been titrated against each other, for it is considered that a close similarity between the forms of the titration curves for this series of homologous drugs, which almost certainly act at common sites, and those of the other drugs described here would provide support for the thesis that all these drugs act and interact at the same sites which are capable of mediating the opposed effects of stimulation and depression.

The titration procedure has been described before<sup>6</sup>. All drugs were injected intraperitoneally in aqueous solution with the exception of the *n*-butyl and *n*-amyl glutarimides, which were administered as a 1 per cent tragacanth suspension. Loss of righting reflex or clonic convulsions in 50 per cent of a group of mice was the titration end-point. The titration curves and regression lines representing the stimulant, depressant or dual stimulant-depressant action of the foregoing glutarimides against each other and against hydroxydione and trimethadione are shown in Figs. 1-3 and Table 1. Regression lines

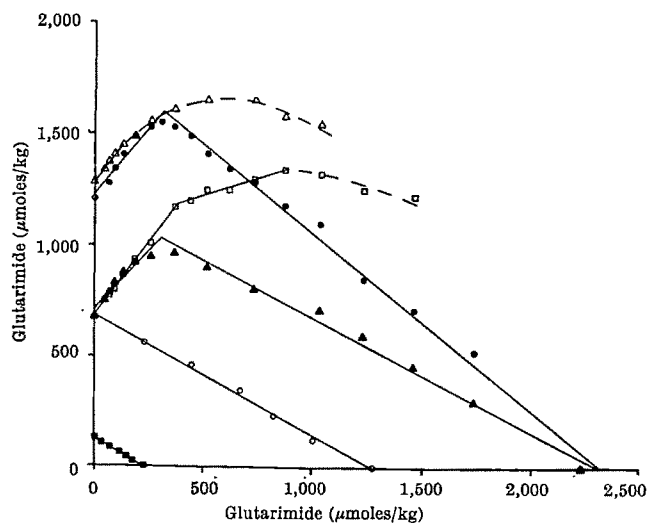


Fig. 1. Titration curves and least squares regression lines (solid lines) representing titration of  $\beta$ -methyl- $\beta$ -ethylglutarimide against  $\beta$ -methyl- $\beta$ -n-butylglutarimide ( $\Delta$ ) and  $\beta$ -methyl- $\beta$ -n-amylglutarimide ( $\square$ );  $\beta$ -methyl- $\beta$ -n-propylglutarimide against  $\beta$ -methyl- $\beta$ -n-amylglutarimide ( $\blacktriangle$ );  $\beta$ -methyl- $\beta$ -n-butylglutarimide against  $\beta$ -methyl- $\beta$ -n-amylglutarimide ( $\bullet$ );  $\beta$ -methyl- $\beta$ -n-butylglutarimide against  $\beta$ -methyl- $\beta$ -n-amylglutarimide ( $\circ$ ). The titration end-point is hypnosis (or clonic convulsions in the case of  $\beta$ -methyl- $\beta$ -n-propylglutarimide against  $\beta$ -methyl- $\beta$ -ethylglutarimide) in 50 per cent of a group of mice. Each titration point is the mean of at least two concordant estimations.

representing titrations with pentobarbitone sodium<sup>6</sup> are also included in Table 1 for comparison. Relative stimulant and depressant potencies are shown in Table 2. The combined stimulant action of bemegride ( $\beta$ -methyl- $\beta$ -ethylglutarimide) and its *n*-propyl homologue and the combined depressant action of each hypnotic pair shown in Table 1 are represented by regression lines (of statistically significant difference,  $P < 0.001$ ). Further, as in titrations against pentobarbitone sodium<sup>6</sup>, the curves representing titration of the agonist  $\beta$ -methyl- $\beta$ -ethylglutarimide or partial agonist  $\beta$ -methyl- $\beta$ -n-propylglutarimide by their *n*-butyl or *n*-amyl homologues or by hydroxydione or trimethadione have the form of hyperbolae, but approximate relative stimulant and depressant potencies have been calculated where possible by fitting the titration points in each case by two least squares regression lines ( $P < 0.001$ ) which intersect and are probably different in slope ( $P < 0.001$ ). For each bemegride curve, the lower regression lines were calculated using all titration points which gave a regression line and an intercept with the *y* axis which showed least deviation from the (0,  $HD_{50}$ ) titration point (not more than 1.7

Table 1. LEAST SQUARES REGRESSION LINES REPRESENTING TITRATIONS OF THE CNS DEPRESSANTS PENTOBARBITONE SODIUM (PB), HYDROXYDIONE (HYD),  $\beta$ -METHYL- $\beta$ -N-BUTYLGLUTARIMIDE (MBG),  $\beta$ -METHYL- $\beta$ -N-AMYLGLUTARIMIDE (MAG) AND TRIMETHADIONE (TRM), THE CNS STIMULANT BEMEGRIDE ( $\beta$ -METHYL- $\beta$ -ETHYL GLUTARIMIDE, MEG) AND  $\beta$ -METHYL- $\beta$ -N-PROPYLGLUTARIMIDE (MPG) WHICH HAS MIXED CNS STIMULANT-DEPRESSANT ACTION

Pair of drug titrants	Regression lines representing stimulant action of <i>x</i>	Intersection points ( <i>x</i> , <i>y</i> )	Regression lines
<i>x</i>	<i>y</i>	( $\mu$ moles/kg) for the	representing
		stimulant regression lines	depressant action of <i>x</i>
MEG/PB*	$y = 0.2149x + 110.7$	(420.7, 200.8)	—
MEG/HYD	$y = 0.1931x + 51.3$	(446.7, 109.0)	—
MEG/MAG	$y = 1.286x + 687$	(384.3, 1,181)	—
MEG/MBG	$y = 1.343x + 1,280$	—	—
MEG/TRM	$y = 17.090x + 8,081$	(371.9, 14,437)	—
MPG/PB*	$y = 0.1027x + 112.9$	(525.7, 166.9)	$y = -0.0898x + 214.1$
MPG/HYD	$y = 0.0538x + 53.1$	(515.4, 80.8)	$y = -0.0438x + 103.4$
MPG/MAG	$y = 1.052x + 709$	(304.5, 1,029)	$y = -0.5119x + 1,185$
MPG/MBG	$y = 1.166x + 1,227$	(315.1, 1,595)	$y = -0.7949x + 1,845$
MPG/TRM	$y = 9.453x + 7,950$	(473.4, 12,455)	$y = -6.852x + 15,699$
MBG/PB*	—	—	$y = -0.0835x + 108.0$
MBG/HYD	—	—	$y = -0.0412x + 50.4$
MBG/MAG	—	—	$y = -0.5409x + 688$
MBG/TRM	—	—	$y = -6.118x + 7,890$
MPG/MEG	$y = -0.5709x + 126.3$	—	—
HYD/PB	—	—	$y = -1.847x + 103.8$
MAG/PB*	—	—	$y = -0.1578x + 106.7$
TRM/PB	—	—	$y = -0.0126x + 97.8$
TRM/HYD	—	—	$y = -0.0070x + 7.723$

$P < 0.001$  in all cases. The titration end-point is hypnosis (or clonic convulsions in the case of MPG/MEG) in 50 per cent of a group of mice.

\* See ref. No. 6.

† Because of the curvature, a realistic regression line for the higher dose range was not calculable.



standard deviations). The intercepts of the positive slope regression lines of  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide were all within 2.58 standard deviations from (0,  $HD_{50}$ ).

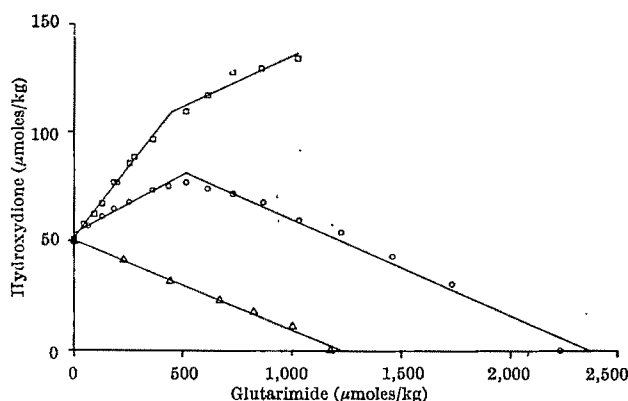


Fig. 2. Titration curves and least squares regression lines representing titration of  $\beta$ -methyl- $\beta$ -ethylglutarimide ( $\square$ ),  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide ( $\circ$ ) and  $\beta$ -methyl- $\beta$ -*n*-butylglutarimide ( $\Delta$ ) by hydroxydione. The titration end-point is hypnosis in 50 per cent of a group of mice. Each titration point is the mean of at least two concordant estimations.

The form of the titration curves of bemegride against the hypnotics, glutarimide, hydroxydione or trimethadione, is similar to that against pentobarbitone sodium<sup>6</sup> (Figs. 1-3); further, the calculated anaesthetic dose at the point of intersection of each pair of lines is similar (Table 1). It has been suggested<sup>3,6</sup> that the deflexion of a hypnotic-anaesthetic titration curve marks the commencement of virtual saturation by the titrants of the common responsive sites and that the hypnotic has the higher affinity for the sites when the deflexion is towards the anaesthetic axis. It is concluded on this basis that bemegride has a lower affinity than its hypnotic homologues, hydroxydione or trimethadione. The similarity of the anaesthetic doses in the region of deflexion of the various curves supports the suggestion that each hypnotic may be associated with about the same number of responsive sites in producing the  $HD_{50}$  end-point. Similar findings and conclusions can be drawn from the titration curves of the partial agonist  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide with hydroxydione or trimethadione. It should be noted, however, that, although the form of the curves for the hypnotic glutarimides is the same as that of the pentobarbitone sodium curve, the dose of the *n*-propylglutarimide at the point of intersection is less for the pairs of lines representing titration with the glutarimide hypnotics than that for those representing titration with the other three hypnotics (Table 1). We do not know why this is so.

The continuity and reversal in the slope of all the partial agonist titration curves (Figs. 1-3) provides, especially in the case of the homologous hypnotic glutarimides, good evidence for the suggestion that stimulation and depression are opposed responses initiated at common sites on responsive central neurones.

The reversal in slope obtained in titrating the highest doses of bemegride by its *n*-butyl or *n*-amyl homologue or by trimethadione (Figs. 1 and 3) requires special comment. This decrease in hypnotic requirement in the presence of very large doses of agonist is clear-cut, the  $HD_{50}$  titration end-point precise and readily reproducible, and the recovery of righting reflex rapid and uncomplicated, suggesting that the same basic mechanism is still operating at the neuronal level although in a modified fashion. This phenomenon has been observed previously in titrating a variety of hypnotics by large doses of anaesthetics<sup>8</sup>. It should be noted that in all such titrations the safe and clear-cut phase of decreased hypnotic requirement was invariably followed, as the dose of anaesthetic was still further increased, by one in which the mice showed persistent signs of stimulation in association with loss of righting reflex, followed later by death by respiratory depression, apparently of stimulatory origin. The nature of this fall is at present unknown, but it suggests that bemegride is not a pure agonist tending to show, when present in high doses, depressant action similar to that of its partial agonist homologue.

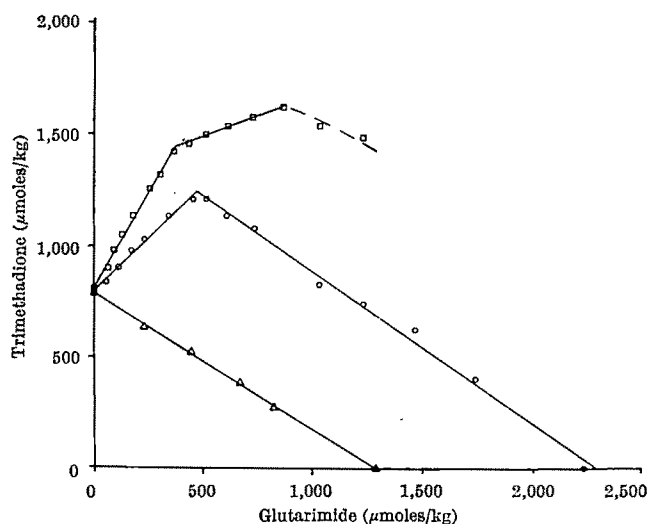


Fig. 3. Titration curves and least squares regression lines (solid lines) representing titration of  $\beta$ -methyl- $\beta$ -ethylglutarimide ( $\square$ ),  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide ( $\circ$ ) and  $\beta$ -methyl- $\beta$ -*n*-butylglutarimide ( $\Delta$ ) by trimethadione. The titration end-point is hypnosis in 50 per cent of a group of mice. Each titration point is the mean of at least two concordant estimations.

Examination of the appropriate regression lines (Table 1) shows that there is a close relationship between the ability of low doses of the partial agonist  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide to enhance the convulsant action of the agonist bemegride and the ability of the antagonist  $\beta$ -methyl- $\beta$ -*n*-butylglutarimide to increase the depressant action of its *n*-amyl homologue. The addition of one methylene group to the  $\beta$ -ethyl side chain of bemegride or to the  $\beta$ -*n*-butyl

Table 2. RELATIVE POTENCIES OF PENTOBARBITONE SODIUM (PB), HYDROXYDIONE (HYD), TRIMETHADIONE (TRM),  $\beta$ -METHYL- $\beta$ -*n*-AMYLGLUTARIMIDE (MAG),  $\beta$ -METHYL- $\beta$ -*n*-BUTYLGLUTARIMIDE (MBG),  $\beta$ -METHYL- $\beta$ -*n*-PROPYLGLUTARIMIDE (MPG) AND BEMEGRIDE ( $\beta$ -METHYL- $\beta$ -ETHYLGLUTARIMIDE, MEG)

Depressant	Depressant potency relative to pentobarbitone sodium						
	From titration by MEG	From titration by MPG stimulant action	From titration by MPG depressant action	From titration by MBG	From titration by PB	From titration by TRM	From $HD_{50}$ values
HYD	1.7	1.9	2.1	2.0	1.8	1.8	2.2
MAG	0.16	0.10	0.18	0.15	0.16	—	0.16
MBG	0.16	0.09	0.11	—	0.08	0.08	0.09
MPG	—	—	—	0.11	0.09	0.09	0.05
TRM	0.013	0.011	0.013	0.014	0.013	—	0.014
Stimulant	Stimulant potency relative to bemegride						
	From titration by HYD	From titration by PB	From titration by TRM	From titration by MAG	From titration by MBG	From titration by MEG	From $CD_{50}$ values
MPG	0.42	0.48	0.55	0.82	0.87	0.57	0.56

Relative potencies were calculated from lower stimulant or depressant regression lines shown in Table 1.

side chain of  $\beta$ -methyl- $\beta$ -*n*-butylglutarimide appears to decrease the stimulant potency of the former drug and increase the depressant potency of the latter by a factor of about two in each case. These results agree with those obtained for titration of the glutarimide homologues against pentobarbitone sodium, hydroxydione and trimethadione (Table 2) and with the ratio of the  $CD_{50}$  values of the two stimulant glutarimides (1.8) or of the  $HD_{50}$  values of the two depressant glutarimides (2.0) (ref. 6). The relative depressant potency of the *n*-amylglutarimide and its *n*-butyl homologue (1.6), calculated from the negative regression coefficients representing their titration by the *n*-propyl homologue, also agrees with the foregoing estimates. The *n*-amyl and *n*-butyl homologues, however, are about equipotent in the positive slope region of the curves representing their titration by the *n*-propylglutarimide and also in the lower line region of the bemegride titration curves. Similarly, bemegride and the *n*-propylglutarimide seem to have about equal stimulant potency when titrated against both the *n*-butyl and *n*-amyl homologues. These exceptions may reflect in part the inadequacy of fitting regression lines to titration points which so obviously fall on a curve (Fig. 1); however, it seems more likely that other factors are important.

A comparison of the depressant activities of the hypnotics, from all relevant regression lines, shows that generally hydroxydione is twice as active as pentobarbitone sodium, which is in turn about 5–10 times more active than the glutarimide hypnotics and eighty times more active than trimethadione (Table 2). A similar two-fold difference in the depressant activities of hydroxydione and pentobarbitone sodium has been found in titrating these hypnotics against the anaesthetics,  $\beta$ -spirocyclopentaneglutarimide, pentylenetetrazol, diethadione and picrotoxin, again using the  $HD_{50}$  response of a group of mice as the end-point (our unpublished work).

With the exception of  $\beta$ -methyl- $\beta$ -*n*-amylglutarimide, the agreement in the magnitudes of the relative depressant activities of this series of glutarimide and non-glutarimide hypnotics, when calculated from the lower regression lines which represent the stimulant action of the partial agonist  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide or the negative slope regression lines representing its depressant action, provides support for the view that this series of drugs acts and interacts at the same central sites. This evidence is convincing when it is observed that the relative depressant activities of this series of hypnotics, calculated in this manner, are so similar to those calculated from the regression lines representing their titration by the agonist bemegride or antagonist  $\beta$ -methyl- $\beta$ -*n*-butylglutarimide, homologues to which the partial agonist  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide is intermediate both in structure and pharmacological action (Table 2).

The regression lines which represent the combined depressant action of  $\beta$ -methyl- $\beta$ -*n*-propylglutarimide and the hypnotics pentobarbitone sodium, hydroxydione,  $\beta$ -methyl- $\beta$ -*n*-amylglutarimide and trimethadione are parallel to those which represent combined depressant action of  $\beta$ -methyl- $\beta$ -*n*-butylglutarimide and each of these hypnotics (Table 1, Figs. 1–3); in other words, an equivalent increment of the *n*-propyl- and *n*-butylglutarimides in these circumstances produces an equivalent increase in depression. In terms of "rate theory" of drug action<sup>9</sup>, the parallelism suggests that the differences which exist between the rate constants of association ( $k_1$ ) and dissociation ( $k_2$ ) of the drug-site complexes and the relative penetrabilities to the responsive sites of these adjacent homologues are such that when the *n*-propylglutarimide, with the larger  $k_2$ , interacts in the presence of a limiting number of responsive sites, its depressant potency is equivalent to that of the *n*-butylglutarimide, which has a lower  $k_2$  and produces depression in conditions of freely available responsive sites. In producing equivalent depression, the *n*-propylglutarimide must involve a much

larger number of responsive sites than its *n*-butyl homologue because the  $k_2$  of the *n*-propylglutarimide is higher and a dosage of approximately 1,000  $\mu$ moles/kg is required to overcome its own stimulant action before depression, which shows as potentiation of hypnosis, is observed.

This quantitative investigation of the pharmacological interactions of pairs of closely related  $\beta$ , $\beta$ -dialkylglutarimide homologues with each other or with a series of structurally related or unrelated depressant drugs provides good evidence for the idea that the stimulant, depressant or dual stimulant-depressant actions produced by these drugs in the CNS of the mouse all arise at common sites.

Evidence has been presented here and elsewhere<sup>3,23</sup> from titrations using a large variety of CNS stimulant or depressant drugs with similar or widely divergent structures that the responsive sites lack specificity in the structural and polar requirements of drugs to which they will respond and it is now probable that the effects of a large variety of drugs which show predominantly stimulant or depressant action in low doses and the reverse type of action in high doses may all be mediated, although not exclusively, at these sites; the type of response produced is predominantly a reflection, in terms of "rate theory" of drug action<sup>9</sup>, of the relative drug concentrations presenting at the responsive sites and the relative magnitudes of the association and dissociation rate constants of the drug-site complexes<sup>3,6</sup>. Examples of drugs with dual stimulant and depressant action in the CNS have been reported widely among the glutarimides and related drugs<sup>3</sup>, volatile and local anaesthetics<sup>10–13</sup>, analgesics<sup>14,15</sup>, antihistaminics<sup>16,17</sup>, sterols<sup>18</sup>, and a variety of psychoactive drugs<sup>19,21</sup>. Titration of a number of such substances with the series of drugs described in this article are in progress and seem to support the proposition that such compounds show, in appropriate circumstances, the properties of partial agonists in the CNS; preliminary investigations relating to the apparently paradoxical actions<sup>1</sup> of 'Dilantin' and sodium 5-ethyl-5-(1,3-dimethylbutyl)barbiturate have been reported<sup>22</sup>.

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## LETTERS TO THE EDITOR

## PLANETARY SCIENCE

## Cosmic Ice Residuum associated with an Astrobleme?

MANY circular to approximately polygonal structures on the Earth's surface have been described by geologists<sup>1</sup> as astroblemes—the result of impact between the Earth and extraterrestrial bodies—although not all geologists<sup>2</sup> accept this view of their origin. Some astroblemes have the form of craters and are associated with fragments of meteoritic iron. Others, more deeply dissected, are represented by a circular patch of sedimentary rock that originally filled the crater. Still others<sup>3</sup>, which include most astroblemes greater than 10 km in diameter, have a central uplift in which the strata are highly fractured and may be overturned. Surrounding this uplift is an annulus of impact breccia.

The ring structure at Gosses Bluff, a prominent topographic feature 150 km west of Alice Springs in the Northern Territory, Australia, has been described by the author<sup>4</sup> as an astrobleme. The ring structure exhibits the essential features of large astroblemes; the physiographic prominence of Gosses Bluff comprises the central uplift. The margins of the structurally disturbed area are obscured by younger surficial deposits, but outcrops of breccia and subsurface seismic and gravity data<sup>4</sup> suggest that the structure is about 14 km in diameter.

During a recent seismic survey<sup>5</sup> of the region in which Gosses Bluff is situated, gas was observed to blow from nineteen shallow seismic shot holes situated in the vicinity of the Bluff. Their distribution is indicated in Table 1.

Distance (km radius) from centre of Gosses Bluff	Total number of holes	Number of holes that blew gas
7	48	6
10	119	8
13	206	11
16	318	13
24	612	18

The more distant holes tend to cluster. Given the known occurrence of petroleum gas in the Alice Springs region, these shallow occurrences can be interpreted conventionally as pockets of petroleum gas, perhaps related to structurally controlled migration paths. An alternative explanation may, however, be tenable. The gas was detected only after a charge had been fired. The duration of the gas blows varied from a few seconds to more than 18 h. The stratigraphic unit in which the gas occurs, the Pertnjara Formation, was deposited under fluvial conditions and is "tight", having little porosity or permeability. The Pertnjara Formation is normal neither as a source rock nor as a reservoir rock for natural gas. The mode of release of the gas indicates that the gas is not trapped in interconnected pore space in the manner normal for natural gases. Were it normally entrapped, the gas would have blown from the seismic shot holes before the charge had been exploded, because the drill bit would have penetrated the interconnected system of pores. Fracturing of the rock by explosion is evidently necessary to release the gas, suggesting that it is located in non-connected pores and fractures. This is somewhat surprising in that the strata in the vicinity of the Bluff are known to be strongly fractured because of their involvement in the periphery of the central uplift.

One of the problems associated with astroblemes having a central uplift is the apparent absence of any meteoritic material associated with them. It does not appear sufficient to invoke normal processes of erosion to remove all traces

of meteoritic iron from such structures. Although their surface form is invariably considerably modified, debris from the impact is preserved in the annulus of breccia surrounding the central uplift. If a meteorite were to be disseminated explosively on impact, one might expect fragments of it to be incorporated in the impact breccia which is formed largely by comminution of the country rock. In fact, no examples of meteoritic material such as nickel-iron are known to me from breccias associated with astroblemes having a central uplift.

It is of interest therefore to consider the possibility that, in the case of Gosses Bluff, the bolide may have been a small comet with a nucleus composed of cosmic ice. Explosive dissemination of the nucleus on impact might be expected to result in the incorporation of some of the cosmic ice in the impact breccia and the forceful injection of some of the cosmic ice, or vapour derived therefrom, together with air under high pressure, into the shattered rock within and around the impact site. Entrapment would occur on the settling of material following the explosion. Such may be the origin of the near-surface natural gas encountered in the seismic shot holes in the vicinity of Gosses Bluff.

The possibility of such an origin for the gas may be checked first by determining the probability of impact between the Earth and a comet, and second, by comparing the composition of the gases encountered with those described from the tails and coma of comets.

Öpik<sup>6</sup> has estimated the likely frequency of collisions between the Earth and comets of various sizes. By taking the diameter of the Gosses Bluff astrobleme as 14 km, using the graph derived by Innes<sup>7</sup>, and assuming the impact velocity to be 20 km/sec, and the density of the comet as 1.3 g/cm<sup>3</sup> (ref. 8), the mass and the diameter of the comet can be calculated as 0.5 km. The Gosses Bluff astrobleme is believed to have formed during the Mesozoic<sup>1</sup> and for the purposes of this calculation the age will be taken as  $130 \times 10^6$  yr. During this span of time Öpik's figures suggest that 100 collisions between the Earth and comets of the size calculated may have taken place. The Australian land mass occupies about 1.5 per cent of the Earth's surface, and should therefore have received at least one 0.5 km diameter comet during the last  $130 \times 10^6$  yr. The Gosses Bluff astrobleme could thus well be the result of impact by a comet.

Wurm<sup>8</sup> lists the non-metallic neutral molecules identified within the heads of comets as CN, C<sub>2</sub>, C<sub>3</sub>, NH, OH, NH<sub>2</sub>, CH and O, and the ionized molecules identified in the gas tails and the region of the head as CO<sup>+</sup>, N<sub>2</sub><sup>+</sup>, CO<sub>2</sub><sup>+</sup>, CH<sup>+</sup> and possibly OH<sup>+</sup>. Whipple<sup>9</sup> considers that ammonia, water and CH<sub>4</sub>.6H<sub>2</sub>O may also be present in the heads of comets. No reliable quantitative estimates of cometary compositions are available.

Comparison of these data with those from the natural gases is difficult. Because encounter with gas in such shallow holes was unexpected, adequate sampling equipment was not available. Samples were collected by holding polythene bottles, which are permeable to hydrocarbons, over the blowing hole and also by displacement of water. Also the gases collected may have been contaminated by products of the explosions.

Seven gas chromatographic analyses of samples from separate holes have kindly been provided by Magellan Petroleum (NT) Pty. Ltd. A typical analysis of a sample from within 7 km of the Bluff gave hydrogen=2.8 per cent, oxygen=11.2 per cent, nitrogen=78.5 per cent, carbon monoxide=2.6 per cent, carbon dioxide=4.2 per cent, methane=0.7 per cent, helium—not detected (<50 p.p.m.); higher hydrocarbons—not detected (<20 p.p.m. methane equivalent). A sample collected 10 km from the Bluff 18 h after the gas flow commenced gave oxygen=16.7 per cent, nitrogen=74.2 per cent, carbon dioxide=6.7 per cent, water=1.5 per cent, methane=0.85 per cent, ethane=420 p.p.m., propane=20 p.p.m., butane—present (<2 p.p.m.), higher hydrocarbons and

hydrogen sulphide—not detected (<1 p.p.m.). The samples from holes remote from the Bluff are depleted in oxygen and nitrogen relative to carbon monoxide, and carbon dioxide; for example, hydrogen = 6.4 per cent, oxygen = 5.35 per cent, nitrogen = 46.8 per cent, carbon monoxide = 14.7 per cent, carbon dioxide = 22.2 per cent, methane = 4.45 per cent.

Air is apparently an important component of these natural gases. The elements present in combined form are also present in comets, sometimes in similar compounds. The occurrence of carbon monoxide in a natural gas is most unusual, and might suggest a cometary origin. The nitrogen compounds present in comets, however, are absent from the natural gas.

Without entire uncontaminated samples and some knowledge of the volumetric composition of cometary cosmic ice and the transformations that it might undergo during entrapment, further comparisons cannot be made. The similarities appear sufficient to warrant retention, for the present, of the theory that a significant part of the gas encountered is cosmogenically derived.

*Note added in proof.* Some other occurrences of shallow natural gas have recently been reported from the Alice Springs region in close association with concealed anticlines and faults in an area remote from Gosses Bluff. Analyses are not available.

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## Surface Pressures in the Martian Highlands and Lowlands

CONFLICTING opinions have been expressed on the question of elevation of the Martian dark areas (maria) relative to the bright areas (deserts).

Wells, from his detailed survey of the Martian clouds<sup>1</sup>, has reported observations of stationary white clouds adjacent to (and presumably downwind from) the dark areas. Analysing this phenomenon, he expressed the opinion that the maria are elevated mountain ranges<sup>2</sup>. On the other hand, Miyamoto, mainly on the basis of his own observation of clouds at the Kwasan observatory correlated with observation by Dollfus, reached an opposite conclusion<sup>3</sup>: "the belt of deserts situated south of the maria belt and including Hellas and Argyre may be a highland belt" . . . and that "Dioscuria-Cydonia region may be a highland plateau or a mountain range". Thus Miyamoto supports the opinion of Tombaugh, that the deserts are elevated—an opinion largely based on a relative affinity for whitening and in part on radiometric measurements<sup>4</sup>.

Direct evidence concerning the relative elevation of the maria and the deserts has not been available<sup>5</sup>. Recently, measurements by more direct methods have been published, but the results do not yet appear conclusive.

As a result of analysis of *Mariner IV* data, Fjeldbo *et al.*<sup>6</sup> report that the immersion over desert Electris occurred at a highland relative to the emersion from occultation over Mare Acidalium. This result is based both on the pressure determination which is by about 70 per cent higher at emersion, and also on the occultation

radii measured from the Martian centre of mass. The radius at immersion (Electris) is calculated to be 3,384 km, and at emersion (Mare Acidalium) 3,380 km.

Sagan and Pollack<sup>7</sup>, on the other hand, point out that both at immersion and emersion the tangency point could lie in a bright area adjacent to a dark area. The measured pressures would in both cases be biased towards low values, if the two types of areas were characterized by differences in elevation.

From an analysis of radar returns at 12.5 cm, Sagan, Pollack and Goldstein<sup>8</sup> drew the conclusion that dark areas are elevated. They consider that the quasi-specular component, when displaced in longitude from a dark area, is more likely to originate on mountain slopes close to the subterrestrial point (which corresponds to the elevated maria hypothesis) than on the far slopes of depressions (which would be required if maria are depressions).

Sagan and Pollack<sup>7</sup> also mention the evidence of dust storms rising in the bright areas which, in some cases, were apparently deflected in their paths by major dark areas. Elevations on the Earth are cooler than the neighbouring lowlands, but an opposite situation could exist on Mars. The fact that maria temperatures reach a peak some 8° above the temperatures in the desert is explainable in terms of lower bolometric albedo<sup>9</sup>.

In view of the very recent spectroscopic determination of the Martian surface pressure<sup>9,10</sup>, if large altitude differences do exist, it is very likely that the surface pressures bracket the 6 mbar of the triple point of water, that is, pressure is higher than that in the lowlands and lower than in the highlands. Taking Tombaugh's figure of 20,000 ft. as a possible topographic difference<sup>4</sup>, and using a scale height of 9 km as determined by Kliore *et al.*<sup>11</sup> in the *Mariner IV* occultation experiment, a possible ratio of surface pressure in the lowlands to that in the highlands is obtained which is greater than two. Using a value of  $P_s$  of 8 mbar as determined by Spinrad *et al.*<sup>10</sup> for  $T_s = 200^\circ$  K as the median pressure, surface pressures can be visualized for the lowlands of  $P_L = 11$  mbar and for the highlands of  $P_H = 5$  mbar.

While the question of the diverging views of Wells and Sagan *et al.* on one hand and Miyamoto, Tombaugh, Fjeldbo *et al.* on the other is not yet definitely settled, the idea of the lowland maria with pressure greater by a factor of two than in the deserts offers an intriguing explanation within the framework of the "micro-hill" hypothesis for the Martian wave of darkening<sup>12</sup> of why the maria are subject to the seasonal darkening and the deserts are not.

The micro-hills are formed by the heaving action of moisture, when it freezes. Their creation is thus predicated on the absorption of moisture by the soil. This could conceivably occur at the lowest possible pressures mentioned for the Martian surface, below the pressure at the triple point of the  $H_2O$  phase diagram, by two separate mechanisms. One is the direct absorption by the soil of wind-carried water in the form of vapour. The second mechanism is the appearance of liquid water under an ice crystal, which is plausible in a small volume of a crevice not ventilated to the atmosphere. In other words, an ice crystal can be sublimating on top and melting from underneath. These two mechanisms, however, are not as likely to provide appreciable amounts of moisture to the soil as the mechanism of melting when ambient pressure is above the triple point of  $H_2O$ .

It is thus intriguing to attribute the association of the maria with the creation of micro-hills and the resulting darkening to the possible surface pressure difference of, say, 11 mbar in the maria as compared with 5 mbar in the deserts.

It should be made quite clear that standing water in the maria is quite unthinkable because the required water-vapour pressures cannot be maintained for any appreciable time. The  $H_2O$  liquid stage would be transitory even at 11 mbar, because the process of almost

instantaneous drying would be competing with the absorption by the soil.

If the absorption process does take up a large part of the vanishing ice crystal, the elimination of the whitening will progress more quickly in the maria than in the deserts—the specific heat of melting being much smaller than the heat of sublimation. The relative absence of whitening in the maria might thus be due not so much to the infrequency of depositions, but to the fact that they vanish more quickly. Especially if the ice crystals are formed in strong upward convection during the early afternoon and if deposition occurs on the still warm surface of the maria, the process of melting and vanishing can be quite fast.

The temporary invasion of the bright areas by the darkening process can be explained by the coincidence of  $H_2O$  deposition and a locally prevailing high pressure. According to circulation simulation by Loevy and Mintz (personal communication), variations in the surface pressure of the order of  $\pm 10$  per cent can occur. The temporary invasion by the darkening would thus be limited to regions of only the lowest deserts, that is, flatlands in the deserts which are only 1,000 or 2,000 ft. higher than the highest maria.

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### Comparison of Relative Radionuclide Ratios in Debris from the Third and the Fifth Chinese Nuclear Test Explosions

Of the five first Chinese nuclear test explosions, No. 3 and No. 5 detonated on May 9, 1966, and December 28, 1966, respectively, have been the most powerful. According to the U.S. Atomic Energy Commission<sup>1</sup> both explosions were in the lower end of the intermediate range, that is, they corresponded to a few hundred kilotons of TNT.

Because a weapon in the intermediate range might be a thermonuclear device, special interest has been focused on the nuclear debris from these two tests.

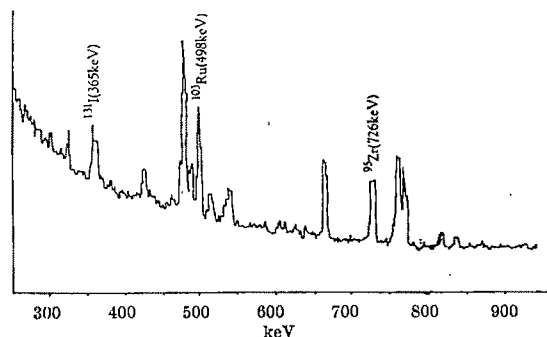


Fig. 1. Air filter collected June 6, 1966, measured June 10, 1966.

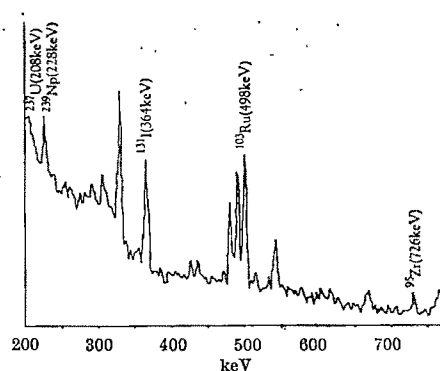


Fig. 2. Air filter collected January 19, 1967, measured January 24, 1967.

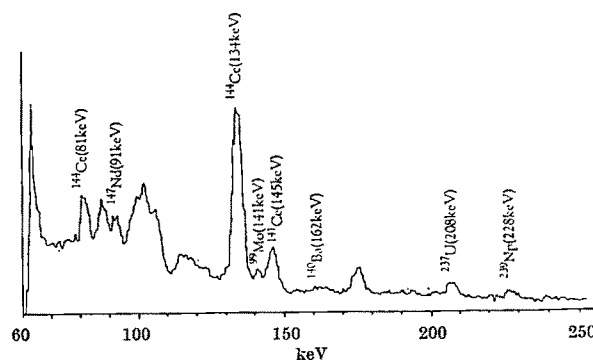


Fig. 3. Smear test collected May 23, 1966, measured May 26, 1966.

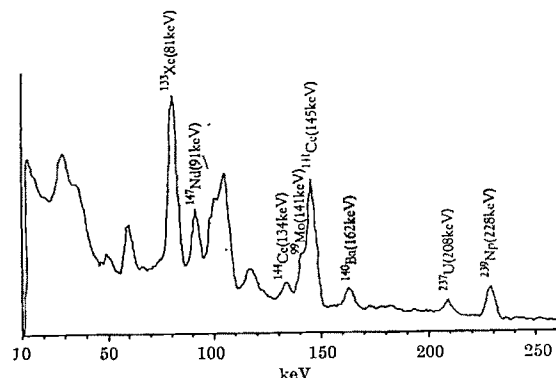


Fig. 4. Smear test collected January 9, 1967, measured January 15, 1967.

Two sets of samples were collected after each of the two test explosions: airfilter (A) and smear tests (B). The airfilters were collected at ground level at Risø. Each sample consisted of a glass fibre filter (Whatman GFA) which weighed approximately 16 g, and which in 2–3 days had been passed by approximately 40,000 m<sup>3</sup> air. The smear tests were obtained by wiping the front parts of the DC-8 aircraft with pieces of cottoncloth when the aeroplanes arrived at Copenhagen. Thus there was a considerable difference between the two sampling methods, and a difference in sampling location, altitude and time after the explosion at which the debris was collected.

Four samples were used in the present study: two air samples A<sub>1</sub> and A<sub>2</sub> and two smear tests B<sub>1</sub> and B<sub>2</sub>. A<sub>1</sub> was collected on June 6, 1966, and A<sub>2</sub> on January 19, 1967, B<sub>1</sub> on May 19, 1966, and B<sub>2</sub> on January 9, 1967. These samples were selected because they showed the highest levels within each sample group.

The use of a lithium drifted germanium detector<sup>2,3</sup> connected to a 256 channel TMC analyser has made it possible to measure a number of  $\gamma$ -emitting radionuclides in several samples in a relatively short time without lengthy radiochemical analyses. Figs. 1–4 show some



examples of the spectra found in the samples. Only the photo-peaks used in the following were indicated at the figures.

The measurements of the *A* samples were all referred to 32 days after the date of explosion and the *B* samples to 14 days after. Table 1 shows the counting rates for the different nuclides in the photopeaks at these reference dates.

Table 1

Nuclide	Photo-peak keV	Half-life days	$A_1$ c.p.m.	$A_2$ c.p.m.	$B_1$ c.p.m.	$B_2$ c.p.m.
Zirconium-95	726	65	1.03	0.21	—	—
Molybdenum-99	144	2.79	—	—	1.37	6.32
Ruthenium-103	498	40	1.32	1.09	—	—
Iodine-131	364	8.05	0.90	0.70	—	—
Barium-140	162	12.8	1.22	1.62	0.105	1.91
Cerium-141	145	32.5	24.55	12.75	1.56	9.16
Cerium-144	134	284	5.87	0.92	7.31	0.47
Neodymium-147	91	11.1	7.52	2.01	0.77	3.50
Uranium-237	208	6.75	2.02	0.29	0.72	1.63
Neptunium-239	228	2.35	0.16	0.13	0.78	6.64

From replicate measurements the relative standard deviation of a single determination was determined at 10–15 per cent.

To compare the debris from the two tests cerium-141 was used as a reference nuclide, partly because it was rather abundant in all samples, and thus showed a small measuring error, and partly because this nuclide takes up a mean position as regards volatility<sup>4</sup>, that is, the 141-chain is neither very volatile nor very refractory. Table 2 shows the ratios between the cerium-141 nuclide ratios for the two types of samples.

Table 2 does not reveal any significant difference between samples *A* and *B* as regards ratios between the fresh debris from the May and the December tests. The table shows, however, that barium-140, and also probably neptunium-239, iodine-131 and ruthenium-103, were relatively more abundant, as compared with cerium-141, in the debris from the December test than in the May test debris. Zirconium-95 and uranium-237, on the other hand, were relatively more abundant in the May debris than in the debris from December. In all samples from the fifth Chinese explosion (compare Fig. 4) a prominent 81 keV peak was present. The half-life of the peak was determined at approximately 5 days and after an incineration of the sample at 600° C in 5 h the peak completely disappeared. Thus the peak was attributed to xenon-133 with a half-life at 5.3 days. This nuclide was not found in any of the samples from the May explosion.

It is concluded that the debris from the fifth Chinese explosion in comparison with the third was enriched in nuclides belonging to volatile decay chains (neptunium-239, ruthenium-103, iodine-131 and barium-140) as compared with the less volatile (molybdenum-99, neodymium-147 and zirconium-95). It is further evident that uranium-237 was relatively more abundant in the third than in the fifth Chinese test. While the first observation could be attributed to fractionation phenomena<sup>5</sup> it is not possible to explain the uranium-237 difference in this way because neptunium-239 and uranium-237 do not fractionate from each other, as the precursor of neptunium-239 is 23.5 min uranium-239. The explanation for the higher uranium-237 content in the May debris might be some additional production of uranium-237 by some other process than a (2n)-capture in uranium-235, perhaps by a (n,2n)-capture in uranium-238. The difference could, however, undoubtedly also be explained by differences in the weapon design (J. H. Harley, personal communica-

tion). The occurrence of xenon-133 in the smear test as well as in the air samples from the December explosion is another indication of the relative abundance of volatile nuclides in the debris from this test. How this noble gas is captured and contained in the two sample types is, however, still a subject of controversy.

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### Search for Very High Frequency Radiation resulting from the Passage of Comet 1965f through the Solar Corona

ON October 21, 1965, a rare solar event occurred. The "Sun-grazing" Comet 1965f (Ikeya-Seki) passed through the solar corona at a perihelion distance of less than 500,000 km from the Sun's surface, or 1.66  $R_{\odot}$  from its centre, where  $R_{\odot}$  is the optical solar radius<sup>1</sup>. The rarity of such an event is shown by the fact that in the 23 yr from the discovery of radio emissions from the Sun in 1942 to the time of the present investigation, only two other known comets passed within 6  $R_{\odot}$  of the centre of the Sun. These were Comet du Toit (1945 VII), the perihelion distance of which on December 18, 1945, was 1.35  $R_{\odot}$ , and Comet Pereyra (1963 V), the perihelion distance of which on August 23, 1963, was 1.11  $R_{\odot}$  (ref. 2).

In view of this the Radio and Space Research Station's satellite telemetry reception equipment at Sembawang, Singapore (lat. 1° 25' N., long. 103° 49' E.), which operates at 136 Mc/s (2.2 m), was used to monitor solar radiation to find out whether or not the interaction of the comet with the region of the solar corona in which very high frequency radiation normally originates would produce any detectable increases. (The radio radius of the quiet Sun at 2.2 m is normally about 1.5  $R_{\odot}$ .)

The aerial system used consisted of a steerable array of eight Yagi aerials, which had a gain of 18 dB and with half-power beamwidths of 17° and 21° respectively in planes parallel and perpendicular to the individual elements. The output of the array was fed into a pre-amplifier at the base of the aerial tower and thence to a 136 Mc/s superheterodyne receiver. Because of the station's primary function as a satellite radio observatory it was not possible to obtain records of control data over long periods. The solar radiation was monitored continuously, however, from 02.50 to 04.40 U.T. on October 20 and from 03.05 to 04.20 U.T. on October 22. On the day on which the comet passed closest to the Sun (October 21) recording was carried out from 01.50 to 08.30 U.T., the time of perihelion being within about 2 min of 04.24 U.T.<sup>1</sup>. The comet was within 3  $R_{\odot}$  of the Sun for a period of about 1.5 h on either side of this time. Records were also made of the outputs of receivers tuned to 20 Mc/s and 40 Mc/s, connected to dipole aerials, in order to find the times when atmospherics or other local interfering noise were present.

Although a considerable number of atmospherics were recorded in these periods, on only one occasion was there any disturbance that could be attributed to the Sun. This was at 07 h 19 min 22 sec U.T. on October 21, when a 4 dB increase in signal level lasting 15 sec occurred (corresponding total peak flux density  $\approx 1.6 \times 10^{-21}$  W m<sup>-2</sup> (c/s)<sup>-1</sup>). By this time the comet had receded to a distance

Table 2

Ratio: <sup>141</sup> Ce/nuclide	$A_1$ ratio/ $A_2$ ratio	$B_1$ ratio/ $B_2$ ratio
<sup>141</sup> Ce/ <sup>239</sup> Np	1.6	1.4
<sup>141</sup> Ce/ <sup>237</sup> U	0.3	0.4
<sup>141</sup> Ce/ <sup>147</sup> Nd	0.5	0.8
<sup>141</sup> Ce/ <sup>140</sup> Ba	2.6	3.1
<sup>141</sup> Ce/ <sup>131</sup> I	1.5	—
<sup>141</sup> Ce/ <sup>103</sup> Ru	1.6	—
<sup>141</sup> Ce/ <sup>99</sup> Mo	—	0.8
<sup>141</sup> Ce/ <sup>95</sup> Zr	0.4	—

S.D.  $\sim$  30 per cent.

of  $4.7 R_0$ , and thus it seems unlikely that it could have resulted in the observed enhancement. We conclude that the interaction of the comet with the solar corona did not produce any significant enhancement of emission at 2.2 m wavelength.

This work was carried out as part of the programme of the Science Research Council Radio and Space Research Station. The observations were made by the staff of the Radio and Space Research Station at Singapore under the direction of A. Moorat and C. R. Carter. M. P. Candy, Director of the Comet Section of the British Astronomical Association, provided valuable information on cometary orbits.

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### Amino-acids in Recent Sediments off South-east Devon, England

RECENT results<sup>1-3</sup> on the amino-acid content of marine sediments have related to basin deposits; the diagenetic alteration of these may not be strictly comparable with analogous changes in shelf sediments.

A sequence of drowned freshwater, intertidal and shallow water marine sediments from off the coast of south-east Devon has recently been described<sup>4</sup>; these sediments were largely deposited in association with the Flandrian (Post-glacial) transgression. The total, combined<sup>5</sup> amino-acid content of some of these sediments has been determined by a method described elsewhere<sup>5</sup>. Accuracy was generally better than  $\pm 20$  per cent; some amino-acids such as proline could not be quantitatively

determined when encountered, but errors were minimal from such losses.

Work was largely confined to two gravity cores that were collected from latitude  $50^{\circ} 30' 30''$  N., longitude  $3^{\circ} 25' W.$ , at a depth of 75 ft. (23 m) below O.D. Decca position fixes showed that the two sample localities were within 600 ft. (183 m) of each other. The principal compositional features of the cores are shown on Fig. 1, which also indicates the area studied.

Core 1 contains a thin peat bed near the base (Fig. 1) that has been dated by radiocarbon analysis at

$$8,580 \pm 830 \\ - 755 \text{ years B.P. (NPL-86)}$$

This date confirms the results of pollen analyses of sediments in core 1, which indicate a zone VI b age for the peat and the overlying intertidal sediments<sup>4</sup>. The muddy sand overlying the intertidal mud in cores 1 and 2 was probably deposited in the sub-littoral zone after the inundation of the contemporary coastal region by the transgressive shoreline; the rate of rise of sea level at this time was about 5 ft./century. Assuming a contemporary mean tidal range of 10 ft. (an approximate mean of the present-day tidal range off Teignmouth) and a depositional zone of  $-5$  to  $-25$  ft. below mean sea level, then deposition of the muddy sand was probably completed within 500 years of the establishment of marine conditions at any point. The suggestion is that the muddy sand in cores 1 and 2 may be assigned an age of 7,500 years B.P., with a probable error either way of not more than 500 years.

Fig. 2 indicates the amino-acid content of the muddy sand in cores 1 and 2, and also shows the results of simple humic acid extractions carried out on core 2 (humic acid was dissolved in 0.3 normal sodium hydroxide solution and then precipitated by adjusting the pH to 1). Semi-quantitative analyses of the intertidal mud and the peat bed in core 1 indicate that these sediments contain about 3,500 p.p.m. total amino-acids, the following being present in both deposits: phenylalanine, lysine, arginine,

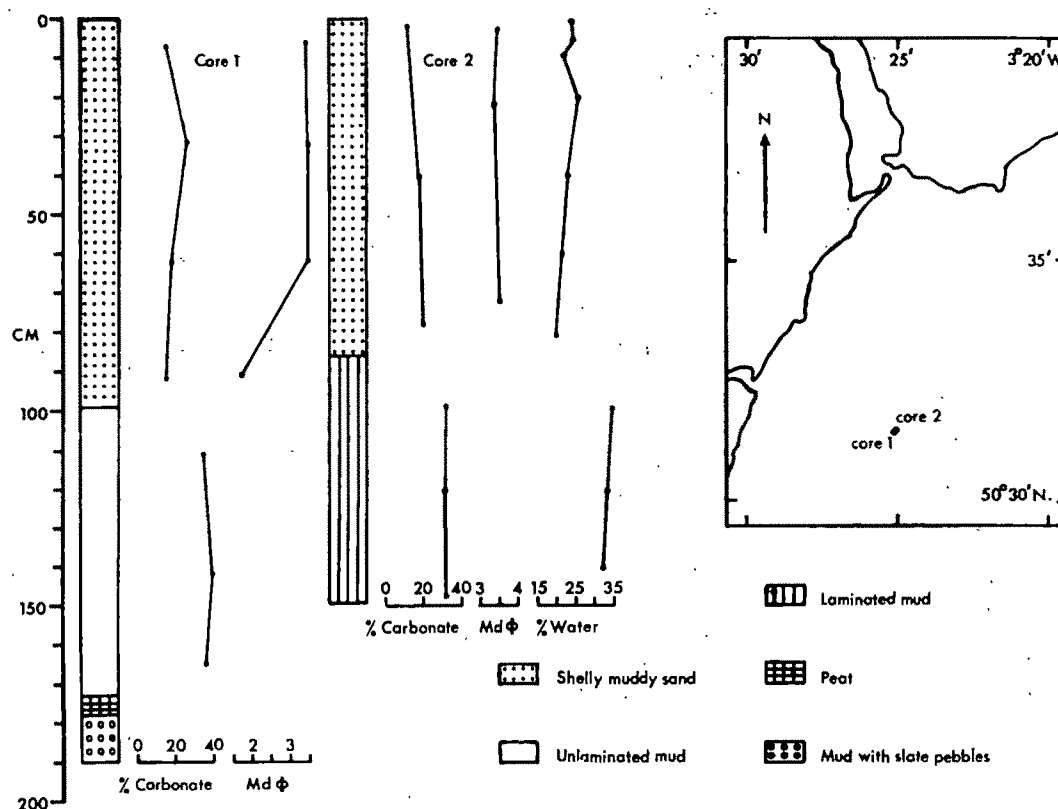


Fig. 1. Compositional characteristics of cores 1 and 2, together with a sketch map of the sampling locality. Water content of core 2 is expressed as a percentage of the wet weight of the sediment.  $Md\phi = \phi_{50}$  percentile where  $\phi = -\log$ , (diameter in mm).

glutamic and aspartic acids, glycine, serine, valine, alpha alanine, leucine, threonine and cysteine. Traces of proline were found in the mud sample (139–141 cm, core 1), and traces of tyrosine occur in the peat. All results are expressed as a proportion of the dry weight of the sediment.

Samples from core 2 were collected and analysed 1 week after the collection of the core; core 1 was analysed after storage in a darkroom for 6 months. Both cores were collected and stored in impervious fibreglass liner tubes which retained the interstitial water of the sediments. After a few weeks storage, microbial activity in the muddy sand led to the formation of black hydrotroilite in core 1. Black patches in some other muddy sand cores from the same area indicate that this reduction process is locally in operation on the sea-bed at present. Pyrite, evidently authigenic, fills some larger foraminiferal tests that are present in the mud and the muddy sand in cores 1 and 2. Pyrite formation in the muddy sand is largely confined to that part of the sea-bed that is underlain by considerable (more than 10 ft.) thicknesses of superficial sediment<sup>4</sup>, and is possibly associated with the expulsion of oxygen-depleted water from these (compacting) sediments. The lowermost increase in the abundance of amino-acids in the muddy sand in core 2 (Fig. 2) may also be associated with the expulsion of interstitial water (and amino-acids in solution) from the intertidal sediments beneath—this process has apparently been of negligible importance in core 1, where the intertidal sediments have a proven thickness of only 74 cm.

The amino-acid profiles for the two cores are similar (Fig. 2). The results for the top of core 2 may have been complicated somewhat by the presence of a *Turritella communis* Risso, and a sipunculid worm in the water-filled space above the core; these animals were alive and may have been feeding. The marked increase in the abundance of lysine in the upper few centimetres of both cores is possibly the result of microbial synthesis<sup>6</sup>. The

rapid fall-off of abundance with depth for both cores is probably not analogous to the superficially similar data for San Diego Trough sediments<sup>2</sup>, mainly because the muddy sand was deposited during a short period of time; depth in the muddy sand cannot be equated with age in the same way as it can in the San Diego Trough sediments.

Assuming a sub-littoral depositional environment for the muddy sand<sup>4</sup>, it might be expected that the net rate of deposition would be rapid initially and then decrease in proportion to the declining turbulence of the environment as the sea level rose and the shoreline migrated landwards. If another assumption can be accepted—that the supply of proteinaceous material (and humus) to the environment remained fairly constant during the deposition of the muddy sand—then it is clear that the amino-acid profiles can be explained on this basis. The apparent lack of humic acid below 10 cm in core 2 may also be a result of rapid deposition of the sand below this depth and a consequent dilution of the supply of humic material to values less than the detection limit of the extraction method (about 0.01 per cent).

If the amino-acid profiles in the muddy sand are a direct consequence of the mode of deposition, it is clear that diagenetic changes involving the organic constituents of the muddy sand have been slight since deposition ceased about 7,500 years ago. Mild reducing conditions, established within the sediments soon after deposition had ceased, probably assisted in the protection of the organic material (compare ref. 1).

The carbonate content of the muddy sand averages about 15 per cent (Fig. 1) and consists largely of whole or broken molluscan shells, together with a small contribution from microfungal tests. In order to test the hypothesis that the proteinaceous material in the muddy sand might be largely confined within the structure of the shell debris<sup>7</sup>, semi-quantitative analyses of some of this material were carried out.

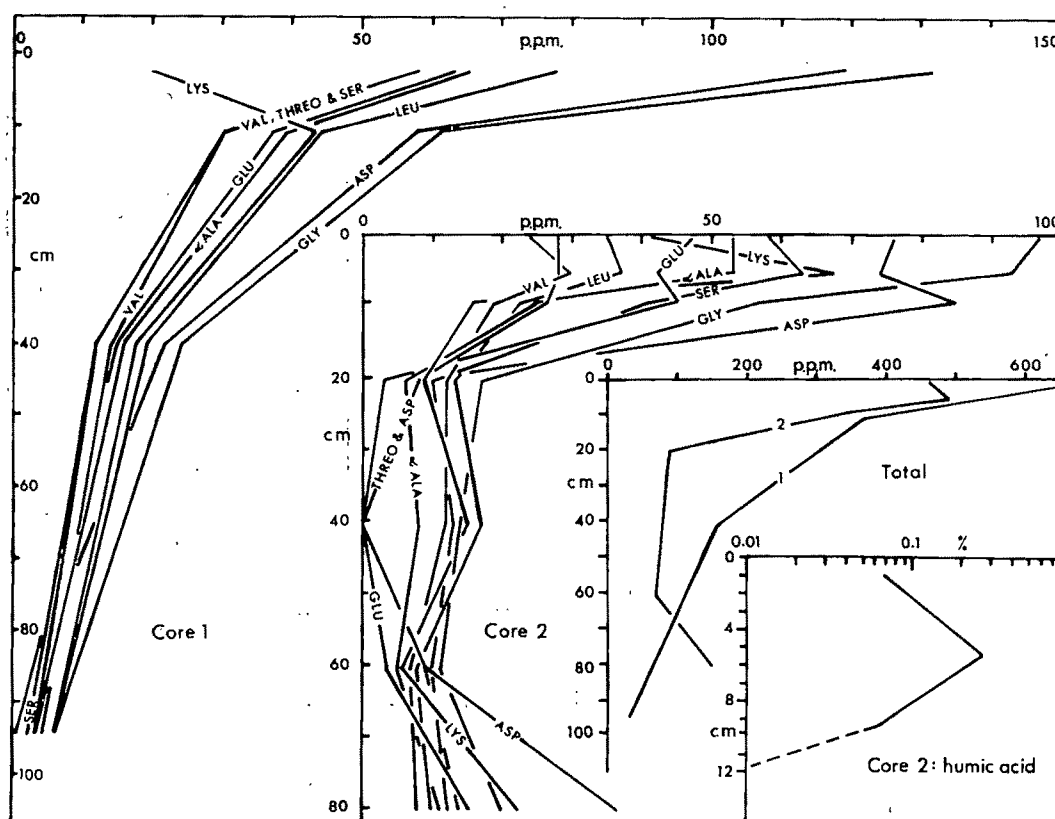


Fig. 2. Individual amino-acid content of cores 1 and 2 (abbreviations as in ref. 2) expressed as p.p.m. of the dry weight of the sediment. Also shown are the total abundance of amino-acids in cores 1 and 2, and the humic acid content of core 2.

An analysis of *Turritella communis* shells from a muddy sand core from latitude  $50^{\circ} 27' 30''$  N., longitude  $3^{\circ} 25'$  W., depth 90 ft. (27 m), revealed the presence of traces of cysteine, proline and tyrosine, and about  $10 \pm 5$  p.p.m. of the following: phenylalanine, lysine, arginine, glutamic and aspartic acids, glycine, serine, valine, alpha alanine and leucine, giving a total content of amino-acids of about  $80 \pm 30$  p.p.m. The absence of threonine is notable; otherwise the assemblage is similar to that in the bulk sediment samples. The abundance of amino-acids in these shells is too low to make a significant contribution to the bulk content of the muddy sand.

A bulk sample of foraminiferal tests was taken from several samples of muddy sand with carbon tetrachloride flotation. Material soluble in acid weighed 1.62 g; insoluble parts such as pyrite and sand were removed before the acid hydrolysis. The foraminiferal tests contained about  $250 \pm 50$  p.p.m. total amino-acids, the range in abundance being about 20 to 60 p.p.m. The following compounds were identified: glutamic and aspartic acids, glycine, serine, valine, alpha alanine, leucine and threonine. The restricted nature of this assemblage, despite the presence of many species of foraminiferids, is of interest, but clearly the abundance of amino-acids in the foraminiferal tests can only account for a small fraction of the bulk content of the muddy sand.

Having eliminated the shelly material as a possible source of the amino-acid content of the muddy sand, there remains the probability that a large proportion of the amino-acids (or their polymers) are attached to clay minerals, and possibly humic compounds, within the finer fractions of the sediment<sup>2</sup>.

This work was carried out in the Department of Geology at the University of Bristol under the supervision of the late Professor Whittard, and with the support of a research studentship from the Department of Scientific and Industrial Research.

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### Archaeomagnetic and Palaeomagnetic Study of the Magnetic Field of the Earth in the Past 600,000 Years

DIRECT observations provide information about the variation of the Earth's magnetic field only during the past 400 years, but these records can be considerably extended by archaeomagnetic and palaeomagnetic investigations. This article reports new information on variations of the Earth's magnetic field based on data gathered from three sources. These are (1) measurements of the thermoremanent magnetism of baked clay samples, which provide information about the past 6,800 years;

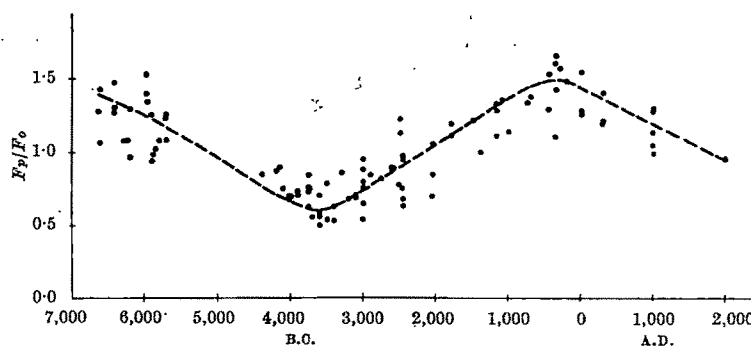


Fig. 1.

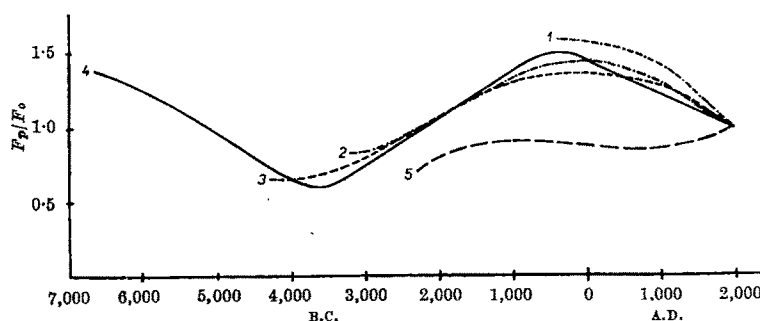


Fig. 2.

(2) measurement of magnetic declination and dip on loess samples from a defile of layers in South Moravia 18 m thick; (3) investigation of the magnetization of baked clays and porcelanites produced by the underground combustion of Tertiary coal deposits in North Bohemia which have acquired high thermoremanent magnetization during the Quaternary—that is, during the last 600,000 years.

Archaeological samples from Czechoslovakia<sup>1</sup> have been taken from baked clays, kilns and potteries for the interval from 4,400 B.C. up to the present time. The age of some has been determined archaeologically and the age of others by means of the carbon-14 method. Also, samples of baked clay (which date from 6,500 B.C. to 5,400 B.C.) from Catal Hüyük in Turkey have been measured in collaboration with A. A. Mellaart. Because the total Earth's magnetic field intensity was investigated by the double heating method it was possible to determine the ratio between the past and present Earth's magnetic field (coefficient  $k$ ), using heating and cooling curves of remanent magnetization<sup>2</sup>. The data from Turkey yield the coefficient  $k$  equal to 1.35 for the samples dated at 6,500 B.C.,  $k = 1.25$  for those at 6,000 B.C. and  $k = 1.15$  for those dated at 5,400 B.C. For the Neolithic samples from Czechoslovakia the values of  $k$  vary from 0.8 to 0.5 depending on the age in the time interval 4,400–3,500 B.C. During the Eneolit a gradual increase of the values  $k$  takes place which continues up to a maximum value of  $k = 1.5$  (400 B.C.). Then the field intensity decreases up to the present. It follows from the curve (Fig. 1) that the changes of the field have an almost periodical character; the length of the period amounts up to 3,500–4,000 yr and the amplitude changes from 0.5 (about 3,500 B.C.) to 1.5 (approximately zero) times the present field intensity.

Previously determined archaeomagnetic curves which represent the changes of the Earth's magnetic field intensity in the world are given in Fig. 2. The comparison shows good agreement among French (1) (ref. 2), Georgian (2-USSR)<sup>3</sup>, Japanese (3) (ref. 4) and Czechoslovak (4) curves. On this basis it would be possible to judge on the planetary character of the changes of the Earth's magnetic field. On the other hand the Indian curve (5) (ref. 5) exhibits a decrease of intensity going back in time; the

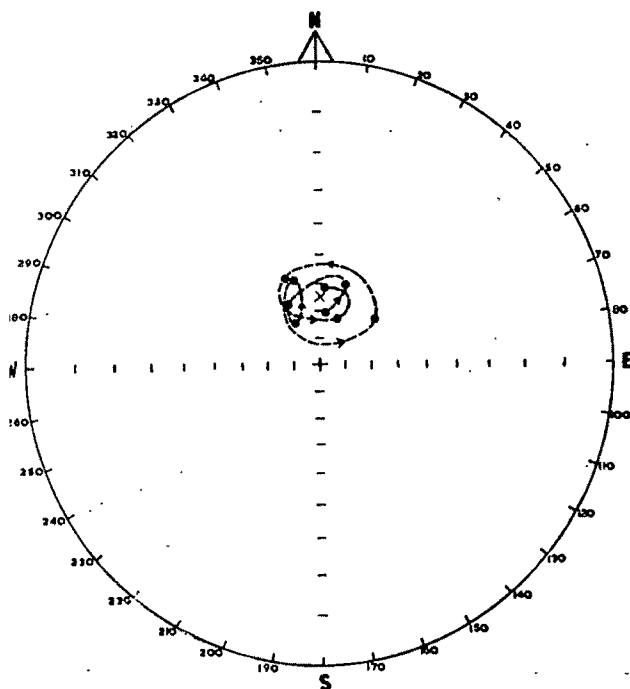


Fig. 3.

suppose that the direction of magnetization of individual layers represents the direction of the field that acted in the time of gradual accumulation of the layers. Besides brown coloured loess and fine grained orange sandstones layers of humus are also present and the magnetization of these is fairly high.

The values of  $D$  and  $I$  are plotted on a stereographic net (Fig. 3). The spiral character of the changes when we proceed from the oldest to the youngest samples is apparent. The spiral continues anti-clockwise, the declination changing in the range of  $-40^\circ$  to  $+53^\circ$  and the dip in the range of  $54^\circ$  to  $75^\circ$ . Naturally, this spiral cannot express quite fully the continuous changes of the Earth's field direction, but from its path (especially in the time interval 55,000–52,000 yr) it is possible to deduce that the changes in direction hardly exceeded the area bordered by the outside contour of the spiral. It is remarkable that the changes occur in the direction opposite to that supposed some years ago; the results concerning this anti-clockwise trend, however, agree with changes of the pole position that Japanese geophysicists have obtained for the period 600 to 1950 yr<sup>7</sup>. Our data may be supposed to characterize a longer temporal process (the secular variation is probably partially averaged in the samples). This means that the shorter temporal changes observed in the course of some hundreds of years at magnetic observatories would superpose the movement obtained by us on the loess. This also follows from the large diameter

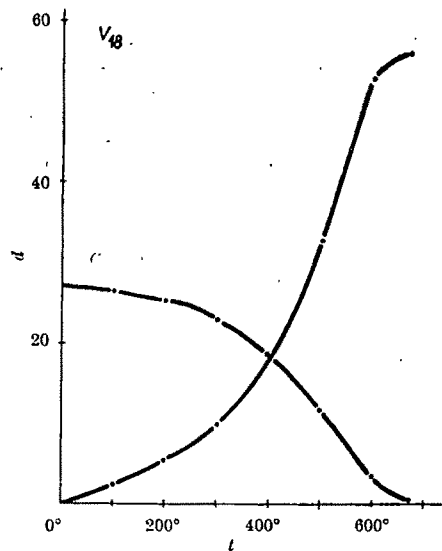
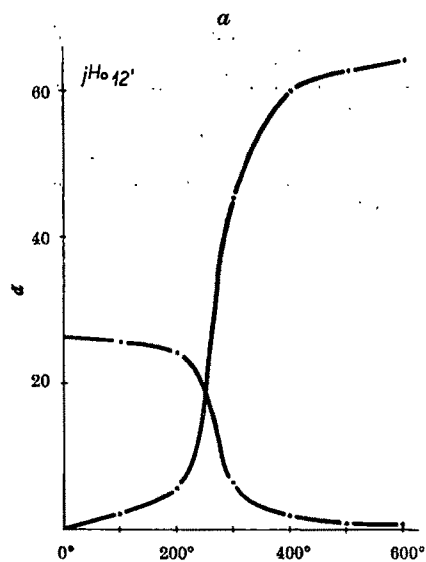


Fig. 4.

maximum about zero A.D. is not apparent. It is possible, therefore, to make a conclusion that the character of the field seems to be similar at the same geographical latitudes, while near the equator the sharper influence of the westward drift changes the course of the curve somewhat.

The magnetic declination and dip of loess deposits up to 60,000 yr. old have been investigated. A profile 18 m thick at Věstonice (South Moravia)<sup>6</sup> represents a basis for a gradual temporal dating of the last glacial series because of the possibility of its detailed stratigraphical division. The main horizons have been dated by means of radiocarbon and the layering was investigated palaeontologically. The age for five suitable horizons was determined to be 55,000; 52,000; 28,100; 18,600; and 14,800 yrs respectively. Cubic samples were taken from the layers and the directions of their remanent magnetization were determined. After measuring the samples the declinations and dips were calculated. Taking into consideration the fact that the rocks are very fine grained, we may



b

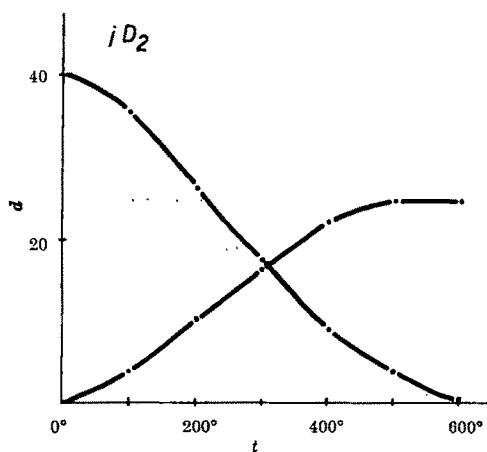


Fig. 5.



of the spiral compared with the observatory data, for example for London.

The determination of the total Earth's magnetic field vector for approximately 25,000 yr B.C. from double heating measurements of ten samples from baked clays and archaeological kilns from Věstonice made it possible to obtain the coefficient  $k=0.5$  which means that the field then had 0.5 times the present day field intensity. The cooling and heating curves for one representative sample are given in Fig. 4.

During the Pleistocene underground fires broke out in the North Bohemian Tertiary coal basin, the cause of which was spontaneous oxidation processes within the coal layers. It resulted in baking the rock cover, especially clays, sandstones, gravels, which were changed into new types of rocks (porcelanites, palaeoslugs). Consequently, the rocks were mineralogically stabilized and thermoremanent magnetization, in most cases with a high stability, was produced. This enables us to use these rock samples for the examination not only of the direction but also the intensity of the Earth's magnetic field at the time of the fires. The progressive double heating method has been used for thirty samples of porcelanites and palaeoslugs from eleven localities.

These rocks can be classified into approximately three groups—the lower, middle and upper Pleistocene. The paths of two typical heating and cooling curves are given in Fig. 5(a) ( $k=0.31$ ) and 5(b) ( $k=1.5$ ). The values of the Earth's magnetic field intensity at the time of the heating of the clays in the different localities ranged from 0.31 to 1.5 times the present day field intensity (see Table 1). For the two oldest groups the age of which is supposed to be lower Pleistocene, we have found reversed remanent magnetization with, for the first group,  $k=1.2$  and for the second one  $k=0.96$ . The mean values of declination and dip for the groups measured are given in Fig. 6 and exhibit clustering not far from the present field direction; the older groups give a somewhat lower dip.

The aim of this investigation was to determine the behaviour of the Earth's magnetic field in time for an interval of some 100,000 yr. The results indicate that the total Earth's magnetic field intensity had a periodical

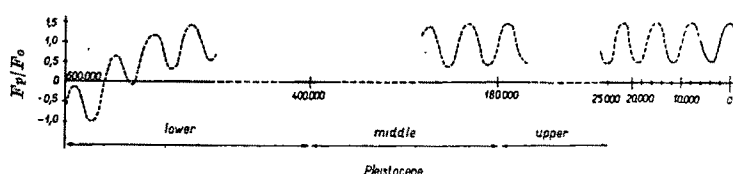


Fig. 7.

character, at least in the last 8,500 yr. This period was approximately 7,500 yr, and the amplitude was between 0.5 and 1.5 times the present day field intensity. In the older epochs (in the Pleistocene) the amplitudes of the Earth's field varied from 0.3 to 1.5 times the present day field intensity. The amplitudes of these intensity variations probably continued to increase going back in time and resulted in reversals of polarity (observed in the lower Pleistocene) at first for shorter time intervals. Later the periodical course of the field would pass to reversed polarity. The probable schematic course of the dynamic changes of the field intensity for the last 600,000 yr is given in Fig. 7. The intensity of the reversed field increased as our results show to approximately the same value as that of the present day field intensity.

Table 1.

Age	Locality	$k = F_p/F_0$	$F_0$ (present field = 0.475 Oe)	Mean value
Upper Pleistocene	D	1.44, 1.38	1.43 1.41	1.42
	N	1.38 1.29	1.30 1.28	1.31
Middle Pleistocene	Za	1.16	1.05	1.10
	Z	0.98	0.90	0.94
	Tg	0.83	0.83	0.83
	B	0.54	0.44	0.49
	Ho	0.31	0.41 0.50 0.46	0.42
Lower Pleistocene	K	1.15	1.20 1.28	1.21
	S	0.80	0.80	0.80
	C	0.58	0.72	0.66
	V	-1.06	-1.11 -1.35	-1.17
	V'	-0.97	-0.96	-0.96

As for the declination and dip, the changes of the Earth's magnetic field direction took place with substantially greater amplitudes some 10,000 yr ago, when the range of changes of declination were 70° and of dip, 25°.

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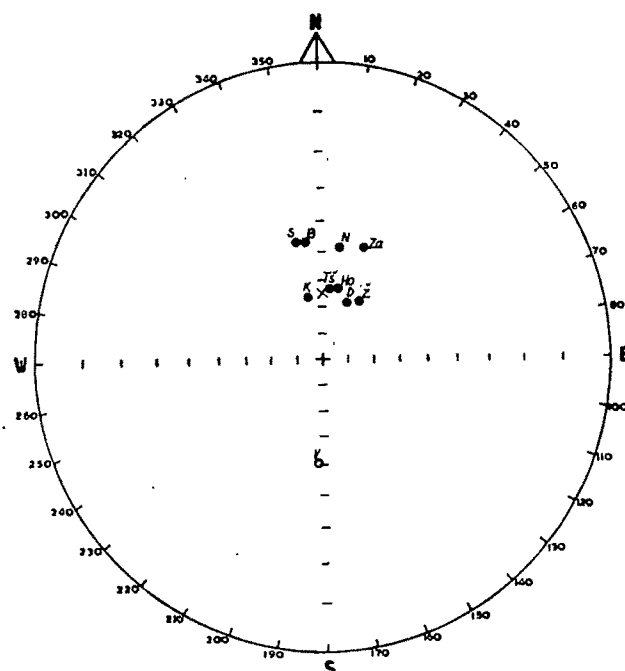


Fig. 6.

## PHYSICS

### Retro-reflexion by Diffusing Surfaces

R. J. SEANEY<sup>1</sup> appears unaware of previous observations over some 30 years<sup>2-4</sup>.

The apparatus which I devised 2 years ago (Fig. 1) enables the retro-reflexion from diffusing surfaces to be seen easily and binocularly. A pseudo-image of the source is seen at X, more or less sharp, according as the retro-reflexion is confined to a small solid angle or not. A pointer previously erected at image-point X helps to

locate it in space. The light source should be as small and as bright as possible. A crisp spot is given by most white paper or card, smoked magnesium oxide, magnesium oxide powder between glass, silica powder between glass (irrespective of graded particle size between 20 nm and 5  $\mu$ m), cotton fabric, and so on. A less crisp spot is given by coarse cardboard, soapstone, aluminium sheet fully etched in hot sodium carbonate solution, and many other materials.

My chief systematic observations are (1) with plane polarized incident light, the spot is polarized in the same plane, although the diffusely reflected light is substantially depolarized; (2) the fraction of total reflected light contributing to retro-reflexion is very small, of order, say,  $10^{-4}$ ; (3) laser (polarized) light shows no behaviour differing significantly from that of ordinary polarized light; (4) many of my observations appear to disagree with Seaney's linear relationship between angular spread of retro-reflexion and reciprocal particle size. For example, the great majority of cubic crystals composing smoked magnesium oxide are only about 200 nm in size, whereas the spot is small and sharp. Perhaps, consistently with observation (2), it could be formed by a minority of much larger crystals which may be present.

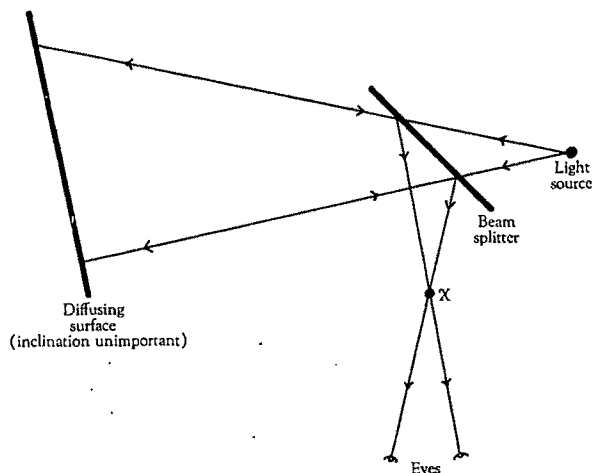


Fig. 1. Apparatus for viewing retro-reflexion from diffusing surfaces. A bright spot is formed at X by, for example, white card, and can be viewed as shown. If a small intense arc is used, and stray light screened off, the spot can be bright enough to be seen on an opaque white screen placed at X.

I can trace no explanation of the effect in the literature. I suggest it is a diminutive kind of *heilighenschein*<sup>5</sup>, the bright aura around the shadow of an aircraft cast by the Sun on a forest, as viewed from the plane. This occurs because the spaces between surface leaves make it possible, in that particular direction, to see a maximum area of illuminated leaves deeper down. In the present case I assume that elementary reflexions from deeper elements in the structure are single surface reflexions of specular type, so accounting for observation (1). Furthermore, on this small scale, diffraction would broaden the divergence of these reflexions, the broadening depending at least as much on the size of spaces between individual solid structural elements as on the size of the elements themselves. It is worth noting that, of the total volume occupied by smoked magnesium oxide, 90 per cent consists of air spaces, while blotting paper, which also shows a crisp spot, has a very open structure. Unfortunately one can hardly hope to see, using a microscope and scaled-down version of my apparatus, individual bright elements which may contribute to the retro-reflexion, because the divergence of the latter is insufficient to afford the necessary resolving power.

I thank Mr. Eric J. Gillham for suggesting the use of a beam-splitter, and for stimulating discussions.

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<sup>5</sup> Minnaert, M., *The Nature of Light and Colour in the Open Air* (English version—Dover Publications paperback).

## Mechanism of Turbulent Friction Reduction in Pipes by Dissolved Additives

GADD<sup>1</sup> has summarized some of the recent work in friction reduction by high molecular weight additives and concludes that the primary mechanism is a thickening of the laminar sublayer. Investigations of the effect of such additives on pipe flow carried out at West Virginia University support this explanation.

Measurements of the decrease in pressure drop<sup>2</sup> using polyethylene oxide as the additive showed that there is a saturated regime corresponding to maximum reduction in friction. This occurred at relatively low concentrations: 30 p.p.m. for material with an average molecular weight of  $2.3 \times 10^6$  and 20 p.p.m. for a molecular weight of  $4.5 \times 10^6$ . Increasing the concentration had no effect until the concentration became large enough ( $\sim 100$  p.p.m.) to change the viscosity. For these saturated solutions the friction coefficient varied as  $R_e^{-2/3}$  as against  $R_e^{-1}$  for laminar flow and  $R_e^{-1/4}$  for normal turbulent flows (where  $R_e$  is the Reynolds number). Similar results have been obtained by other investigators<sup>3</sup> and it appears that the saturated friction curve is the same for all effective additives. The concentration of additive required to obtain the maximum friction reduction can be considered a measure of the effectiveness of the additive.

Measurements of the velocity distribution<sup>4</sup> gave the surprising result that the velocity distribution with additive present was not intermediate between the laminar and turbulent profiles but was actually fuller than for normal turbulent flow. When plotted in the velocity defect form

$$\frac{u_c - u}{u^*} = f(r/r_0) \quad (1)$$

however, the data for flow with additive agreed with that for normal turbulent flow within the limited accuracy of the measurements. This is strong evidence that the effect of the polymer is on the laminar sublayer. It can be shown<sup>5</sup> that when the velocity defect law is valid, the friction coefficient is given by

$$\lambda = 8 (k + u_w/u^*)^{-2} \quad (2)$$

where  $k$  is a constant.

The results for normal turbulent flow in smooth pipes are fitted by taking  $u_w/u^*$  (the velocity at the junction of the turbulent core and the sublayer) as

$$\frac{u_w}{u^*} = 5.25 \log R_e \times 10^3 \quad (3)$$

and the constant as 10.3. It can readily be seen that increasing  $u_w/u^*$  decreases the friction coefficient and gives a fuller profile.

If the effect of the additive is on the laminar sublayer then it should be possible to reduce the amount of additive needed by injecting the additive through the wall at the pipe inlet. The observation of Davies and Ponter<sup>6</sup> that friction reduction persists when water flows through a pipe previously wetted by additive indicates that diffusion of the additive from the sublayer into the core is relatively slow, so that the required concentration will

be maintained in the sublayer for an appreciable length of pipe.

A possible physical explanation of the mechanism by which the additive affects the laminar sublayer may be found in the work of Deryaguin, who developed the blow off method for investigating the viscosity of thin film. He states<sup>7</sup>: "The results of these studies published so far leave no shadow of doubt that the viscosity in polymolecular boundary layers of polar liquids differs from that of the bulk (being sometimes lower but more frequently higher). On the whole there is a clear tendency for the thickness to increase with increasing length of the liquid molecules. In some cases, that is, that of hexaethoxy-decane, the viscosity is several times higher in a boundary layer about  $0.3\mu$  thick near the wall." Investigations of solutions of additives by Deryaguin's technique might explain the mechanism of friction reduction in terms of the thickness of the anomalous layer and the change in viscosity.

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## Geometry of Condon Loci of Molecular Spectra

It is well known that if the positions of the most intense ( $v'$ ,  $v''$ ) bands of a diatomic molecular spectrum are indicated on the usual Deslandres diagram the axes of which are the upper ( $v'$ ) and the lower ( $v''$ ) quantum numbers involved, then the set of points ( $v'$ ,  $v''$ ) so plotted trace out a set of nested, open-limbed curves called the primary and subsidiary Condon loci, some aspects of the geometry of which have been previously discussed<sup>1,2</sup>. In particular, it was suggested<sup>2</sup> that the position of the primary locus could be predicted from a consideration of those vibrational wave functions for which two respective antinodes are located at the same internuclear separation  $r$ . It was further suggested that the subsidiary loci could be located from a similar consideration of those wave functions for which one primary antinode and one appropriate subsidiary antinode, respectively, lie at the same value of  $r$ . These criteria ensure significant overlap between the two wave functions and thus a relatively large value of the Franck-Condon factor (square of the vibrational wave function overlap integral) which controls all transition probability and intensity parameters of the band.

The vibrational wave function  $\Psi_v(r)$  possesses  $(v+1)$  antinodes which are numbered in pairs inwards from the terminal antinodes. The  $p'$ th antinodes are designated  $r_{p'}^1(p)$  and  $r_{p'}^2(p)$ . The terminal antinodes are similarly designated  $r_v^1(1)$  and  $r_v^2(1)$ . Further,

$$r_{v'}^1(1) < r_{v'}^2(p) < r_e < r_{v'}^2(p) < r_{v'}^2(1)$$

$r_e$  is the equilibrium internuclear separation. It may thus be expected that the primary Condon locus passes through or near those  $v'$  and  $v''$  values which satisfy

$$r_{v'(1 \text{ or } 2)}(1) = r_{v''(1 \text{ or } 2)}(1) \quad (1a)$$

or more simply

$$r'_{(1 \text{ or } 2)}(1) = r''_{(1 \text{ or } 2)}(1) \quad (1b)$$

Similarly, the  $p'$ th subsidiary locus should pass through or near  $v'$  and  $v''$  values which satisfy

$$r'_{(1 \text{ or } 2)}(1 \text{ or } p) = r''_{(1 \text{ or } 2)}(p \text{ or } 1) \quad (2)$$

It is the purpose of this communication to record a recent extensive examination<sup>3</sup> of the ability of equations (1) or (2) to predict the positions of the primary and subsidiary Condon loci for a large number of band systems for which vibrational wave functions and Franck-Condon factors are available.

The three alternatives for the primary locus implied in equation (1) for the case where  $r_e' > r_e''$  are

$$r_1'(1) = r_1''(1) \quad (3a)$$

$$r_1'(1) = r_2''(1) \quad (3b)$$

$$r_2'(1) = r_2''(1) \quad (3c)$$

Fig. 1a and b illustrate (for  $r_e' > r_e''$ ) the three segments of the primary locus predicted by equations (3a)–(3c). Fig. 1a traces (in the  $v$ - $r$  plane) the location of the principal antinodes of wave functions for each of the two potentials involved in the transition. The three vertical lines on it correspond, respectively, to the conditions of equations (3a)–(3c). Fig. 1b represents the corresponding

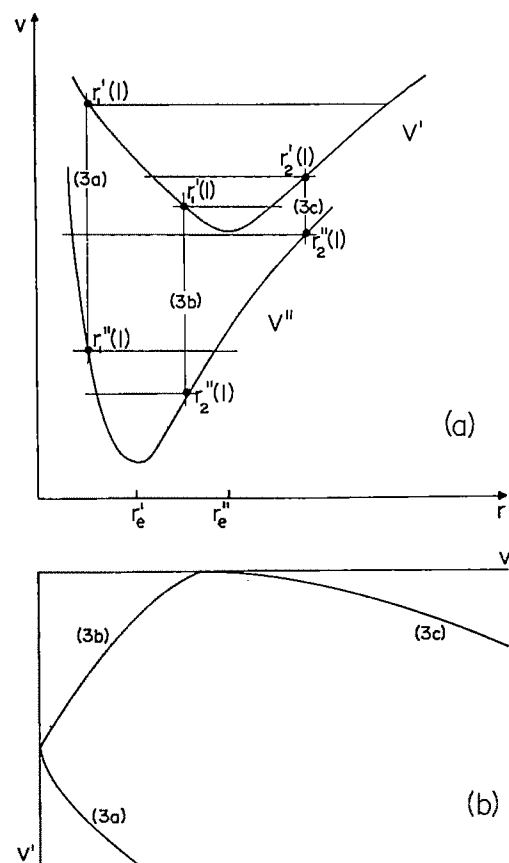


Fig. 1. Conditions giving rise to primary Condon locus. (a) Pairs of terminal antinodes located at equal internuclear separation. The curves are loci of terminal antinodes in  $v$ - $r$  plane. (b)  $v'$ - $v''$  Deslandres plot from co-location of terminal antinodes. Designations (3a) etc. relate to equations in text.

location of the three segments of the primary locus in the  $v'-v''$  plane.

Possible segments of the  $p'$ th subsidiary locus are those implied in equations 4a-4f which cover all possibilities of equation (2). The cases  $r'_2(1) = r'_1(p)$  and  $r'_2(p) = r'_1(1)$  are excluded by the hypothesis  $r'_e > r''_e$ .

$$r'_1(1) = r''_1(p) \quad (4a)$$

$$r'_1(p) = r''_1(1) \quad (4b)$$

$$r'_1(1) = r''_2(p) \quad (4c)$$

$$r'_1(p) = r''_2(1) \quad (4d)$$

$$r'_2(1) = r''_2(p) \quad (4e)$$

$$r'_2(p) = r''_2(1) \quad (4f)$$

Fig. 2a and b illustrate the situation. Fig. 2a traces in the  $v-r$  plane the location of the principal and  $p'$ th antinodes of the wave-functions for each of the two potentials involved in the transition.

The vertical lines correspond to the respective conditions of equations (4a)-(4f). Fig. 2b illustrates the corresponding possible locations of segments of the  $p'$ th subsidiary locus.

A direct comparison between the Condon loci and the predicted position of the segments has been carried out for many band systems using the parabolic and Morse models of molecular potentials. For the parabolic model, Hermite wave functions are known analytically and have been tabulated by Pillow<sup>4</sup>, while extensive tables of overlap integrals have been computed by Aiken<sup>5</sup>. For Morse potentials, extensive Franck-Condon factor arrays are available<sup>6</sup> together with tabulations of wave functions.

Comparisons were made between loci segment predicted by equations (3a)-(3c) and (4a)-(4f) and the actual loci evident both in computed Franck-Condon arrays and also

in intensity distributions for thirteen representative band systems the  $\Delta r_e$  of which varied from nearly zero (gallium iodide) to 0.4 Å (molecular oxygen). Excellent agreement was found between the location of calculated segments and actual Condon loci in all cases except for those of very small and very large values of  $\Delta r_e$ , for both of which principal contributions are made to the overlap integral from more than one pair of overlapping wave-function loops. The size of each segment and its orientation and position depend largely on  $\Delta r_e$ , and to a lesser extent on other molecular constants.

The subsidiary loci closely follow the location of segments  $a$ ,  $c-d$  and  $f$  when  $r'_e > r''_e$ , for there is additional constructive overlap between many of the wave functions.

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## THE SOLID STATE

### Domain Wall Motion in Double Nickel-Iron Films

Middelhoek and Wild<sup>1</sup> have recently measured mobilities for domain wall motion in double nickel-iron alloy which are significantly greater than those reported earlier by Patton and Humphrey<sup>2</sup> for similar films. Both groups have published mobility data for single layer films which are in good agreement<sup>3,4</sup>. In an attempt to clarify the situation concerning double films, mobility measurements have been made for two of Middelhoek and Wild's double films, each with two 400 Å nickel-iron layers (80 per cent nickel) separated by 100 Å of silica, using the technique reported previously<sup>2,4</sup>. For these films, a wall mobility value of  $1.2 (\pm 0.3) \times 10^4$  cm/sec-Oe was obtained. Middelhoek and Wild's mobility of  $4.1 \times 10^4$  cm/sec-Oe for double films (440 Å nickel-iron layers separated by 100 Å of silica) is significantly larger than the present determination. These differences are too large to be explained by the 40 Å difference in nickel-iron layer thickness or the scatter in the data. The differences in mobility determination for similar films from the same laboratory, as measured by different laboratories, can only be due to differences in the experimental technique.

For both techniques, single isolated domain walls are moved across the film using a number of short easy-axis field pulses of known duration and amplitude. Middelhoek's technique uses a large number (about 500) of very short pulses (20-800 nsec). The technique used by Patton and Humphrey<sup>2,4</sup>, on the other hand, uses only a small number (1-50) of longer (10-100 μsec) pulses to produce the wall displacements. Presumably, for sufficiently short pulses, effects involving the wall mass and elastic wall displacements would make the velocity a function of pulse length. If these effects were important, Middelhoek's technique (using shorter pulses) would be expected to yield lower, not higher, mobilities than those reported by Patton and Humphrey. Both authors have, however, reported that the results do not depend on these parameters. For Middelhoek's technique, the wall geometry in the film is controlled by the field itself, produced by a stripline passing over the film. As wall motion proceeds

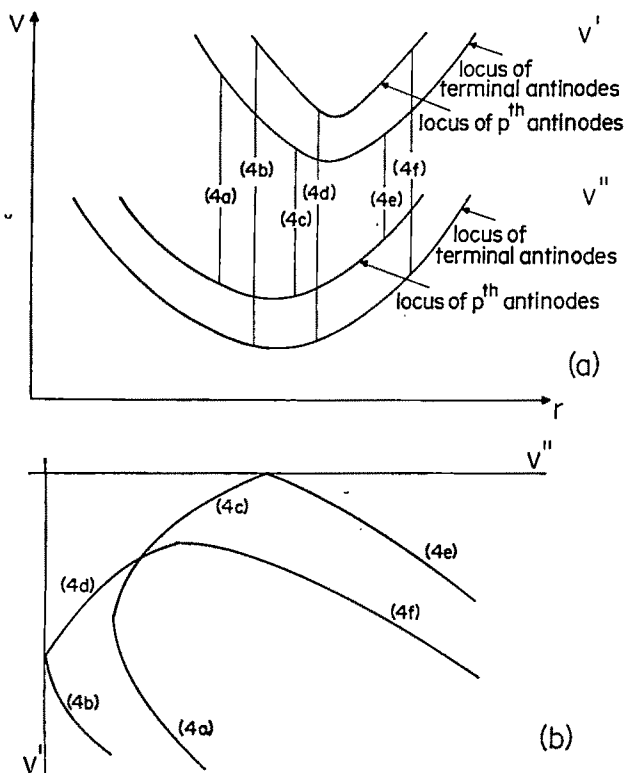


Fig. 2. Conditions giving rise to  $p'$ th Condon locus. (a) Terminal and  $p'$ th subsidiary antinodes at same internuclear separation. The curves are antinode loci in the  $v-r$  plane; (b)  $v'-v''$ , Deslandres plot from co-location of terminal and  $p'$ th antinodes. Designations (4a) etc. relate to equations in text.

additional growth of a zig-zag wall structure occurs at both ends of the wall section under observation, at those points where the stripline field falls below the coercive force. For Patton and Humphrey's technique, the wall geometry is controlled by quality of the film. The easy-axis drive field is produced by a Helmholtz pair and is uniform over the entire film. The wall length must remain approximately constant (limited by the film edge) and no additional nucleation or growth of walls can occur in order for a meaning of mobility determination to be made.

At present, it is not clear why the two sets of mobility data for double films are different. Because the two techniques yield different results even for similar samples from the same laboratory, it appears that the differences result from the measurement technique. Because the mobility data for single layers are in agreement, however, these differences must involve some aspect of the technique peculiar to layered films.

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## CHEMISTRY

### Formation of Polycyclic Aromatic Hydrocarbons in Pre-mixed Acetylene-Oxygen Flames

AN investigation has been made of the polycyclic aromatic hydrocarbons associated with the soot in pre-mixed acetylene-oxygen flames operating at a pressure of 20 mm of mercury. The burner, diameter 5.125 in., incorporated a water-cooled porous metal disk and enabled a flat-flame to be stabilized above it with an extended reaction zone. Water-cooled stainless steel grids were inserted into the flame at varying heights above the burner and samples of soot were collected.

At the specified pressure, an acetylene-oxygen ratio of about 0.95 just began to produce soot. Flames employed in the present work corresponded to acetylene-oxygen ratios of 1.2 and 1.5 respectively. The apparent temperatures (uncorrected) measured by means of a silica coated thermocouple probe of platinum and platinum/rhodium (87:13), the wires being 0.008 in. diameter, were

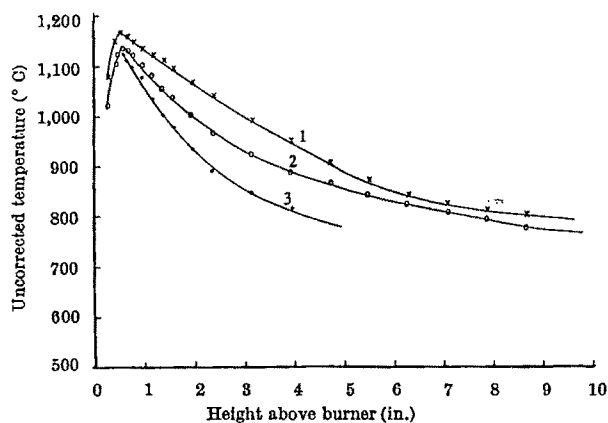


Fig. 1. Flame 1. Temperature of flame versus height above burner surface. 1, Axis of burner; 2, 1.1 in. from axis; 3, 2.0 in. from axis.

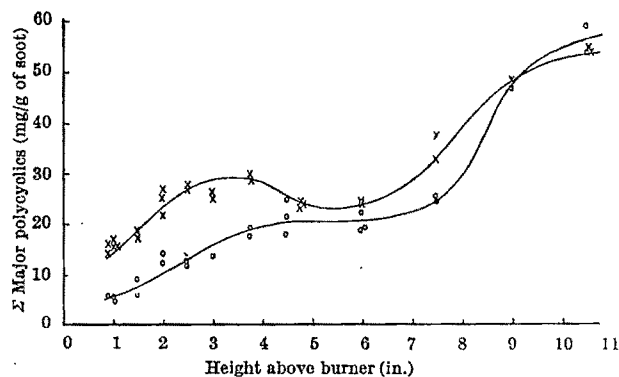


Fig. 2.  $\Sigma$  Major polycyclics in soots from flame 1 (O) and 2 (X) versus height above the burner.

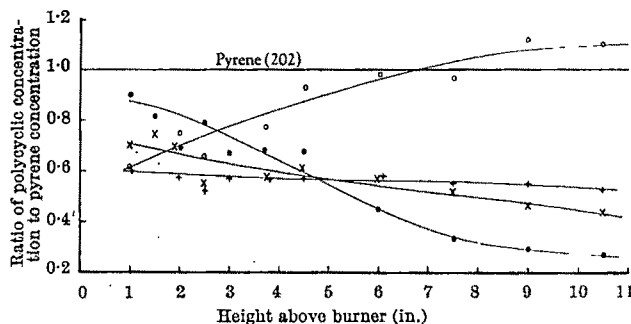


Fig. 3. Individual polycyclics in soot, expressed as ratio to pyrene versus height above the burner. Flame 1. O, Chrysene (228)+1,2-benzanthracene (228); +, 2,13-benzfluoranthene (226); X, fluoranthene (202); ●, anthracene (178)+phenanthrene (178). Numbers after names are molecular weights.

recorded at varying heights and the results are plotted for flame 1 (acetylene/oxygen ratio = 1.2) in Fig. 1.

Soot was removed from the collecting grid and extracted for 4 h with chloroform in a Soxhlet apparatus with a stream of nitrogen passing through it to prevent any air oxidation of extracted material. The extract was transferred to a 1 ml. flask using carbon disulphide as solvent.

Previous work in this laboratory had indicated that 12 ft. columns, 0.25 in. diameter, containing 10 per cent 'SE-52' silicone gum rubber on 60-80 mesh 'Chromosorb W' as a stationary phase, were capable of giving good separations of individual polycyclic aromatic hydrocarbons when using an 'F and M 810' research gas chromatograph with temperature programming from 190°-300° C at 4° C/min. Normally, flame ionization detectors were used for quantitative determinations, but for the identification of individual polycyclic aromatic hydrocarbons thermal conductivity detectors were used and the eluted hydrocarbons were isolated by bubbling the effluent helium carrier gas which contained them through spectroscopic grade cyclohexane. Their ultra-violet absorption spectra were then recorded.

Fig. 2 represents a plot of the sum of the concentrations of the principal polycyclic aromatic hydrocarbons found in the soot, namely (anthracene + phenanthrene), methyl phenanthrenes, fluoranthene, pyrene, methyl pyrenes, 2,13-benzfluoranthene, (chrysene + 1,2-benzanthracene) versus height above the burner, for flames 1 and 2. This corresponds to acetylene/oxygen ratios of 1.2 and 1.5 respectively.

The concentrations of a number of polycyclic aromatic hydrocarbons in the soot relative to that of pyrene are plotted against height above the burner for flame 1 in Fig. 3.

Bonne, Homann and Wagner<sup>1,2</sup>, who investigated a similar flame to that used in the present work, found that



the number of soot particles reaches a maximum early in the flame and then slowly decreases, growth in the size of the particles being caused by agglomeration. The particle size as well as the number of particles became nearly constant at heights of more than about 5 cm above the burner. By mass spectrometer investigations, Homann (discussion on "Elementary Steps in Gas Phase Oxidation", University of Liverpool, 1965) later found a number of polycyclic aromatic hydrocarbons containing 5- and 6-membered rings to be present in the burned gas. Such compounds had already been detected in the soluble part of soot<sup>3</sup>. The concentrations of these aromatic compounds were claimed to increase steadily in the burned gas, which suggested that they cannot be important intermediates in the first phase of carbon formation, as otherwise its rate would not decrease while the concentration of these compounds are still increasing.

Our results in Fig. 2 show that between heights of 1 and about 4 in. in the flame there is a gradual increase in the concentration of polycyclic aromatic hydrocarbons in the soot but a considerably greater increase occurs later in the flame at heights of 6–10 in. above the burner. This corresponds to a much lower temperature region than that in which, according to Bonne, Homann and Wagner, most of the soot is formed.

Badger and co-workers in their extensive investigation of the formation of polycyclic aromatic hydrocarbons by pyrolysis have reported that there is an optimum temperature range for the formation of polycyclic aromatic hydrocarbons, for example 660°–740° C in the pyrolysis of *n*-butylbenzene<sup>4</sup>, although some formation occurs at higher temperatures. It must be borne in mind that the residence time in a flame is much shorter than in the pyrolysis experiments.

Assuming that the concentration of polycyclic aromatic hydrocarbons in the soot reflects the state of affairs in the flame, we support the conclusions of Homann. The formation of polycyclic aromatic hydrocarbons appears to occur principally in a lower temperature region of the flame than that corresponding to soot formation, thus also supporting the earlier evidence of Ray and Long<sup>3</sup> that these compounds are not intermediates in carbon formation in the flames investigated.

Of the polycyclic aromatic hydrocarbons pyrene occurs in the greatest concentration in the soot up to approximately 7 in. above the burner after which 1:2-benzanthracene predominates. (Although the gas chromatographic peak represents chrysene and 1:2-benzanthracene unresolved, spectrophotometry of this fraction indicates only a small proportion of the former.) The relative concentrations of individual hydrocarbons change with height above the burner as can be seen from Fig. 3. In this connexion, Lang, Buffleb and Zander<sup>5</sup> have provided illustrations of the formation of higher condensed ring systems by the pyrolysis of relatively low molecular weight polycyclic aromatic hydrocarbons and such processes appear to be occurring in the flame.

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## Structures of *Tris*-oxalato-complexes of Trivalent Metals

THE *tris*-oxalato-complexes of the general formula  $K_3[M(C_2O_4)_3] \cdot xH_2O$  (I), where *M* can be aluminium, vanadium, chromium, iron, cobalt, rhodium, iridium and several other metals [*x* varies, being 3 for aluminium, vanadium, chromium and iron; 3.5 for cobalt; and 4.5 for rhodium and iridium], have played a leading part in many aspects of co-ordination chemistry during the past 50 years. A great deal of work refers<sup>1</sup> to their spectroscopic behaviour (where they are normally considered as *tris*-chelate compounds), their kinetic behaviour in solution, and, in some cases, their resolution into enantiomers.

Though it has been generally accepted, the assumption of formula (I) as written is not, however, well justified, because a good deal of evidence relates to the importance of the *bis*-chelated form  $[M(C_2O_4)_2(HC_2O_4)(OH)]^{3-}$ , both in the solid state and in solution. The question of the part played by water in the crystal lattice has now been investigated by several methods to attempt to distinguish between salts which are properly formulated as (I), containing water of crystallization, and others, in which the water plays a less obvious part. The racemic potassium salt of the rhodium (III) complex, " $K_3[Rh(C_2O_4)_3] \cdot 4.5 H_2O$ ", is a good example of the latter category; it has been reformulated<sup>2</sup>  $K_6[Rh(C_2O_4)_3][Rh(C_2O_4)_2(HC_2O_4)(OH)] \cdot 8H_2O$  on the basis of broad-line proton resonance measurements.

The salts of the kinetically labile complex anions of aluminium, vanadium and iron have similar structures because they are isomorphous. The racemic complex of chromium (III) is also isomorphous with this series. All have *x*=3. The crystal and molecular structure of a typical member, potassium *tris*oxalatovanadate (III) trihydrate, has been determined by three-dimensional X-ray analysis (using 1,350 independent reflexions) and refined by the method of least squares (*R* is 0.15). The monoclinic green crystals have space group  $P2_1/c$ , *a*=7.81, *b*=20.01, *c*=10.35;  $\beta$ =108° 33', *Z*=4,  $\rho_{calc}$ =2.102,  $\rho_{obs}$ =2.09 g/cm<sup>3</sup>. The complex ion is shown in Fig. 1.

Each vanadium ion is surrounded by three chelating oxalate ions, the complex anion having approximately *D*<sub>3</sub> symmetry. The three molecules of water of crystallization fall into two classes, neither class being strongly hydrogen bonded (the infra-red spectrum shows three bands in the region due to stretching modes of water). All the water is readily lost on heating; the thermogravimetric analysis curve shows a single weight loss at about 110° C, and differential thermal analysis confirms that this process is endothermic.

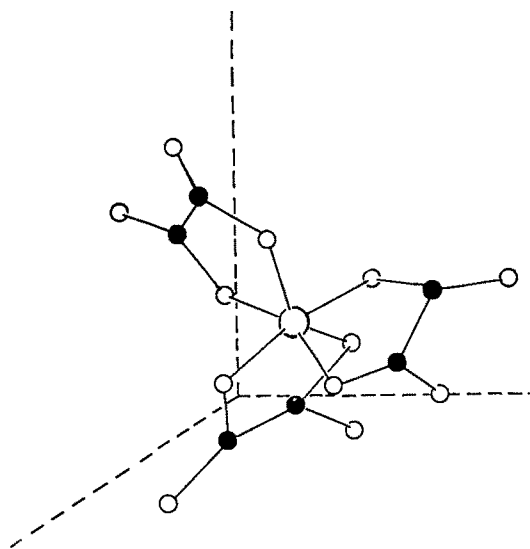


Fig. 1. The complex anion  $[V(C_2O_4)_3]^{3-}$ . O, Vanadium; ●, carbon; ○, oxygen.

The formulation (I) is thus justified for the trihydrates,  $K_3[M(C_2O_4)_3 \cdot 3H_2O]$ , where  $M$  is vanadium (and, by isomorphism, aluminium, iron and chromium). Crystals of these compounds contain *tris*-chelated complex anions of approximately  $D_3$  symmetry, and distinct water of crystallization. The present results agree well with some earlier crystallographic work. The salt  $K_3[Cr(C_2O_4)_3] \cdot 3H_2O$  was investigated<sup>3</sup> by two-dimensional X-ray analysis, and found to belong to the space group  $P2_1/c$  with a structure similar to that found here for the complex of vanadium. The complex of iron (III) in its potassium salt was also found<sup>4</sup> to contain *tris*-chelated complex ions.

The racemic salts of the kinetically inert transition metal ions cobalt (III) and rhodium (III) are entirely different. They are not isomorphous with the trihydrates of formula (I), nor with each other. Their infra-red spectra and thermogravimetric behaviour differ completely from the cases of the trihydrates. It seems likely that, in the cases of the complexes of cobalt and rhodium (and in the racemic complex of iridium, which is isomorphous with that of rhodium), some of the "water of crystallization" is constitutive (that is, involved in the co-ordination sphere). Further investigations are in progress on the factors influencing the molecular structures of complex metal oxalates.

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## IMMUNOLOGY

### Correlation between Net Charge of Antigens and Electrophoretic Mobility of Immunoglobulin M Antibodies

EARLIER we reported that the net electrical charge of rabbit antibodies of the immunoglobulin G class to various natural and synthetic antigens was inversely related to the net electrical charge of the antigens<sup>1</sup>. Thus, under a certain set of conditions, antibodies to acidic antigens were found to predominate in the first chromatographic fraction eluted from diethylaminoethyl-'Sephadex', whereas antibodies to basic antigens were mostly in the second fraction. Immuno-electrophoresis revealed that at pH 8.6, the second fraction moved faster towards the anode than the first fraction. This effect was shown to be independent of the immunological specificity of the IgG antibodies investigated, as the electrophoretic mobility of anti-dinitrophenyl (DNP) antibodies elicited by DNP conjugates of various acidic and basic proteins and synthetic polypeptides was related to the net charge of the macromolecular carrier.

Many differences have been detected between the structural, biosynthetic and biological characteristics of IgM and IgG proteins<sup>2-5</sup>. It was, therefore, of interest to determine whether the effect of the net charge of antigens on the IgG class of antibodies was also characteristic of the IgM antibodies. The preparation in this laboratory of immunoadsorbents using bromoacetyl cellulose permitted the isolation of IgM and IgG antibodies of the same

immunological specificity in a highly pure form<sup>6</sup>. The data presented here show that the net charge of the antigen influenced the electrophoretic mobility of IgM antibodies in a manner similar to that observed for the IgG antibodies.

Randomly bred rabbits were immunized by injecting 5-10 mg of the antigens listed in Table 1, emulsified in Freund's adjuvant at multiple intradermal sites. Purified antibodies were isolated by adsorption from the antisera with immunoadsorbents prepared by the chemical reaction of the respective antigens and bromoacetyl cellulose<sup>7</sup>, followed by acid elution<sup>8</sup>. Anti-DNP immunoglobulins were purified with DNP-human serum albumin cellulose as the immunoadsorbent. Antibodies of the IgM and IgG class were separated by gel filtration using 'Sephadex G-200' (ref. 8). Analytical ultracentrifugation revealed a single component for each class of antibody<sup>9</sup>.

The electrophoresis was carried out in a standard apparatus ('Immuno-for', LKB Produkter, Sweden) using 0.05 molar sodium barbital buffer, pH 8.6, in the electrode tanks and for the preparation of the agar (1.5 per cent special Noble agar, Difco, Michigan). The proteins were separated by applying 220 V, 31 m.amp, to eighteen slides for 100 min. After the electrophoresis the albumin zone of the normal serum, used as a reference for each slide, was located and pierced with a capillary pipette. Goat anti-rabbit immunoglobulin sera were applied to the troughs and, after precipitin arcs had developed, the slides were washed with 0.15 molar sodium chloride and stained with naphthol blue black dye<sup>9</sup>.

The migration of the serum albumin was chosen as the distance between the cathodal end of the precipitin arc of the normal serum IgG and the mark in the gel. The centre of the precipitin arc of the normal immunoglobulins and the individual antibodies was located and the distance determined from this point to the cathodal end of the normal serum IgG. The  $M_r$  values<sup>10</sup> listed in Table 1 were obtained by dividing the distance of migration of the normal and antibody immunoglobulins by the distance of migration of serum albumin. The  $M_r$  for normal IgM was  $0.537 \pm 0.007$  and for normal IgG was  $0.270 \pm 0.007$ . All samples were examined in duplicate or triplicate.

Table 1. RELATIVE  $M_r$  VALUES OF RABBIT IgM AND IgG ANTIBODIES

Immunogen	$M_r$ of IgM		$M_r$ of IgG	
	Normal serum	Antibody	Normal serum	Antibody
(A) Acidic				
(1) Diphtheria toxoid	0.51	0.43	0.24	0.14
(2) (T, G)-A-L <sup>1</sup>	0.53	0.43	0.25	0.12
(3) DNP-(T, G)-A-L <sup>1</sup>	0.51	0.41	0.24	0.12
(B) Basic				
(1) Lysozyme	0.55	0.54	0.26	0.24
(2) Ribonuclease	0.56	0.56	0.25	0.25
(3) Uridine-A-L <sup>11</sup>	0.54	0.55	0.25	0.27
(4) DNP-poly-L-lysine <sup>1</sup>	0.55	0.53	0.26	0.28

IgM and IgG antibodies, elicited by the immunogens listed in the first column, were isolated from immune sera with immunoadsorbents prepared using bromoacetyl cellulose<sup>8</sup>. Individual immunoglobulin samples were electrophoresed with a normal serum specimen on the same slide. Following the electrophoresis the albumin zone of normal serum was located and its position marked in the agar. Goat antiserum to rabbit IgM or the  $F_2$  fragment was used to develop the precipitin arcs. The distance between the cathodal edge of the normal serum IgG and the albumin was determined. The  $M_r$  values of the antibodies and their normal serum counterparts were calculated by dividing the distance between the middle of the precipitin arc of these immunoglobulins and the cathodal edge of the normal serum IgG by the distance of the albumin migration.

The results of the immuno-electrophoretic runs are summarized in Table 1. Two representative patterns of IgG and IgM antibodies, elicited by the acidic synthetic antigen (T, G)-A-L<sup>1</sup> and by the basic uridine-A-L<sup>11</sup>, are shown in Fig. 1. It is clear from Fig. 1 and from the  $M_r$  values in Table 1 that the electrophoretic mobility of both the IgM and the IgG antibodies depends on the net charge of the antigens. The centre of the precipitin arc of the anti-(T, G)-A-L IgG preparation (Fig. 1) was chosen in the region where the precipitin arc was most intense. This region represents 79 per cent of the total IgG antibody<sup>1</sup>.

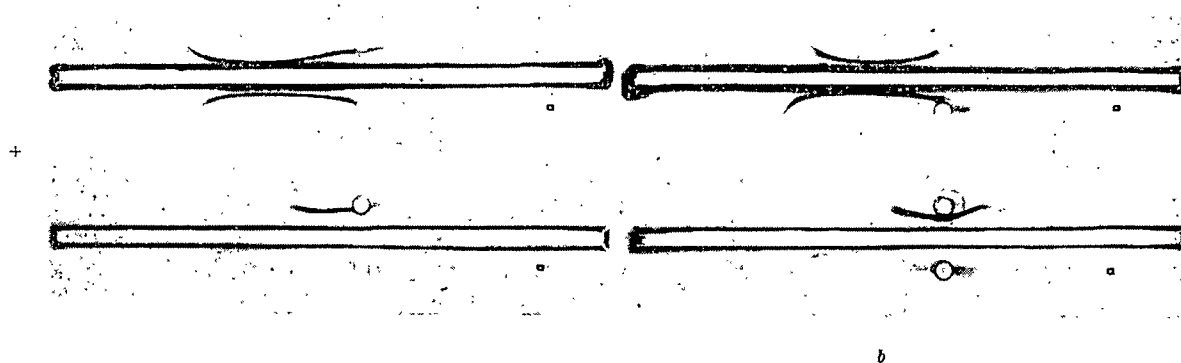


Fig. 1. Immunoelectrophoresis of purified IgM (top) and IgG (bottom) anti-uridine-A—L (a) and anti-(T, G)—A—L (b) antibodies. The normal serum was placed in the upper hole and the antibody applied to the lower hole of each slide. The proteins were electrophoresed and the albumin zone of the normal serum pierced with a capillary pipette (□). A goat anti-rabbit immunoglobulin serum was added to the trough and the slides washed with 0.15 molar sodium chloride and stained after the precipitin arcs were developed.

The inverse relationship between the charge of the antigen and the electrophoretic mobility of the IgM as well as the IgG antibodies was also demonstrable for the basic proteins, ribonuclease and lysozyme, and the acidic protein, diphtheria toxoid. As previously reported<sup>1</sup>, this difference in electrophoretic mobility was independent of the specificity of the antibody. Thus, IgM antibodies with anti-DNP specificity had their electrophoretic mobility directed by the net charge of the carrier molecule, similarly to the IgG anti-DNP antibodies.

Antibodies isolated from individual rabbit sera yielded the same results as were observed with the pooled samples. The electrophoretic mobility also did not change when antibodies were isolated at different times during the course of immunization.

It is apparent from the data presented here that the effect of the charge of an antigen on the electrophoretic mobility of the antibodies it elicits is demonstrable not only in the IgG class but also in the IgM class of antibodies. The simplest explanation would seem to be that there is a process of selection by the antigen, at cellular or subcellular level, of the loci at which the antibody is synthesized<sup>1</sup>. One possibility may be that an initial step in the biosynthesis of the antibody is the reaction of a charged area on the antigen with some complementary receptor sites. These sites may be complementary either to antigenic specificity determinants or to other areas of the antigenic molecule.

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### Apparent Absence of Immunological Cross-reactivity between Human and Simian Gonadotropic Hormones as determined by Radioimmunoassay

It has been reported that crude monkey luteinizing hormone (LH) and follicle stimulating hormone (FSH) preparations cross-react immunologically with an antiserum to human FSH as measured by the haemagglutination inhibition technique<sup>1</sup>. Professor Carl Gemzell (personal communication) was also able to show, using the same technique, that a partially purified rhesus monkey pituitary LH preparation, supplied by us, reacted with an antiserum to human chorionic gonadotropin (HCG). This information, together with the well established immunological and physiological similarities between human and simian growth hormone<sup>2</sup>, prompted us to attempt the development of radioimmunoassays for the measurement of circulating LH and FSH in the rhesus monkey using the more readily available human gonadotropins as antigens.

The interaction of purified preparations of human and simian (rhesus monkey) luteinizing hormone and follicle stimulating hormone with antibodies against several human gonadotropins was studied with a modification of the radioimmunoassay devised for insulin by Herbert *et al.*<sup>3</sup>. Human pituitary LH (HLH) and human pituitary FSH (HFSH) were iodinated with iodine-131 according to the method of Greenwood *et al.*<sup>4</sup>. The HLH had a biological activity of 5.3 times NIH-LH-S1 in the ovarian ascorbic acid depletion assay of Parlow<sup>5</sup>. The HFSH had 156 times the potency of NIH-FSH-S1 as measured by the assay of Steelman and Pohley<sup>6</sup>. Antisera produced in rabbits against four gonadotropin preparations were used: HCG (7,000 IU/mg), HCG (2,600 IU/mg), pituitary HLH (same relative potency as NIH-LH-S1) and pituitary HFSH (relative potency of fifteen times NIH-FSH-S1). Antigen-antibody reactions were carried out for 5 days at 5° C in a barbitone buffer containing bovine serum albumin. Iodinated hormone bound to antibody (B) was separated from free iodinated hormone (F) by adsorption of the latter to charcoal coated with dextran.

Three different simian pituitary LH (SLH) preparations with biological activities of 0.06, 0.3 and 0.4 times NIH-LH-S1 were tested for their ability to compete with HLH-<sup>131</sup>I for the various antibodies to HCG and HLH. One simian pituitary FSH (SFSH) preparation with a biological activity ten times NIH-FSH-S1 was similarly tested against HFSH-<sup>131</sup>I using the HFSH antiserum. Each simian gonadotropin preparation was assayed, at least twice, against the appropriate antiserum. The amounts of the SLH and SFSH preparations are expressed in terms of their biological activity relative to that of the HLH and HFSH preparations with which they are compared rather than in terms of weight in order to

eliminate the extent of purification as a variable (for example, see Fig. 1).

When HLH was reacted with the two HCG antisera or with the HLH antiserum, a significant displacement of the radioiodinated HLH from the antibody was observed with 0.1  $\mu\text{g}$  of the hormone. Unfortunately, however, amounts of SLH as high as 36  $\mu\text{g}$  equivalents of HLH produced very little displacement of iodinated HLH from the antibody (Figs. 1 and 2). Varying dilutions of rhesus monkey pituitary homogenate (1/160–1/10 of a single gland) also failed to produce a significant displacement of the iodinated HLH. Identical results were obtained with homogenates of squirrel monkey pituitary glands. Similarly, SFHSH at a level of 32  $\mu\text{g}$  equivalents of HFSH failed to effect a significant displacement of the iodinated HFSH from the HFSH antibody while 0.1  $\mu\text{g}$  of the HFSH produced a significant depression of the ratio of  $B:F$  (Fig. 3).

These results demonstrate the relative absence of immunological interaction between the rhesus monkey pituitary gonadotropins and antibodies produced against HLH, HFSH or against HCG. Whether larger amounts of SLH and SFHSH than those used in the present investigation would at some point give a dose-response curve with the human antibody has not been determined. The data presented, however, lead to the conclusion that the successful application of radioimmunoassays to the measurement of circulating gonadotropins in the rhesus monkey will require antisera against FSH and LH isolated from this species and that antigens of human pituitary and chorionic origin cannot, unfortunately, be used for this purpose.

In contrast to the relative absence of immunological cross-reaction between human and simian gonadotropins, ovine, equine and human luteinizing hormone preparations show full interstitial cell stimulating activity in hypophysectomized male rhesus monkeys<sup>7</sup>.

The apparent discrepancy between the results of the haemagglutination inhibition assay and the radioimmunoassay in assessing the immunological cross-reactivity of human and simian gonadotropins cannot be fully evaluated at the present time.

We thank Dr. A. F. Parlow for the LH antiserum and for the bioassays of our simian and human gonadotropin preparations, and Dr. B. B. Saxena for the HFSH antiserum.

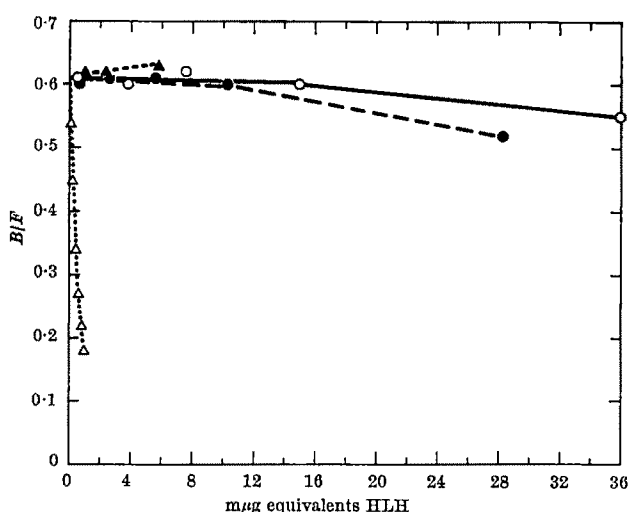


Fig. 1. Relative affinity of a human pituitary LH preparation and of three simian LH preparations for an antibody to HCG as measured by the displacement of  $^{125}\text{I}$ -HLH from the antibody. This displacement is expressed as the ratio of antibody-bound ( $B$ ) to free ( $F$ ) radioactivity.  $\Delta$ , HLH;  $\bullet$ , SLH;  $\circ$ , SLH;  $\blacktriangle$ , SLH. The numerical values on the abscissa represent the biological activity contained in the simian preparations expressed in terms of the amounts of the HLH containing equivalent biological activity. For example, 36  $\mu\text{g}$  equivalents of  $\circ$  represent 500  $\mu\text{g}$  of this preparation containing the same biological activity as 36  $\mu\text{g}$  of the HLH preparation.

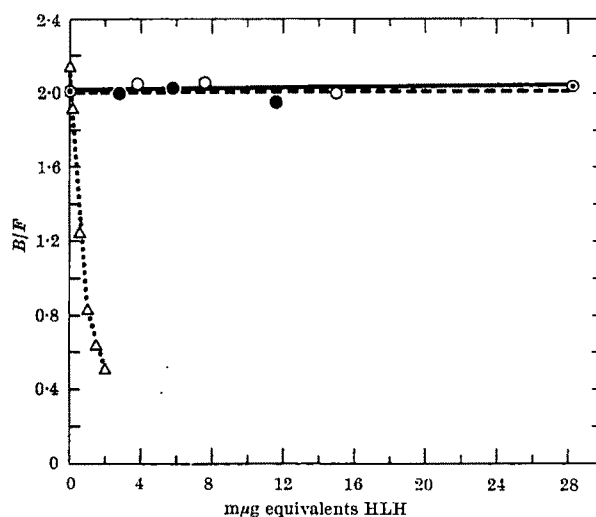


Fig. 2. Relative affinity of HLH and of two SLH preparations for an antibody to HLH. Symbols as in Fig. 1.

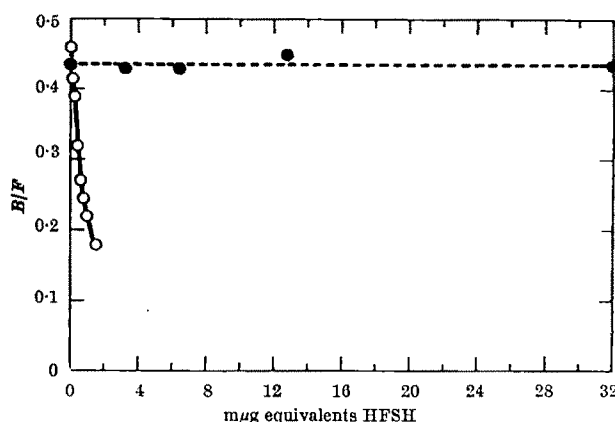


Fig. 3. Relative affinity of HFSH ( $\circ$ ) and of SFHSH ( $\bullet$ ) for an antibody to HFSH.

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### Further Studies on the Adjuvant Principle of Pertussis Vaccine for the Mouse

We have already presented evidence that the capacity of pertussis vaccine to enhance antibody formation in mice to unrelated protein antigens is labile to heat and therefore, contrary to the view of many workers, cannot be attributed

to the heat-stable endotoxin of the pertussis organism<sup>1</sup>. We postulated that the protective antigen is not only capable of sensitizing mice to histamine but may also be responsible for this typical adjuvant effect associated with pertussis vaccine. We further proposed that the autonomic nervous system may be involved in an enhancement of the immune response in mice mediated by pertussis. The object of this communication is to describe certain experiments designed to determine the validity of these postulations.

The experiments involve the final precipitate and supernatant of our fractionation scheme<sup>2</sup> formed by adding ammonium sulphate to the clarified vaccine lysate to 35 per cent saturation. We have shown that this precipitate contains most of the immunogen and the histamine sensitizing factor (HSF) whereas the supernatant, although it contains the bulk of the organic bacterial substance, has only small amounts of these activities. The object of the experiment was to compare these two fractions in mice with respect to all three activities—immunogenicity, histamine-sensitization, and adjuvance for tetanus toxoid. A graded three-dose design based on non-dialysable nitrogen, identical for each activity, was adopted for their biometric estimation. The dosage for "precipitate" was selected to span the previously determined median sensitizing dose of HSF. These doses were 2.3, 6.9 and 20.7  $\mu$ g nitrogen. The highest feasible dose of "supernatant", constrained by volume and toxicity considerations, was about ten times the highest "precipitate" dose, and was scaled down by half instead of by one third. Thus this dosage was 56, 112, and 224  $\mu$ g nitrogen. The mouse intracerebral immunogenicity test and the HSF test were described earlier<sup>2</sup>. The test for adjuvance involved intraperitoneal injection of groups of ten mice with the three dosages of the pertussis fractions, and subcutaneous injection of all these mice with the constant dose of 5 Lf of fluid tetanus toxoid. One group of mice received toxoid only and another group served as uninoculated controls. All mice were challenged in 7 days with an intramuscular injection of 2 minimum lethal doses of tetanus toxin. Observation of the time of death and symptoms was scored by a modification of the method of Ipsen<sup>3</sup>. The mean score of the mouse group expressed as a percentage of the highest possible score comprised the response metameter, designated "percentage of immunity".

Table 1. PARALLEL FRACTIONATION OF THE IMMUNOGENIC, THE HISTAMINE SENSITIZING AND THE ADJUVANT ACTIVITIES OF PERTUSSIS VACCINE

Pertussis vaccine fraction	Mouse dose ( $\mu$ g N)	Immunogenicity (survival rate)	Histamine sensitization (death rate)	Adjuvance (mean per cent immunity for 10 mice, 5 Lf tetanus toxoid each)
35 per cent SAS supernatant	56 112 224	1/11 0/13 2/11	0/16 0/16 3/16	30 38 33
35 per cent SAS precipitate	2.3 6.9 20.7	6/10 6/10 10/10	3/16 11/15 14/16	32 62 72
Controls	None	0/10	0/15	0
Toxoid only	None	—	—	37

The results are given in Table 1 in terms of mouse survival rates for the immunogen, death rates for histamine sensitization, and percentage of immunity for adjuvance. It is apparent that the supernatant showed a very low level for all three of these activities. At the highest dose administered, the survival rate for intracerebrally challenged mice was only two in eleven, the death rate from histamine challenge only three in sixteen, and the apparent tetanus toxoid immunity did not differ significantly from that of mice receiving toxoid alone. On the other hand, the groups receiving "precipitate" in nitrogen dosage ranging from 1/100 to 1/10 that of the highest "supernatant" dosage showed fair to good dose-response regression for all three activities. These results demonstrate "parallel fractionation" of the activities in question which, when considered in conjunction with previous reports that these activities are labile to 100° C heat for 30–40 min<sup>1,4,5</sup> and

that at least two of them, the immunogen and the HSF, can be inactivated by proteolysis<sup>6-7</sup>, suggest that a single substance is implicated.

Fishel *et al.*<sup>8</sup> have suggested that blockade of the  $\beta$ -adrenergic receptors of the autonomic nervous system may be the mechanism by which pertussis vaccine enhances the susceptibility of mice to histamine and serotonin. We have suggested that this may also be so with vaccine enhancement of peptone shock<sup>9</sup>. Fishel *et al.*<sup>10</sup> have demonstrated a converse phenomenon; namely, that injection of an  $\alpha$ -adrenergic blocking agent, dibenzyline, cancelled the capacity of vaccine to increase sensitivity to some of these stresses. After failing to demonstrate adjuvance when the  $\beta$ -agent, propranolol, is substituted for vaccine, possibly because it is metabolized before it can influence the prolonged course of the primary immune response, we decided to attempt the converse type of experiment in relation to adjuvance. We were successful and repeated the experiment.

A group of sixty mice was injected subcutaneously with 1 Lf of tetanus toxoid, and a similar group with 5 Lf. Sub-groups of twenty mice were at the same time injected intraperitoneally with: (1) pertussis vaccine ( $19 \times 10^9$  cells in 1 ml.); (2) the same plus 1 mg dibenzyline in the contralateral site; (3) no injection additional to the toxoid.

A group of twenty mice was set aside as uninoculated controls. After 7 days half of each group of mice was challenged by intramuscular injection of 2 minimum lethal doses of tetanus toxin, and 7 days later the remaining half was challenged with 10 minimum lethal doses. A scoring system appropriate for each challenge was applied to survival time and symptoms and the percentage immunity calculated as already described. The results are shown in Fig. 1. It is apparent that the  $\alpha$ -blocking agent, dibenzyline, partially suppressed the adjuvance of pertussis vaccine, in the same way as the findings for other activities. In a secondary experiment of similar design we found that dibenzyline partially suppressed the immune response of mice to tetanus toxoid given without an associated pertussis vaccine injection.

Recently, Floersheim has reported that dibenamine, a haloalkylamine  $\alpha$ -adrenergic blocking agent closely related chemically to dibenzyline, could depress the tuberculin reaction in sensitized guinea-pigs<sup>11</sup>. Obviously, it is difficult to demonstrate that these apparent immunosuppressive effects result exclusively from the neurologic blocking activities of the molecule, rather than from some other property. Both dibenamine and dibenzyline, for

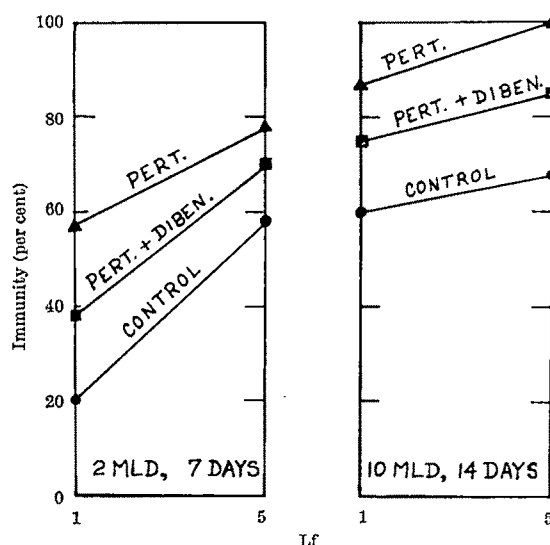


Fig. 1. Partial suppression by dibenzyline of the adjuvant effect of pertussis vaccine for the immune response of mice to 1 and 5 Lf doses of tetanus toxoid. Each point represents the mean response of ten mice.



example, are known to have antihistaminic effects, and to bear certain chemical resemblances to the nitrogen mustards. Hadnagy *et al.*<sup>12</sup> showed, however, that antihistaminics had no effect on the formation of antibodies. Moreover, whereas the mustard alkylating agents can suppress the immune response of rabbits, Rosenblatt and Johnson found that dibenzylamine did not depress the production of antibodies in that species<sup>13</sup>. In fact, the haloalkylamine blocking agents have few effects in laboratory animals that cannot be attributed to their capacity to block the  $\alpha$ -adrenergic receptors of the autonomic nervous system<sup>14</sup>. One might suggest that the observed immunosuppression might be non-specific in nature or a function of a "generalized toxicity", but it should be noted that many drugs, even when given in near lethal doses, have no effect on the immune response<sup>15,16</sup>.

Several investigators have recently reviewed the altered responses induced in mice and rats to a variety of stresses or stressor agents by pertussis vaccine<sup>17-19</sup>. We propose that the substance responsible is the immunogen itself and that many, if not all, of these reactions are primarily mediated through autonomic nervous mechanisms.

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### Variation in Tolerance Dose Requirements as related to Heterogeneity of the Immune Response

By varying widely the dose of a protein antigen it is possible, in a given system, to vary the response to this antigen from formation of both IgM and IgG antibodies (in the medium dose range<sup>1</sup>) to lack of formation of either type (repeated massive doses). In between, a regimen of antigen administration can be found which stimulates the formation of IgG and not of IgM. This dissociation is reflected in the cellular changes involved.

Seven to eight week old chickens were immunized by intravenous injection of commercial human serum albumin (HSA). The titre of antibodies was evaluated by means of passive haemagglutination with diazobenzi-

dine-conjugated erythrocytes. Distinction between IgM and IgG antibodies was based on specific inhibition by 0.2 molar 2-mercaptoethanol of the former<sup>1</sup>. The cellular response was followed in spleen imprints by the indirect immunofluorescent method of Coons. Counts of antibody containing cells (ACC) were made from 50-100 microscope fields and standardized for fifty fields (about  $5 \times 10^4$  cells). The morphological classification followed the accepted convention<sup>2</sup>; large blast cells were omitted from differential counts. Details and representative examples of the cell types have been given elsewhere<sup>3</sup>. The capacity of the immunofluorescent method employed to detect intracellular antibodies even in the presence of a high level of circulating antigen has already been demonstrated<sup>3</sup>.

Four chickens were killed on each of several days after immunization with various doses of HSA, and the antibody in their serum and spleen was examined as described here. The results are summarized in Fig. 1.

After a relatively small dose (5 mg of HSA/kg of body weight) the first ACC appear after 48 h, their number increases exponentially and after reaching a peak declines rapidly (open circles). During the first phase the majority of ACC are small immature cells with a narrow rim of cytoplasm, mostly distinguishable as lymphocytes. In the succeeding two phases immature and mature plasmocytes appear and gradually predominate. During the exponential phase, serum antibodies are of the IgM type (open triangles), whereas the IgG type (solid triangles) coincides with the appearance of plasmocytes.

With doses more than 2,000 mg, the picture is different. After a dose of 2,500 mg, ACC do not appear until on the fourth day; their number increases slowly to maximum of only about ten ACC per fifty fields (solid circles). Furthermore, the cells are exclusively plasmocytes. The serum antibody is only IgG, and IgM antibodies are absent<sup>1</sup>.

An experiment was carried out to inhibit the immune response completely. If an initial dose of 2,000 mg of HSA is followed by daily doses of 1,000 mg, no ACC appeared in twelve chickens examined on days 4, 7 and 12 (solid squares).

To find whether in animals showing no IgM antibody response its production is totally inhibited, a corresponding group of chickens was injected with a similar dose of 2,500 mg and challenged 7 weeks later with 10 mg. In this group also the IgM antibodies were almost completely absent, even though there is a marked IgM antibody response to 10 mg of HSA in chickens which had received no previous injection or were pretreated with lower doses (0.5-50 mg).

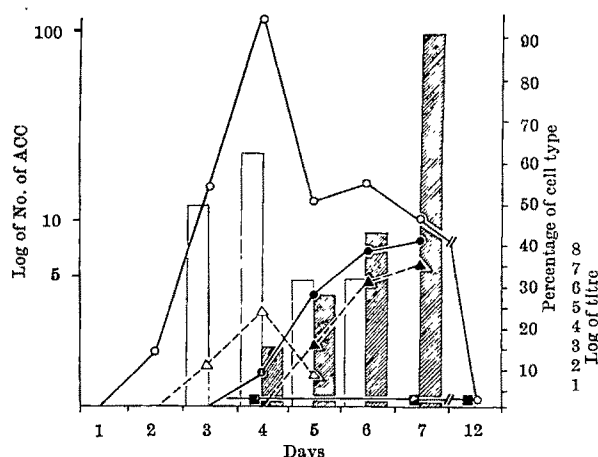


Fig. 1. Response after 5 mg of HSA. —○—, Log number of antibody containing cells (ACC); columns, mean percentage of given cell types (white column, lymphocyte cells, black column, plasmocytes); —△—, values of log titre of IgM (—△—) and IgG (—▲—) antibodies; —●—, log number of ACC after a dose of 2,500 mg (all are of plasmocyte type); —■—, log number of ACC after repeated massive doses (no response).

During the exclusively IgG response elicited by large amounts of antigen the initial exponential proliferation of antibody producing cells is absent, even though it occurs typically, and parallels the serum IgM antibody titre, when both types of antibodies are formed. The complete suppression of any IgM response was confirmed by its absence even when a secondary anamnestic response of IgG type could be induced.

We found, as before<sup>5</sup>, that a much smaller amount of antigen (less than 500 $\gamma$  of HSA) induces the formation exclusively of IgM antibodies; it seems that the IgM response has a low threshold both for induction and inhibition compared with the IgG response, which was only inhibited by repeated massive doses of antigen.

The cellular changes observed probably indicate a functional separation of the IgM and IgG responses. The early phase of differentiation of antibody-forming cells (morphologically of lymphocyte character), which coincides with the IgM response, is possibly accomplished by cells already capable of reacting with antigen at the time of immunization. It may represent a primitive regulatory stage in the development of immunity. The direction of differentiation and the recruitment of further cells to this population may be controlled by the concentration of antigen. This possibility is suggested by the finding<sup>3</sup> that a series of massive doses given during the development of response to a small dose affects proliferation and accelerates differentiation to plasmocytes; here again, the IgG type of response is less easily inhibited by such additional doses than the IgM response.

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## CYTOLOGY

### Chromosome Deterioration in Ageing Eggs of the Rabbit

COITUS-INDUCED ovulation in the rabbit ensures that fertilization of nearly all eggs is initiated within 2 or 3 h of their arrival in the fallopian tube. When ovulation was induced by hormone injection, and coitus permitted at the time of ovulation (10 h after injection), fertilization began about 5 h later and continued for a further 3–5 h, eventually involving a little over 70 per cent of the eggs<sup>1</sup>. As a result, therefore, of the delay in the time of coitus, eggs underwent 6 or 7 h ageing before fertilization was initiated; fertilization nevertheless occurred in all but about 30 per cent of eggs. Despite this relatively small drop in fertilization rate there is evidence that fertility in the rabbit is seriously impaired by the delay of coitus. Chang<sup>2</sup> observed that 60 per cent of eggs failed to implant and that more than half the implanted embryos died before term, and Hammond<sup>3</sup> recorded an 80 per cent reduction in the mean number of young born. To some extent, the reduced fertility can be attributed to the occurrence of polyspermy and polygyny, conditions that lead to the highly lethal state of triploidy in the embryo, but this would not account for embryo loss of more than about 16 per cent<sup>4,5</sup>.

Observations were made on unfertilized eggs and on blastocysts. The unfertilized eggs were recovered at 3 and 7 h after gonadotrophin-induced ovulation; they were examined in the whole state, after fixation and staining under a coverslip. Blastocysts were recovered on the sixth day from rabbits which had been artificially inseminated at the same time that they received an injection of human chorionic gonadotrophin ("normal insemination") and also from rabbits that had been inseminated 8 h after the injection ("delayed insemination"). (With the latter time relations, it is estimated on the basis of previous work<sup>1</sup> that fertilization began 3–7 h after ovulation.) The blastocysts were prepared for karyotype analysis by a modified blood-leucocyte technique. In addition, implanted embryos were recovered at 10 days of pregnancy; the cells were disaggregated with protease and karyotypes again analysed.

Thirty-four eggs from four rabbits were recovered 3 h after ovulation; all exhibited metaphase chromosomes of normal appearance, arranged individually and in a regular manner at the equator of the spindle. Sixty-seven eggs from eight rabbits were recovered 7 h after ovulation. In only twenty-nine eggs could the chromosome group be regarded as normal; twenty eggs had chromosomes that were plainly pycnotic, and coalescing to different degrees, and in eighteen eggs there were signs of chromosome scatter with one, two or even three chromosomes detached from the rest of the group (Fig. 1).

Eleven rabbits were killed on the sixth day after normal insemination. These yielded 81 blastocysts, or 7.4 blastocysts per rabbit. Fourteen rabbits killed after delayed insemination provided 52 blastocysts, or 3.7 blastocysts per rabbit.

Chromosome counts, on from eight to thirty-six spreads for each embryo, were made on seven blastocysts arising from normal insemination and sixteen from delayed insemination. The blastocysts from normally inseminated rabbits all gave counts that were predominantly diploid ( $2n=44$ ), but of which 26 per cent were hypodiploid (38–43). Cells from the 16 blastocysts recovered after delayed insemination, however, yielded 63 per cent hypodiploidy. On the other hand, when the 10-day implanted embryos were examined, results obtained in 23 embryos arising from normal insemination were similar to those in 19 embryos from delayed insemination, hypodiploidy being shown by 33 and 23 per cent, respectively, of the counts made. Thus, though the proportion of hypodiploid cells in the delayed insemination group appeared to be elevated at 6 days, the difference had disappeared in the implanted embryos. Triploidy was evident in two 10-day embryos from normal insemination and in five from delayed insemination.

The findings described here showed that (a), by so short a time as 7 h after ovulation, dispersal or pycnosis had taken place among the second metaphase chromosomes in nearly 60 per cent of unfertilized rabbit eggs; and (b),

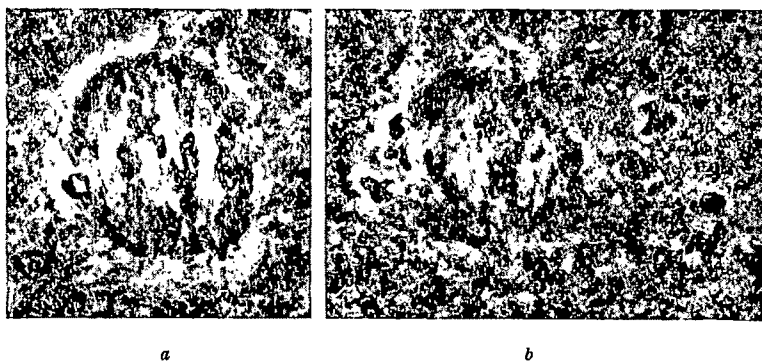


Fig. 1. a, Normal second metaphase chromosome group, at 3 h after ovulation; b, group showing break-up of the spindle and dispersal of chromosomes 7 h after ovulation.

when fertilization began 3–7 h after ovulation, because of delayed insemination, many embryos died during cleavage and the number of blastocysts at 6 days was reduced by half. It is inferred that the chromosome derangements seen in eggs at 7 h after ovulation attested to ageing effects that were responsible for considerable preimplantation losses. Most of the faulty embryos were thus apparently eliminated, and there was no evidence that ageing of the eggs produced errors of chromosome number, other than triploidy, in post-implantation embryos.

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## MICROBIOLOGY

### *In vitro* Growth of *Plasmodium knowlesi* and *P. falciparum*

IMMUNOLOGICAL investigations of the malaria parasite have indicated that effective protective immunity is acquired only against the erythrocytic parasites as they reach maturity. It has been shown<sup>1,2</sup> that chronic malarious sera only agglutinate the mature schizont and that the most effective antigen is derived from the mature erythrocytic form<sup>3</sup>. Gel precipitin reactions have also shown that stronger reactions are obtained against schizont rather than trophozoite antigen of *P. falciparum* when these are diffused against immune sera<sup>4</sup>. Furthermore, passive transfer of acquired immunity is more effective against mature than immature forms<sup>5</sup>.

In some species of malaria parasite, mature erythrocytic stages can be collected from the peripheral circulation but in *P. falciparum* this is not possible because the erythrocytic cycle is concluded in the deep tissues. The only source of parasite antigen has been the collection of schizonts from infected placentae. An obvious prerequisite for large scale investigations of acquired immunity to *P. falciparum* is a relatively simple technique whereby trophozoites obtained from the peripheral blood can be grown to the schizont stage *in vitro*. This paper reports the use of a simplified method for the *in vitro* growth from ring to schizont stage of malaria parasites. Initially *P. knowlesi* was used as a model for the later experiments with natural infections of *P. falciparum* in The Gambia, West Africa.

The medium was a modification of that developed in the Harvard laboratories<sup>6</sup>, with the number of stock solutions reduced to four (solution I, balanced salt solution<sup>6</sup>, solution II, glucose-acetate concentrate<sup>7</sup>, solution III, vitamin concentrate<sup>8</sup>, and solution IV, purines and pyrimidines<sup>8</sup>). The purines and pyrimidines were added at a concentration ten times greater than that of the original medium<sup>8</sup>. Folic acid and vitamin B<sub>12</sub> were added to the vitamin concentrate at a concentration of 100 µg/l.<sup>9</sup> and ascorbic acid was added fresh to the final medium at a concentration of 5,000 µg/l. The amino-acid source was lactalbumin hydrolysate at a final concentration of 0.75 per cent<sup>10</sup>. Reduced glutathione was added to the medium at a concentration of 0.1 per cent. Penicillin was added to give a final concentration of 50 U/ml. and phenol red indicator as 0.2 ml. of a 0.1 per cent solution/100 ml. of medium. The final medium

was sterilized by 'Millipore' filtration and the pH adjusted to 7.4 by bubbling through carbon dioxide.

Infected blood, containing ring stage parasites, was taken aseptically by cardiac puncture from monkeys experimentally infected with *P. knowlesi* and intravenously from Gambians naturally infected with *P. falciparum*. The blood was mixed with sterile 1:1,000 heparin/Ringer in the ratio of one part of heparin/Ringer to ten parts of blood. Initially 4 oz. screw capped medicine flats were used as culture vessels, containing 3 ml. of medium. The bottles were filled with 5 per cent carbon dioxide/95 per cent air mixture to equilibrate with the medium and 1 ml. of heparinized blood added to give a final parasite density of 5,000–50,000 parasites/mm<sup>3</sup> and an erythrocyte density of approximately 1.0–1.5 × 10<sup>6</sup>/mm<sup>3</sup>. Growth of the parasites was evaluated by observing the appearance of the parasite in thin smears, fixed in methanol and stained in Giemsa. Samples were taken from the cultures at the beginning of the experiment and at the end of the incubation period, usually 24 h. Differential counts were made of 200 parasites found in successive microscope fields. A parasite was called a schizont if it possessed three or more nuclei and irrespective of whether it had reached the segmenter stage.

With synchronous *P. falciparum* infections, between 70 per cent and 90 per cent of large ring stages (Fig. 1), taken from the host before their disappearance from the peripheral circulation, developed into schizonts, possessing three to twenty-four nuclei (Fig. 2). The number of abnormal forms was 3 per cent. When very small rings were inoculated into the medium the period of incubation required for growth to mature schizonts was increased to 36–48 h and development was less synchronous. A lower percentage of schizonts, 30 per cent to 50 per cent, were obtained after 36 h of incubation and an increase in the abnormal forms up to 10 per cent was observed. The

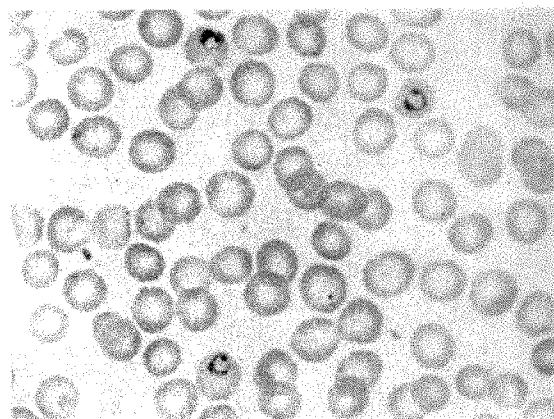


Fig. 1. Culture (0 h) ring-stage of *P. falciparum* (× 700).

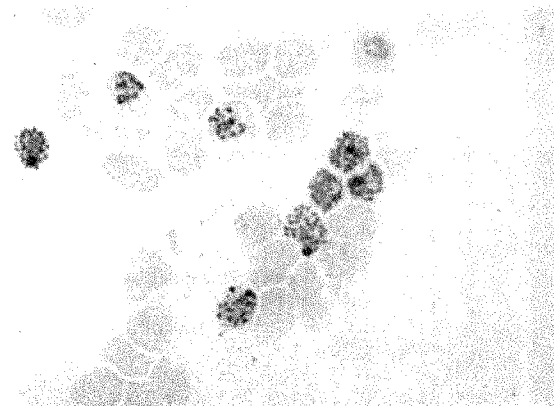


Fig. 2. Culture (24 h) schizont of *P. falciparum* (× 700).

decrease in the yield of schizonts after this longer incubation period was probably caused by the depletion of glucose by the parasites with the subsequent increase in lactic acid and drop in pH in the cultures<sup>11</sup>. A similar effect was observed during a 24 h incubation period with parasite densities of 40,000–50,000/mm<sup>3</sup>. In experiments with *P. knowlesi*, this was shown to be partially overcome by increasing the ratio of medium to parasitized cells from 3:1 to 6:1.

For large scale work involving *P. falciparum* 1 l. bugged Erlenmeyer flasks, containing 25 ml. of medium and 8.3 ml. of heparinized blood, were used. After 24 h incubation consistently good results were obtained with up to 90 per cent of the parasites reaching the schizont stage with < 3 per cent abnormal forms.

It has previously been noted<sup>11,12</sup> that in culture the maturing *P. falciparum* parasite causes the host erythrocyte to become progressively more "sticky" with the subsequent formation of clumps. This was observed in eight out of ten experiments using blood taken from patients more than 3 yr old. Agglutination, however, was absent in two experiments performed with parasitized blood taken from patients approximately 6 months old. The clumping of parasitized cells may be due to either the transfer of agglutinins or histones<sup>13</sup> from the host's serum into culture or to a fibrinogen effect<sup>14</sup>. Agglutination was prevented by replacing the host serum with normal AB serum after thorough washing of the erythrocytes in the medium (one experiment). By using this culture method, it is possible to obtain large numbers of schizonts which can be used to extend investigations on metabolic and antigenic problems in human malaria.

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### Metabolic Activity of Purified Suspensions of *Rickettsia rickettsi*

THE independent metabolic activities of some of the obligate intracellular bacteria, such as rickettsiae and chlamydiae<sup>1</sup>, have been extensively investigated<sup>2</sup>. Such work has rarely included *Rickettsia rickettsi*, the agent of Rocky Mountain spotted fever, because of the difficulty of obtaining sufficient material of adequate purity and stability. The only data on respiration of *R. rickettsi* are those given in a preliminary report by Price<sup>3</sup>, who carried out his experiments with the collection from several thousand infected yolk sacs and tested the micro-organisms immediately after the final step of purification.

The present work was prompted by the opportunity that now exists to compare the biochemical activities of

*R. rickettsi* with those of other nutritionally fastidious bacteria and by the progress in biochemical technology which has occurred during the 13 yr since the report by Price. We have shown that *R. rickettsi* is amenable to relatively simple methods of purification and investigation. Its metabolic activity resembles that of typhus rickettsiae.

Optimal yield of *R. rickettsi* and greatest specific metabolic activity were obtained by following the recommendations of Stoenner *et al.*<sup>4</sup>: yolk sacs of chick embryos, 4–5 days old, were inoculated with sufficient rickettsiae (Bitterroot strain) to kill most embryos in 4–5 days. The eggs were incubated at 33.5° C for 6–7 days and the yolk sacs were collected 36–48 h after the death of the embryos. The initial suspending diluent was SP 25 (sucrose 0.25 molar, potassium phosphate buffer 0.02 molar, pH 7.0). Glutamate, included in the common rickettsial diluent SPG (ref. 5), was omitted because of the report<sup>4</sup> that it adversely affected the virulence of *R. rickettsi* for the chick embryo. The yolk sacs suspended in SP 25 were maintained at –70° C until they were processed further.

The host cells were macerated with glass beads and the rickettsiae were purified by a procedure adapted from those commonly used for agents grown in yolk sac<sup>6,7</sup>. Host cell components were eliminated by cycles of high and low speed centrifugation, adsorption on to 'Celite', precipitation by bovine plasma albumin, and digestion with the proteolytic enzyme 'Pronase'. When partially purified micro-organisms, suspended in diluent KSC (0.15 molar potassium chloride, 0.015 molar sodium citrate, pH 7.0), were treated with 0.1 per cent 'Pronase' for 1 h at 25° C, their infective and metabolic activities were unaffected, although the remaining host components were removed. 'Pronase' or trypsin was not applied during early steps in the purification procedure, because of the experience with typhus rickettsiae (unpublished results of E. Weiss) which indicated that rickettsiae, in contrast to chlamydiae and *Wolbachia persica*, when in crude suspensions, were highly susceptible to the action of trypsin. Final suspensions were made in diluent K 36 (ref. 8), 0.1 molar potassium chloride, 0.015 molar sodium chloride, 0.05 molar potassium phosphate buffer, pH 7.0, to which was added crystalline bovine plasma albumin ('Pentex', final concentration 3 mg/ml.). Concentrated suspensions did not lose metabolic activity when maintained in these conditions at 0° C overnight or even for 2 days.

The metabolic activities of the purified suspensions were determined in 25 ml. Erlenmeyer flasks to which were added the rickettsial suspension, usually containing 0.2–1.0 mg of rickettsial protein, 2.5 × 10<sup>–3</sup> molar magnesium chloride, 5 × 10<sup>–4</sup> molar manganese chloride and substrate labelled with carbon-14 (8.3 × 10<sup>–3</sup> molar) in a total volume of 2.4 ml. The flasks were fitted with small plastic cups suspended from rubber stoppers to which were added 0.2 ml. aliquots of 1 molar 'Hyamine' to trap the carbon dioxide<sup>9</sup>. The flasks were incubated for 2 h at 32° C in a metabolic shaker and the reaction was terminated by the injection of 0.6 ml. of 25 per cent trichloroacetic acid/flask. After an additional 0.5 h of incubation, the cups were placed in liquid scintillation vials and to each were added 2.5 ml. of methanol and 15 ml. of toluene scintillant. The radioactivity of the carbon dioxide was determined in a liquid scintillation counter and the amount of carbon dioxide produced was calculated on the basis of the specific activity of the original substrate. Carbon dioxide produced in the absence of micro-organisms was subtracted. Normal yolk sac preparations, subjected to the same procedures of purification, showed negligible activity. Variation among triplicate tests with rickettsiae was usually less than 5 per cent.

For electron microscopy purified suspensions were centrifuged twice and the pellets were suspended the



first time in 0.26 molar ammonium acetate, the second time in the same solution plus formaldehyde to a final concentration of 0.37 per cent. After 48 h the suspensions were centrifuged again, the pellets were washed with distilled water and suspended in a 1 per cent aqueous solution of osmium tetroxide for 1 h. The specimens were dehydrated through several changes of 'Cellusolve' mixtures increasing from 50 per cent to the pure reagent. After being left overnight in 1:1 'Epon-Cellusolve' mixture, the specimens were embedded in 'Epon', sectioned and stained for 7 min with uranyl acetate and for 5 min with lead citrate.

The average metabolic experiment required the processing of 100 to 150 yolk sacs. Approximately 4 per cent to 25 per cent of the original infectivity was recovered in the final purified suspension or 0.05–0.1 mg of rickettsial protein for each yolk sac. One milligram of rickettsial protein corresponded to approximately  $5 \times 10^7$  chick embryo  $LD_{50}$ .

Light microscopy or electron microscopy of shadowed preparations revealed little, if any, material of host origin in the final preparation. The degree of purity could best be assessed, however, from electron micrographs of sectioned material. All the structures displayed in Fig. 1, except for a staining precipitate, appear to be of rickettsial origin. The micro-organisms are approximately  $0.5\mu$  wide and up to  $3\mu$  long. Details of structure can be recognized (Fig. 2) as previously described in *R. rickettsi* grown in tissue culture<sup>10</sup> and *R. quintana* grown *in vitro* or in the louse<sup>11</sup>.

Results of typical metabolic experiments are shown in Table 1. Of the substrates tested, glutamate was used most vigorously. Production of carbon dioxide in two experiments shown in Table 1, and other experiments not shown, varied from 0.5–1.0  $\mu$ mole/mg of rickettsial protein for the 2 h period of incubation. Glutamine and pyruvate were utilized less intensely: the ratio of carbon dioxide from glutamate to that obtained from glutamine was approximately 3:2, of glutamate to pyruvate 3:1. Approximately half of the carbon dioxide from pyruvate was derived from carbon 1, and one-quarter each from carbons 2 and 3. Two active preparations, as judged by

glutamate utilization, failed to produce carbon dioxide from glucose.

Table 2 illustrates an experiment carried out by the conventional Warburg technique, as well as the technique described here. With the collection from 230 yolk sacs and 2.4 mg of rickettsial protein/flask it was clearly shown that glutamate stimulated the oxygen consumption of *R. rickettsi*. The metabolic activity, however, was not sufficiently great for an accurate determination of the respiratory quotient.

Table 1. CARBON DIOXIDE PRODUCED BY *Rickettsia rickettsi*\*

Experiment No.	Substrate	$\mu$ moles/mg of protein	
1	Glutamate	0.54	0.60
	Glutamine	0.35	0.35
2	Glutamate	0.86	
	Pyruvate: carbon 1	0.14	
	carbon 2	0.08	
	carbon 3	0.09	

\* The micro-organisms were incubated for 2 h at 32° C. Glutamate and glutamine were randomly labelled with carbon-14; pyruvate was labelled as indicated. Data represent means of triplicate flasks. Experiment 1 was carried out with two concentrations of rickettsiae.

Table 2. GLUTAMATE METABOLISM OF *R. rickettsi*\*

No addition	Glutamate
Oxygen consumed	
0.40	1.50
Carbon dioxide produced	
Not tested	1.95

\* Data are expressed in  $\mu$ moles and represent means of triplicate flasks, each containing 2.4 mg of rickettsial protein and incubated for 2 h at 32° C.

By these metabolic tests, which agree with those of Price<sup>3</sup>, *R. rickettsi* appears to be most closely related to the typhus rickettsiae, which utilize glutamate, glutamine, and pyruvate in the same decreasing order<sup>12–15</sup>. *R. quintana*, which has an ecology similar to that of typhus rickettsiae but grown extracellularly, utilizes succinate and glutamine more vigorously than glutamate<sup>16</sup>. *Coxiella burnetii*, which is a tick symbiote like *R. rickettsi*, has a glutamate dehydrogenase<sup>17</sup>, but metabolizes pyruvate as its chief substrate<sup>18</sup>. Another tick symbiote, *Wolbachia persica*, appears to be unrelated, because in contrast to rickettsiae it respire vigorously in the presence of glucose<sup>19</sup>. In the absence of criteria such as nutritional requirements, the investigation of the metabolic activities

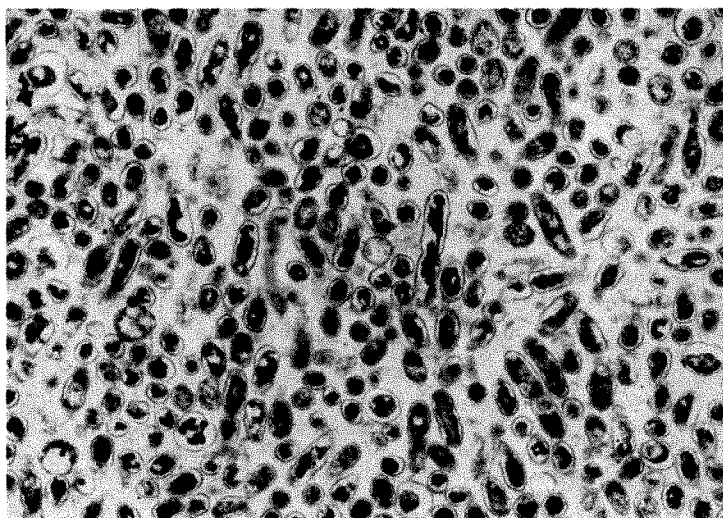


Fig. 1

Fig. 1. An electron micrograph of a section of a purified preparation of *Rickettsia rickettsi*, the agent of Rocky Mountain spotted fever. The micro-organisms were grown in the yolk sac of chick embryos and purified by a procedure which eliminated recognizable host components. Structures analogous to those described in other bacteria can be seen in those rickettsiae which have been sectioned longitudinally through the centre ( $\times$  c. 5,540).



Fig. 2

Fig. 2. A section of *R. rickettsi* clearly demonstrating the cell wall and portions of the cytoplasmic membrane. The light central zone and the two dark polar zones correspond to the DNA strands and the ribosome-filled cytoplasm described in other bacteria ( $\times$  c. 31,540).



of purified rickettsial suspensions appears to be a logical approach to the investigation of evolutionary relationships. This work has established that with *R. rickettsi* this can be done without undue difficulty.

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## BIOCHEMISTRY

### Correlation between the Concentration of Adenosine Triphosphate and the Biosynthesis of Porphyrins in the Livers of Rats Intoxicated with 'Sedormid'

THE well known spontaneous and rapid decrease in the formation of excess porphyrin in several experimental porphyrias after cessation of administration of the toxic agent seemed to us to provide a particularly interesting possibility to assess the role of adenosine triphosphate (ATP) in the quantitative regulation of the biosynthesis of porphyrins. We have observed in a long series of experiments this role of ATP *in vitro* as well as *in vivo*<sup>1</sup>.

For the present investigation, allylisopropylacetyl-carbamide ('Sedormid') porphyria of rats was chosen because there have been several reports that the formation of porphyrins returns to practically normal values in a few days after the administration of the drug has been stopped.

Four groups of female Wistar rats weighing about 200 g and fed a balanced diet were investigated. The rats of the first group served as controls. Those of the second group received by gastric intubation a daily dose of 'Sedormid' (400 mg/kg) during 6 days; they were killed on the morning of the seventh day by decapitation. The rats of the third and fourth groups were intoxicated in the same manner, but were killed 2 and 4 days, respectively, after the last dose of 'Sedormid'.

In the liver removed immediately after death, the concentration of ATP was determined by the luciferase method<sup>2</sup> and that of porphyrins, extractable by ether, by the measure of the optical density of their Soret bands. For quantitative investigations of urinary excretion of  $\delta$ -aminolevulinic acid (ALA) and uroporphobilinogen (PBG), the column chromatographic technique of Mauzerall and Granick<sup>3</sup> was used. Urinary uroporphyrin and coproporphyrin were measured following the technique devised by Rimington<sup>4</sup>.

As is shown in Table 1, we have observed in the rats of the second group a decrease in the hepatic concentration of ATP and an increase in the hepatic concentration of porphyrins as compared with the normal controls. The differences of the means are statistically highly significant.

Table 1.

	Per 100 g of liver (wet weight) ATP ( $\mu$ moles)	Free porphyrins ( $\mu$ g)
Group I (10 rats)	230 $\pm$ 9.7 ( $\pm$ S/ $\sqrt{n}$ )	6.7 $\pm$ 0.68
Group II (14 rats)	150 $\pm$ 9.1	73.0 $\pm$ 13.8
	as compared with group I	
	$t = 6.01$ $P < 0.001$	$t = 4.09$ $P < 0.001$
Group III (13 rats)	185 $\pm$ 7.9	28.0 $\pm$ 6.6
	as compared with group II	
	$t = 2.575$ $P < 0.02$	$t = 2.684$ $P < 0.02$
Group IV (11 rats)	211 $\pm$ 15.3	13.2 $\pm$ 2.7
	as compared with the normal controls (group I)	
	$t = 1.004$ $P > 0.05$	$t = 2.507$ $P < 0.05$

Group I, Normal controls; Group II, rats intoxicated by a daily dose (400 mg/kg) of 'Sedormid' during 6 days; Groups III and IV, rats intoxicated in the same manner, but killed 2 and 4 days, respectively, after the last dose of 'Sedormid'.

In the rats of groups III and IV, in which the administration of 'Sedormid' was stopped 2 and 4 days, respectively, before death, a progressive increase of the hepatic concentration of ATP and a progressive decrease in the concentration of porphyrins were observed as compared with the animals of the second group. In the rats of the fourth group, mean values were quite near to the normal means (Table 1).

Comparison of the hepatic concentration of ATP and of porphyrins in the rats of each group intoxicated by 'Sedormid' shows a statistically significant negative correlation. Thus for group II,  $r = -0.644$  ( $P < 0.02$ ), for group III  $r = -0.598$  ( $P < 0.05$ ) and for group IV,  $r = -0.629$  ( $P < 0.02$ ). (The correlation coefficient  $r$  was calculated from the formula

$$r = \frac{\sum d \cdot d_1}{\sqrt{\sum d^2 \cdot \sum d_1^2}}$$

where  $d$  is the difference between the individual concentration of ATP and the mean for the group and  $d_1$  a similar term for the concentration of porphyrins.)

We observed, in common with others, that the urinary excretion of ALA, PBG, uroporphyrin and coproporphyrin was much raised during the administration of 'Sedormid', but became practically normal about 4 days after the administration was stopped.

The findings reported here show, even more clearly than our earlier observations, a role of ATP in the quantitative regulation of porphyrin biosynthesis. The inverse correlation between the hepatic concentration of ATP and that of porphyrins affords a statistically significant negative correlation between these two concentrations, not only during the production of 'Sedormid' porphyria but also during its spontaneous improvement after withdrawal of the drug. It could be objected that the variations in the hepatic concentrations of ATP and porphyrins might depend on a third unrecognized factor. Militating against this possibility are our earlier observations showing the prevention of 'Sedormid' porphyria (and the other experimental porphyrias) by daily administration of ATP or its precursors such as AMP and inosine<sup>5,6</sup>.

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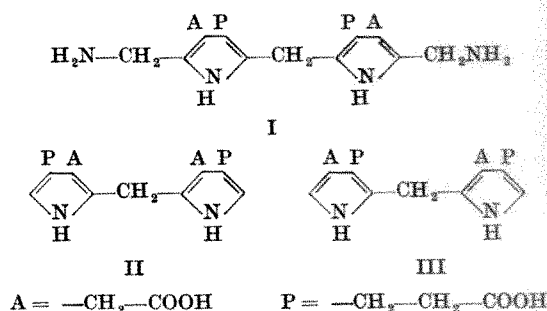
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### Possible Mechanisms for the Enzymatic Condensation of Porphobilinogen

THE problem of the mechanism of the enzymatic condensation of four moles of porphobilinogen (PBG) to form uroporphyrinogen III (urogen III) remains unsolved. Bogorad<sup>1-3</sup> has been able to direct the course of this condensation *in vitro* by using one or two enzyme systems. Porphobilinogen deaminase (PBG-D) or uroporphyrin I synthetase from spinach catalyses the formation of uroporphyrin I (urogen I) from porphobilinogen. Uroporphyrinogen isomerase (U-Is) or uroporphyrinogen III co-synthetase from wheat germ and PBG-D direct the formation of urogen III from porphobilinogen. Kinetic investigations carried out by Bogorad suggest that the action of PBG-D on porphobilinogen is rate-determining and that uroporphyrinogen isomerase participates in a faster step for which porphobilinogen is also a substrate. Uroporphyrinogen isomerase does not act on porphobilinogen alone nor on urogen I (ref. 4). This suggests that some

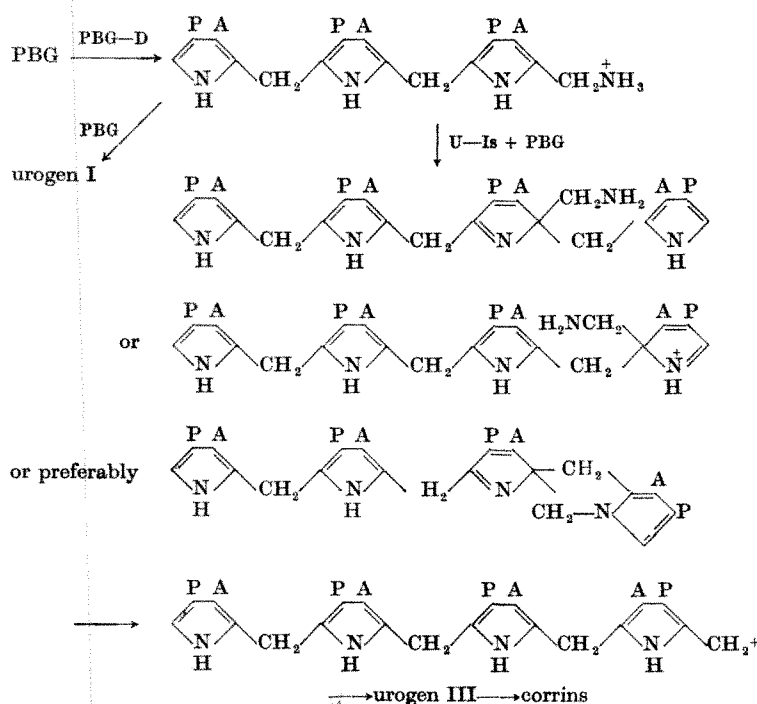
intermediate, the synthesis of which is catalysed by PBG-D, and porphobilinogen are substrates for uroporphyrinogen isomerase.

Of the mechanisms proposed, those amenable to testing have been eliminated. Evidence has been presented against (a) a loss of or exchange of free formaldehyde<sup>4</sup>, and (b) the participation of opsopyrrole dicarboxylic acid<sup>5-8</sup>, porphobilinogen- $\alpha$ -carboxylic acid, cryptopyrrole-dicarboxylic acid, hemopyrrole dicarboxylic acid, porphobilinogen lactam<sup>9</sup>, isoporphobilinogen<sup>10</sup> and the dipyrromethanes, I, II and III (refs. 8 and 10), in purified systems which catalyse the conversion of porphobilinogen to urogen III with one or more enzymes.



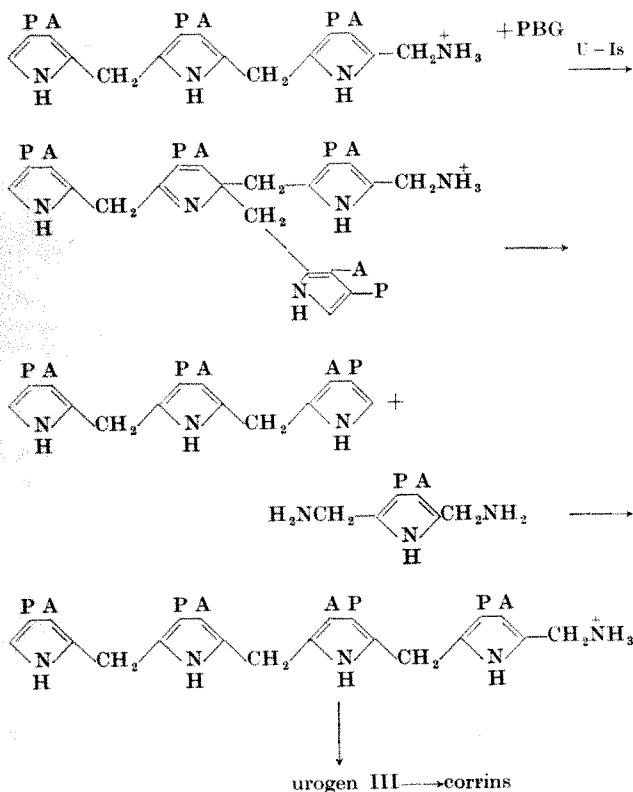
A possible mechanism which needs to be explored is the initial formation of a linear tripyrrole which is catalysed by PBG-D which serves as a substrate for uroporphyrinogen isomerase along with a fourth mole of porphobilinogen. Bogorad<sup>2,9</sup> has detected an Ehrlich-reacting pyrrole intermediate from the reaction of PBG with PBG-D in the presence of hydroxylamine. Uroporphyrinogen isomerase could then direct condensation of PBG on to the substituted  $\alpha$ -carbon of ring C. Intramolecular rearrangement of a one-carbon unit should give a linear tetrapyrrole which would yield urogen III exclusively. Bullock, Johnson, Markham and Shaw<sup>11</sup> have suggested a plausible mechanism for such a rearrangement.

In the absence of the directing influence of uroporphyrinogen isomerase, head to tail condensation would lead to urogen I only.



Porra's work on *Clostridia*<sup>12</sup> indicates that urogen III is probably the precursor for the corrin nucleus.

An alternative suggestion which is worth testing and is somewhat analogous to Bogorad's<sup>9</sup> dipyrromethane proposal is the condensation of PBG with a linear tripyrromethane at ring 2 with the release of  $\alpha$ -aminomethyl-PBG or similarly substituted PBG which then reacts to yield a linear tetrapyrrole precursor for urogen III.



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### DNA during the Development of the American Sea Urchin

We reported recently on the properties of the deoxyribonucleic acids isolated from the egg, sperm and adult diploid tissues of the American sea urchin *Arbacia punctulata*<sup>1</sup>. It was found that the DNA isolated from the two haploid cells had similar buoyant densities of 1.700 g/cm<sup>3</sup>. This can be taken to mean that the DNAs from these sources have identical base compositions<sup>2</sup>. On the other hand, the DNA derived from adult diploid cells exhibited a buoyant density of 1.702 g/cm<sup>3</sup>. If this difference in the density values of the haploid and adult

diploid cells reflects a real shift in base composition, it is indeed a most unexpected finding. The current ideas concerning cellular development and differentiation are based on the belief that just as the adult diploid cells derive from the original zygote by orderly mitotic division, so the DNA from the adult cell is derived from the fertilized egg by exact replicative cycles. Because the DNAs of sperm and egg have identical base compositions, this belief would lead to the prediction that a diploid cell resulting from this union would contain DNA with a correspondingly identical base composition. Implied in this concept is the hypothesis that cellular differentiation is not the result of changes in the DNA but rather that it is controlled by histone-masking and/or "cytoplasmic regulators" which have now been equated with messenger RNA.

In view of the observation that adult diploid cells seemed to possess a base composition not identical with that of the two gametes, the present investigation was undertaken to study the DNA isolated at various stages of embryonic development.

Table 1. BUOYANT DENSITIES OF DNAs ISOLATED AT VARIOUS STAGES OF DEVELOPMENT OF *Arbacia punctulata*

Specimen	Density (g/cm <sup>3</sup> )*	Footnote
Sperm	1.700	a
Egg	1.700	a
Adult diploid	1.702	a
Fertilized egg (pre-cleavage)	1.700	b
2-cell stage	1.700	b
16-cell stage	1.700	b
Morula	1.700	b
Blastula	1.701	c
Gastrula	1.702	d
Pluteus	1.702	c

\* Portions of the echinoderm DNA together with a reference sample (*Micrococcus lysodeikticus* DNA, 1.731 g/cm<sup>3</sup>) were placed in a caesium chloride solution, density 1.70 g/cm<sup>3</sup>, and centrifuged in the 'Spinco' Model E analytical ultracentrifuge at 44,770 r.p.m. for 24 h. The bands formed by these nucleic acids at their buoyant equilibrium positions were photographed with the ultra-violet optical system. The buoyant densities of the samples were calculated from tracings of the photographs obtained with a 'Joyce-Loebl Mark III' microdensitometer.

a. Data taken from a previous study<sup>1</sup>.

b. Two specimens were prepared by a procedure using hot phenol<sup>17</sup>. Each sample was analysed twice.

c. Two specimens were prepared by a procedure using detergents<sup>18</sup>. Each sample was analysed twice.

d. One sample was prepared by the phenol procedure and two of the samples were prepared by the procedure using detergent. Each specimen was analysed twice.

The data summarized in Table 1 indicate that the DNAs isolated from the embryo between the time of fertilization and the beginning of the blastula stage have densities identical with those of DNAs isolated from haploid cells. The DNA isolated during the late blastula stage exhibits a slightly increased buoyant density while DNA isolated after this stage has an even greater density, identical with that of adult diploid tissue.

The data presented here would indicate that the replication of DNA is not exact, which is contrary to Watson and Crick's fundamental theory. If this should be the case, an active role for DNA in cellular differentiation might have to be postulated. (The presence of an excess of cytoplasmic DNA in the unfertilized sea urchin egg is known. The role of this nucleic acid in the phenomenon described here, however, may not have to be taken into consideration, as it has been shown to have a buoyant density identical with that of the nuclear DNA<sup>1,3</sup>.)

In searching for a mechanism that could explain this unexpected finding, the report of Gross *et al.*<sup>4</sup> on the role of messenger RNA synthesis in embryonic development might be germane. Gross and his collaborators found that in the presence of actinomycin D, the development of *Arbacia punctulata* could proceed to the blastula stage but no further. Presumably, continued differentiation was dependent on the availability of new messenger RNA. Because blastulation is also the stage at which a change in the density of the DNA is first noticed, it is possible that messenger RNA might control synthesis of

DNA. Recent work with the RNA-containing Rous sarcoma virus indicates that RNA may indeed transfer information to DNA (ref. 5).

Mention should also be made of the study of Comb<sup>6</sup>, who found that the DNA of the developing sea urchin embryo did not become methylated until the gastrula stage. Although it is unlikely that the extent of methylation would affect significantly the buoyant density of DNA, its contributory effect cannot be ruled out completely.

Actually, evidence of a system somewhat more complicated than an ordered duplication of DNA during successive mitosis can be obtained from recently published studies on the banding properties of DNAs from various animal sources in gradients of caesium chloride. For example, the presence of satellite bands in DNA preparations is well documented<sup>7</sup>. In some cases these bands appear late in the developmental cycle and could not, therefore, have arisen by orderly replication of a gamete in which this special DNA could not be detected<sup>8</sup>. Differences in the buoyant densities of the DNAs of gametes and of somatic cells have been found not only for *Arbacia punctulata* but for the South African "clawed toad" *Xenopus laevis*<sup>9</sup>, the squid *Loligo pealii*, the clam *Spisula solidissima*, and the American oyster *Crassostrea virginica*<sup>10</sup>.

It is also noteworthy in this respect that Mazia and Plaut<sup>11</sup> obtained autoradiographic evidence that the distribution of DNA in the daughter chromosomes of *Crepis* is unequal.

If the DNA of the sea urchin actually "differentiates" during the late blastula stage, a number of biological phenomena become explicable. Spemann<sup>12</sup> and Seidel<sup>13</sup> reported that nuclei isolated from cells during early development are "equipotential" in directing early development. Briggs and King<sup>14</sup> studied this phenomenon further using greatly refined techniques. Their investigations revealed that nuclei taken from late gastrulae are no longer equipotential in directing the development of enucleated eggs. It is perhaps no coincidence that this is the stage when the maximum change in the buoyant density of the DNA is first apparent, although it should be pointed out that the studies dealing with nuclei transplantation were not performed with *Arbacia punctulata*.

Cross-fertilization between certain echinoderms has been reported (for example, *Paracentrotus* × *Arbacia*)<sup>15</sup>. These combinations cleave normally at first, but gastrulation is a fatal event. It is known that the deoxyribonucleic acids derived from the sperm of a number of echinoderms possess identical or very similar base compositions<sup>1,3,16</sup> and it is possible that they even possess some sequence homologies, thus permitting orderly mitosis and DNA duplication to proceed to the completion of blastulation. When gastrulation is about to begin and the specific potentialities of these nucleic acids are expressed in a non-orderly replication, however, this process is either prevented or the new DNA (or the product whose synthesis it directs) is not compatible with survival of the hybrid.

The speculation offered in this paper is based on data obtained mainly from one biological system; however, preliminary results with other species (*Loligo pealii*, *Spisula solidissima*, *Crassostrea virginica*<sup>10</sup>) are in accord with it. If the experimental findings are confirmed, a re-examination of the role of DNA in cellular differentiation may be warranted.

Current studies that are being made of the isotopic distribution of DNA during the different embryonic stages may help to elucidate the mechanism directing the "un-ordered" replication.

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## Nuclear RNA Synthesis in Human Leukaemic Cells

SEVERAL investigators<sup>1-4</sup> have demonstrated RNA synthesis in normal and leukaemic lymphocytes by extraction of RNA from intact cells. To gather more information about RNA synthesis in human leukaemic leukocytes, experiments were designed to characterize nuclear RNA sedimentation patterns. Particular emphasis was placed on the comparison of nuclear RNA synthesis in cells derived from patients with chronic granulocytic leukaemia to those from patients with chronic lymphocytic leukaemia. These leukaemic cell types were chosen for investigation as examples of readily accessible human neoplastic cells of relatively low grade malignancy which appear to originate from two distinct haematopoietic cell lines.

Heparinized whole blood drawn from patients was allowed to sediment for 30-45 min at room temperature; the leukocyte-containing plasma layer was withdrawn, and glucose (3 mg/ml.) and sodium bicarbonate (1 mg/ml.) were added. Aliquots containing approximately  $9 \times 10^8$  cells were incubated at 37° C after addition of uridine-<sup>3</sup>H, to give a final concentration of 1  $\mu$ Ci/ml. (specific activity 3,620 c./mole). Nuclei prepared with citric acid<sup>5</sup> were intact and of high purity as determined by light microscopy. Nuclear RNA was isolated by the method of Salzman *et al.*<sup>6</sup>. In all nuclear RNA extraction procedures, the sodium dodecyl sulphate concentration was 0.34 per cent, and the phenol was freshly redistilled.

The pattern of nuclear RNA synthesis in chronic granulocytic leukaemia cells during 360 min of incubation (Fig. 1) indicated early appearance of an 8S peak, persisting throughout the 90 min of incubation. At 90 min, a 4S peak emerged and the radioactivity was distributed to label a heterogeneous spectrum of RNA. After 90 min of incubation, the 8S peak disappeared, whereas the 4S peak persisted throughout 360 min of incubation. By

180 min, there was labelling in the ribosomal regions, and by 360 min the radioactivity curve approximated the optical density curve. The 8S peak appeared to be an unstable intermediate in nuclear RNA synthesis. Ribosomal RNA was synthesized after the 4S and the polydisperse 16S regions had been labelled.

Table 1. SPECIFIC ACTIVITY (C.P.M./O.D.) OF RNA PEAKS LABELLED AT 6 H

	4S	Peaks	18S	28S
Chronic lymphocytic leukaemia				
Experiment 1	4,150	1,050	1,140	
Experiment 2	4,450	4,000	2,500	
Average	4,300	2,525	1,820	
Chronic granulocytic leukaemia				
Experiment 1	14,800	7,050	4,750	
Experiment 2	6,000	2,560	2,700	
Average	10,400	4,805	3,725	

The pattern of nuclear RNA synthesis in chronic lymphocytic leukaemia cells appeared to be distinctly different from chronic lymphocytic leukaemia. A small rapidly labelled 4S peak appeared which remained throughout the 6 h incubation (Fig. 2). An intermediate (24–26S) peak also formed rapidly and persisted for 90 min, at the end of which some high molecular weight RNA (36S) was also present. After 30 min of incubation, there was progressive formation of ribosomal RNA as well as persistence of higher molecular weight RNA. The rapidly labelled 8S RNA component was not observed in chronic lymphocytic leukaemia nuclei. Quantitation of specific activity in the RNA peaks isolated from the same cells varied considerably in separate experiments (Table 1). When actinomycin D (10  $\mu$ g/ml.) was added after 30 min of incubation, uridine- $^3$ H incorporation into RNA ceased in both chronic lymphocytic and chronic granulocytic leukaemia nuclei. The number of counts in each peak decreased although the pattern of labelling established by

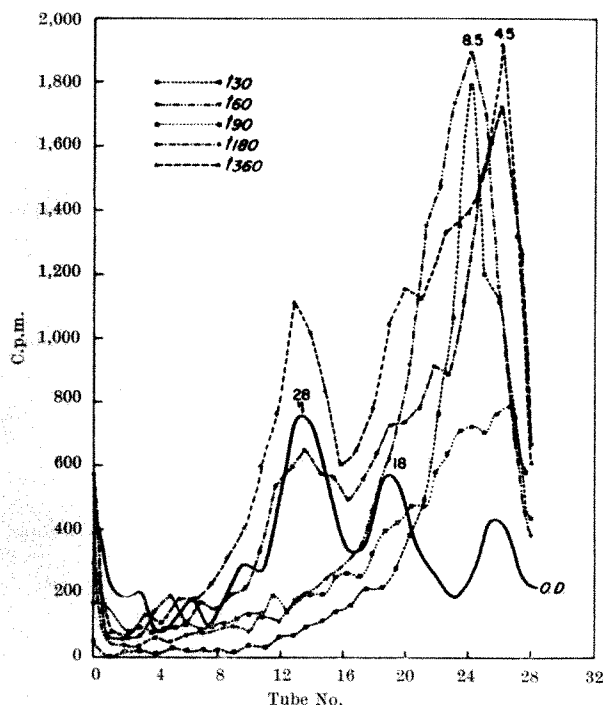


Fig. 1. Nuclear RNA synthesis in chronic granulocytic leukaemia cells. The differential cell count was 50 per cent polymorphonuclear leukocytes, 16 per cent stabs, 8 per cent metamyelocytes, 6 per cent basophils, and 6 per cent monocytes. Leukaemic granulocytes were incubated with uridine- $^3$ H and nuclear RNA was extracted at intervals. Alcohol-precipitable RNA was dissolved in 1 ml. of 0.01 molar acetate buffer (pH 5.1) containing 0.1 molar sodium chloride and 0.001 molar magnesium chloride and layered on to a 5 per cent to 20 per cent sucrose gradient in acetate buffer (magnesium chloride omitted). These gradients were centrifuged at 25,000 r.p.m. for 12 h in a Spinco 'SW-25' rotor. The tube was then punctured and fractions were collected for determinations of optical density at 260 m $\mu$  in a Beckman spectrophotometer. Radioactivity in these fractions was determined by adding Bray's solution and counting in a Beckman liquid scintillation spectrometer.

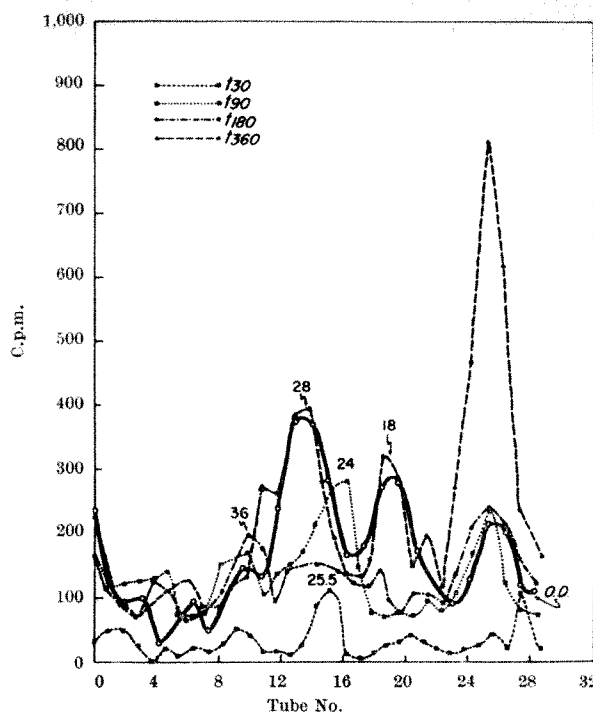


Fig. 2. Nuclear RNA synthesis in chronic lymphocytic leukaemia cells. The differential cell count was 99 per cent abnormal lymphocytes, and 1 per cent polymorphonuclear leukocytes. Procedures are similar to those in Fig. 1.

30 min remained unchanged without progression of label into ribosomal RNA.

These results are not directly comparable with those reported by other investigators<sup>1-4</sup>, because our work involved the synthesis of nuclear RNA rather than total RNA. Granted that other differences exist in the methods of RNA extraction, these results of early labelling of 4S RNA from chronic lymphocytic leukaemia cells are compatible with data reported by Silber *et al.*<sup>1</sup>. The present data, however, differ from their finding of lack of incorporation of radioactivity into the 16S and 28S peaks after 180 min in both chronic lymphocytic leukaemia and chronic granulocytic leukaemia cells.

Rubin and Cooper<sup>4</sup> investigated the sedimentation patterns of total RNA extracted from intact normal resting lymphocytes as well as from lymphocytes stimulated with phytohaemagglutinin (PHA). Lymphocytes stimulated with PHA undergo progressive blastic transformation<sup>8</sup>, and so it is of interest to compare the RNA sedimentation patterns with the RNA patterns of leukaemic leukocytes. After a 6 h exposure to uridine, the sedimentation patterns of total RNA from normal lymphocytes resemble that of mammalian cellular RNA<sup>9</sup>, with the largest amount of label found in the 4S region and smaller amounts in the 18S and 28S peaks<sup>4</sup>. From a comparison of the sedimentation patterns of total RNA extracted from normal lymphocytes and lymphocytes stimulated with PHA with the sedimentation patterns obtained with *n*-RNA from CLL lymphocytes, we made several observations. First, there is a striking similarity in optical density characteristics (28S, 18S, and 4S peaks). Second, the pattern of incorporation in chronic lymphocytic leukaemia *n*-RNA resembles that of the resting lymphocyte more than it does the lymphocyte stimulated by PHA in the synthesis of small quantities of 4S at 30 min followed by progressive but low levels of ribosomal RNA formation. By contrast, the lymphocyte stimulated by PHA incorporated much larger quantities of uridine into all fractions and particularly into polydisperse high molecular weight RNA. Finally, pulse-chase with actinomycin D resulted in loss of the majority of extractable



RNA counts from leukaemic nuclei as with normal and PHA-stimulated lymphocytes. This suggests the presence of large proportions of rapidly labelled RNAs which are unstable, because loss of optical density did not occur on exposure to actinomycin D. In this respect, the lymphocytes appear to differ from the nuclear RNA of HeLa cells, in which 70 per cent of the rapidly labelled RNA is stable<sup>6</sup>. Cooper and Rubin<sup>10</sup>, however, have recently shown that the pattern of labelling of normal lymphocytes can be varied by changing the conditions of incubation.

The rapidly labelled 8S peak from chronic granulocytic leukaemia nuclei was not found in chronic lymphocytic leukaemia nuclei. It is either a ribosomal precursor or messenger RNA. The instability of this fraction would be consistent with both possibilities; the absence of formation of ribosomal RNA following actinomycin D added after 30 min does not provide proof of either. Definition of this peak will depend on characterization of a messenger fraction in leukaemic nuclei in a manner similar to that accomplished in rat liver cells<sup>11-13</sup>, and ultimate identification must depend on stimulation of specific proteins.

Although the results obtained with *n*-RNA may not agree completely with results obtained with RNA extracted from intact cells, it is extremely difficult, with present methods, to extract total undegraded RNA from intact cells, and in particular from intact chronic lymphocytic leukaemia leukocytes. It has been our experience that RNA is extracted with greater ease from leukaemic nuclei because most of the RNase is removed. Nevertheless, there remains considerable variation in the completeness of RNA extraction between duplicate experiments (Table 1). The extent of this methodological variability has not been discussed in other work on leukaemic cells, making quantitative comparisons difficult.

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## Synthesis of RNA in Isolated Cells from *Xenopus laevis* Embryos

INVESTIGATIONS with amphibian embryos are of particular interest because of the recent finding that a sequential synthesis of RNA species takes place during early development: the first, synthesized during very early stages, is soluble RNA, followed by ribosomal RNA, the synthesis of which predominates later<sup>1,2</sup>. These sequential events may provide a parameter of cellular differentiation. The embryo has, however, a definite disadvantage: it needs few exogenous substances except for several inorganic ions which it requires until the feeding stage. For nucleic acid and protein synthesis carbon dioxide is perhaps the

only available precursor which can be incorporated freely into the embryo<sup>3</sup>. We have found that the cells obtained from dissociated embryo actively incorporate a number of radioactive precursors when these are added to the medium and synthesize RNA species sequentially as do whole embryos.

Embryos of *Xenopus laevis* were obtained<sup>1</sup> and kept at 18° C–20° C in the presence of penicillin and streptomycin. Glasses and media used were all sterilized and care was taken to avoid bacterial contamination. Jelly was removed by treatment with 2.5 per cent thioglycollate; dissociation and culture were carried out as previously described<sup>4</sup>.

The cells from 250 dissociated blastulae (stage 8)<sup>5</sup> were incubated in the medium containing 0.25 µc./ml. of uracil labelled with carbon-14 for 10 h and the cells equivalent to fifty embryos were sampled at 2 h intervals for measurements of incorporation into acid-insoluble material. At the same time, 250 sibling embryos were labelled and measured. (When cells were labelled with tritiated-uridine, tritiated-thymidine and protein hydrolysate labelled with carbon-14, it was easy to measure the incorporation of the cells obtained from less than five embryos.) During this period, the blastulae reached stage 14–15. Fig. 1 illustrates the results obtained. The incorporation into the cells rises very sharply throughout the incubation. At the end of the 10 h incubation, the incorporation is more than fourteen times that into the whole embryos. The differences in incorporation between cells and embryos increased with the age of the embryos used, for embryos gradually lost their permeability. After being labelled for 10 h with 3.3 µc./ml. of tritiated-uridine, the cells were collected and RNA was extracted with phenol and detergent-bentonite<sup>2</sup> for methylated albumin column chromatography<sup>6</sup>. The pattern showed all characteristics of that obtained with the whole embryos used as controls; that is, soluble RNA was highly radioactive and the radioactivity peak of ribosomal RNA was low. That ribosomal RNA was eluted as a single peak is of great importance. Ribosomal RNA of bacterial origin is eluted in two peaks while ribosomal RNA from embryos of *Xenopus* and *Rana*<sup>2</sup> shows only a single peak. It is, then, certain from the chromatogram that the synthesized RNA was of embryonic origin. Actinomycin D (0.5 µg./ml.) reduced the incorporation of tritiated-uridine by 83 per cent. Also tritiated-thymidine and protein hydrolysate labelled with carbon-14 incorporated

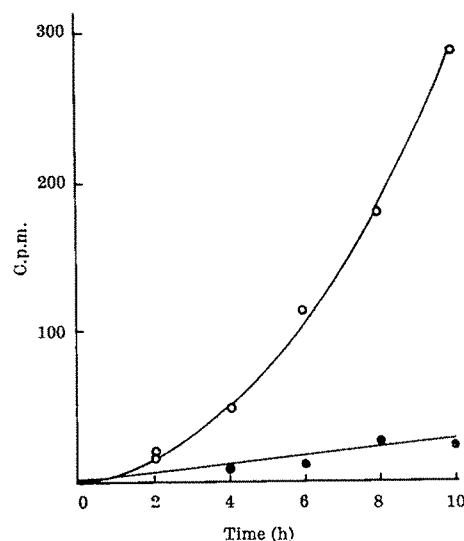


Fig. 1. Incorporation of uracil labelled with carbon-14 into acid-insoluble material of the cells obtained from dissociated blastulae and that of the sibling whole blastulae. The ordinate is c.p.m./fifty embryos or isolated cells equivalent to fifty embryos. ○, Isolated cells; ●, whole embryos.

into acid-insoluble fraction of the cells at constant rates during a 10 h labelling period. Electron microscopic observations revealed that after culture for 10 h the isolated cells retained their normal features; furthermore, nucleoli appeared during this period.

The cells obtained from embryos dissociated at the blastula stage continued to take up tritiated uridine for 25 h. After a 20 h culture, during which the medium was changed three times, the cells were given 2  $\mu$ Ci/ml. of tritiated uridine for 5 h. RNA was purified with phenol, detergent and bentonite and chromatographed on a column of methylated albumin. It was shown that the cells were intensely synthesizing ribosomal RNA as well as soluble RNA. The whole embryos obtained from the same batch were given simultaneously labelled carbon dioxide for 5 h. The pattern which was obtained was essentially the same as that of the dissociated embryonic cells. Again, the elution pattern of ribosomal RNA was a single peak in both cases.

These experiments have demonstrated that the dissociated embryonic cells continued to incorporate a variety of radioactive precursors for a relatively long period and were synthesizing first soluble RNA and then ribosomal RNA in addition. It seems that these isolated cells, instead of whole embryos, can be used to investigate differentiation.

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### Amino-acid Composition of $\alpha_{s1}$ -Casein D

The genetic polymorphism of  $\alpha_s$ -casein was first demonstrated by Thompson *et al.*<sup>1</sup> using the technique of starch-gel electrophoresis. Since that time three genetically controlled variants have been found, which have been denominated as  $\alpha_{s1}$ -casein A, B and C in order of their decreasing electrophoretic mobility.

The amino-acid compositions of these variants have been reported recently<sup>2,3</sup>. The variants B and C of  $\alpha_{s1}$ -casein appeared to differ only by one residue in the amino-acid composition:  $\alpha_{s1}$ -casein B had one more glutamic acid, whereas  $\alpha_{s1}$ -casein C contained one more glycine. The differences found by Gordon *et al.*<sup>3</sup> between  $\alpha_{s1}$ -casein A and B were manifold, and suggest that the former variant cannot be considered as a mutant of  $\alpha_{s1}$ -casein B. More recently Grosclaude<sup>4</sup> reported the discovery of a fourth genetically controlled  $\alpha_{s1}$ -casein, isolated from the milk from one cow of the Flemish breed. According to the recommendations given for the nomenclature of milk proteins<sup>5</sup> it was designated as  $\alpha_{s1}$ -casein D.

The present paper describes the amino-acid composition of  $\alpha_{s1}$ -casein D and compares it with those of the  $\alpha_{s1}$ -caseins A, B and C published earlier<sup>2,3</sup>. A crude sample of  $\alpha_{s1}$ -casein D was obtained from Dr. J. Garnier, Institut National de la Recherche Agronomique, Jouy-en-Josas, France. To demonstrate the differences in electrophoretic mobility, a starch-gel electropherogram of this sample and three samples of whole casein containing the genetic variants of  $\alpha_{s1}$ -casein A, B and C, was made by the method of Wake and Baldwin<sup>6</sup>, using a tray of 50 cm length. Purification of the  $\alpha_{s1}$ -casein D sample was carried out by

column-electrophoresis, as described by Schmidt and Payens<sup>7</sup>. The amino-acid analysis was carried out using a 'Technicon' amino acid analyser as described previously<sup>2</sup>. Determination of the N-terminal group was performed by the dinitrofluorobenzene method of Sanger as described by Fraenkel-Conrat<sup>8</sup>. Identification of the N-terminal amino-acids was effected on thin layers of silica gel by the method of Brenner *et al.*<sup>9</sup>. The identification of dinitrophenyl-arginine was carried out by using high-voltage electrophoresis on thin layers of cellulose in 1 normal ammonia. During electrophoresis a voltage of 1,500 V was applied to the thin layer strip of 20 cm  $\times$  20 cm for about 30 min. The phosphorus content was determined by the method of Griswold *et al.*<sup>10</sup>. Fingerprinting of the tryptic digests of the genetic variants was carried out as described previously<sup>2</sup>.

The starch-gel electropherogram of whole casein samples which contained the genetic variants of  $\alpha_{s1}$ -casein A, B and C, together with a sample of crude  $\alpha_{s1}$ -casein D, is given in Fig. 1. From this pattern it is observed that the mobility of the new genetic variant  $\alpha_{s1}$ -casein D is intermediate between those of  $\alpha_{s1}$ -B and  $\alpha_{s1}$ -A. The relative mobility of  $\alpha_{s1}$ -casein D, calculated according to Wake and Baldwin<sup>6</sup>, is 1.13.

The results of the amino-acid analyses are given in Table 1. It is clear that the calculation of the absolute numbers of residues depends on the molecular weights chosen. Because the exact value of the molecular weight of all the different genetic variants is not yet known, we have based our calculations on a value of 28,600 which was calculated by Gordon *et al.*<sup>3</sup> from the amino-acid composition of  $\alpha_{s1}$ -casein B and C.

Considering the amino-acid composition of  $\alpha_{s1}$ -casein D as a whole, it is obvious that the amino-acid composition of the genetic variants of  $\alpha_{s1}$ -casein D and B fully agree, except for two amino-acids. Compared with  $\alpha_{s1}$ -casein B,  $\alpha_{s1}$ -casein D contains one more residue of proline and one less residue of serine. According to Pelc<sup>11</sup> such mutations are in agreement with a single base substitution in the

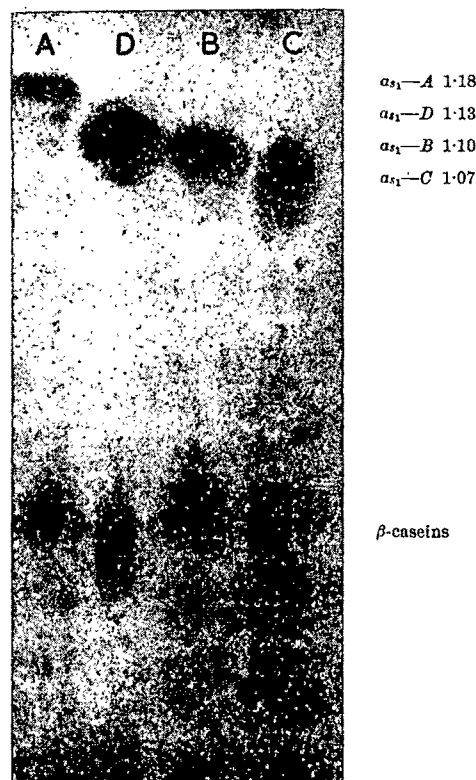


Fig. 1. Starch-gel electropherogram of the genetic variants of  $\alpha_{s1}$ -casein. Only the region containing the  $\alpha_{s1}$ - and  $\beta$ -caseins is shown.

Table 1. AMINO-ACID COMPOSITION OF THE GENETIC VARIANTS OF  $\alpha_{s1}$ -CASEIN  
Residues amino-acid per 28,000

	Gordon <i>et al.</i> <sup>1</sup> A†	Gordon <i>et al.</i> <sup>1</sup> B	De Koning <i>et al.</i> <sup>2</sup> B†	Gordon <i>et al.</i> <sup>2</sup> C	De Koning <i>et al.</i> <sup>2</sup> C†	This paper* D
ASP	17.2	18.1	17.9	18.2	17.6	18.0
THR	6.8	6.0	5.7	6.1	5.8	6.3‡
SER	18.2	17.3	16.8	17.6	16.6	15.8‡
GLU	47.6	46.4	47.0	45.5	45.9	47.0
PRO	21.0	20.3	19.5	20.4	20.0	20.5
GLY	10.9	10.7	10.7	11.8	11.8	10.9
ALA	10.1	10.8	11.0	10.8	11.0	10.4
VAL	12.2	13.4	13.2	13.6	13.4	13.1§
MET	6.0	5.7	5.7	5.7	5.6	5.3§
ILEU	13.9	13.1	12.9	13.3	13.0	13.0§
LEU	17.7	20.3	20.1	20.5	20.1	20.1
TYR	12.4	11.6	11.4	11.7	11.4	11.2
PHE	7.7	9.6	9.4	9.7	9.4	9.6
TRY	2.9	2.7	3.4	2.8	3.5	3.2
LYS	18.5	17.0	16.3	17.0	16.2	16.6
HIS	6.3	6.1	6.0	6.1	6.0	5.8
ARG	6.2	7.2	7.0	7.2	7.0	7.2
NH <sub>2</sub>	27.7	31.1	32.3	29.7	33.1	33.0‡
PO <sub>4</sub> H	—	—	11.5	—	11.7	11.4¶

\* Average of duplicate analysis of hydrolysates at 24, 48 and 72 h.

† Recalculated on a basis of 28,000.

‡ Linearly extrapolated to zero time.

§ Values found at 72 h of hydrolysis.

|| Determined by the method of Spies and Chambers<sup>13</sup>.¶ Determined by the method of Griswold *et al.*<sup>10</sup>.

messenger RNA sequence involving the coding triplets CCA/UCA. The differences between these amino-acids, however, do not explain the higher electrophoretic mobility of  $\alpha_{s1}$ -casein D. Separate determination of the number of amide groups by the method of Stegemann<sup>12</sup> showed an equal value of thirty-one residues in both cases. A difference in the electric charge of the molecule caused by the presence of different numbers of phosphate groups is unlikely, because in  $\alpha_{s1}$ -casein B and D the phosphorus contents appeared to be identical. It is therefore clear that the differences in electrophoretic mobility cannot be explained electrostatically. The N-terminal amino-acid of  $\alpha_{s1}$ -casein D was shown to be arginine, which was also found in the other genetic variants<sup>14</sup>. Fingerprints of the tryptic digests of  $\alpha_{s1}$ -casein B and D showed identical peptide patterns, which present a further indication that these proteins closely resemble each other.

On account of these results it is apparent that  $\alpha_{s1}$ -casein D is to be considered as a mutant of  $\alpha_{s1}$ -casein B. The total number of electric charges in both  $\alpha_{s1}$ -casein D and B is the same, so that the observed differences in electrophoretic mobility may be explained by the different conformations of the genetic variants. This may possibly be the consequence of the excess proline residue in  $\alpha_{s1}$ -casein D.

We thank Dr. J. Garnier for supplying a sample of  $\alpha_{s1}$ -casein D. Also we thank Mr. A. Kok and the Misses P. Both and A. C. J. Heerdt for their skilful technical assistance.

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## Effect of Acetylation on the Biological Activity of Pregnant Mare Serum Gonadotrophin

DURING our recent investigation into the mechanism of action of pregnant mare serum gonadotrophin (PMS)<sup>1</sup>, we prepared a sample of highly radioactive peptide hormone. Radioactive steroid hormones are relatively easy to prepare and have been utilized in investigations on the mechanism of action of these hormones<sup>2</sup>. More recently, radioactive peptide hormones have been reported<sup>3-5</sup> to have similar biological and antigenic properties to the native proteins. Acetylation of bovine growth hormone, utilizing tritiated acetic anhydride, resulted in a sample of hormone biologically and antigenically equivalent to the native hormone<sup>6</sup>, while treatment of ACTH under similar conditions led to a loss of hormonal activity<sup>7</sup>. The results of our attempts to acetylate PMS are now reported.

PMS ('Equinex', Ayerst Laboratories) was acetylated as outlined previously for bovine growth hormone<sup>4</sup>, with slight modification. Each 5,000 IU sample of hormone was suspended in 1.0 ml. of saturated sodium acetate and the pH adjusted to 7.5 by the addition of 0.1 N sodium hydroxide, after the addition of 20  $\mu$ moles of tritiated acetic anhydride. The acetylated hormone was separated from the free acetate by passing the mixture through a 1.4  $\times$  50 cm 'Sephadex G-10' column and eluting the hormone with water. The effluent was collected in 1.5 ml. fractions. Radioactivity in these fractions was measured in a Packard scintillation counter and the hormone detected by its absorption at 280 m $\mu$ . A sample of hormone was carried through the entire procedure without adding the acetic anhydride. Evidence that the hormone had been acetylated was obtained by rechromatography of the labelled hormone and by acid precipitation.

Assay of all PMS samples was carried out by a modification of the previously published uptake investigations for phosphorus-32 (ref. 1). The PMS was administered as a single subcutaneous injection to white Leghorn cockerel chicks 24 h old. Inorganic phosphorus-32 (2.0  $\mu$ Ci/animal) was administered subcutaneously 1 h later. Distilled water served as the diluent for both materials and the total injection volume was 0.2 ml. for each. Chicks were killed by cervical fracture 24 h after hormone treatment, the testes quickly removed and weighed to the nearest 0.1 mg on a 50 mg torsion balance. Radioactivity measurements of whole testes were made on a Nuclear-Chicago gas flow counting system. Total tissue counts divided by gross weight yielded the c.p.m./mg data, which were submitted to analysis of variance.

The results of the purification of the acetylated hormone on 'Sephadex' are presented in Fig. 1. It is evident that the radioactivity completely paralleled the optical density in both chromatograms. This was good evidence that a successful acetylation of the hormone had been carried out. Further proof of this fact was obtained after acid precipitation of the hormone in the presence of carrier serum albumin. It was found that 74 per cent of the radioactivity was insoluble in 5 per cent trichloroacetic acid and the radioactivity remained bound to the protein during repeated acid precipitations.

The results of the biological assays are presented in Table 1. It is evident that acetylation of PMS hormone (PMS-A) abolished its biological activity. This loss in activity was not due to the protein being subjected to treatment with saturated sodium acetate at pH 7.5 to 8.0, as indicated by the results of the assay for the hormone sample PMS-A control.

Until the identity of the acetylated groups in the hormone is known, no reasonable speculation can be offered to explain the lack of activity that resulted. This investigation of acetylated groups may well give some indication of the important functional groups in the PMS molecule. However, acetylation cannot be used to obtain biologically active radioactive hormone. If radioactive

Table 1. BIOASSAY COMPARING UNTREATED AGAINST ACETYLATED PMS

Treatment	Average testis weight (mg)	Standard error	c.p.m./mg	Standard error	Per cent increase*	P†	P‡
Control	7.62	±0.42	14.92	±0.79	—	—	—
10 $\mu$ PMS	11.60	±0.70	34.08	±1.59	128.42	<0.01	—
5 $\mu$ PMS	10.19	±0.71	28.90	±0.66	93.70	<0.01	—
2.5 $\mu$ PMS	10.66	±0.56	29.68	±1.34	98.93	<0.01	—
10 $\mu$ PMS-A Control ¶	10.53	±0.59	33.92	±1.54	127.34	<0.01	>0.01
5 $\mu$ PMS-A Control	10.66	±0.46	28.77	±1.04	92.83	<0.01	>0.01
2.5 $\mu$ PMS-A Control	8.71	±0.60	23.91	±0.27	60.25	<0.01	<0.01
10 $\mu$ PMS-A§	7.79	±0.49	16.16	±0.74	8.31	>0.01	<0.01
5 $\mu$ PMS-A	8.02	±0.33	16.94	±0.66	13.54	>0.01	<0.01
2.5 $\mu$ PMS-A	7.97	±0.43	17.71	±0.70	18.70	>0.01	<0.01

\* Per cent increase in c.p.m./mg.  $n = 20$ /series.

† Probability when all PMS groups compared to control.

‡ Probability when both PMS-A groups compared to PMS equivalent doses.

§ PMS-A — Radioactive acetylated hormone.

¶ PMS-A Control — Non-acetylated PMS carried through reaction procedure.

PMS is required in further investigations, tritium gaseous exchange at 0° C, as outlined for the production of labelled ACTH (ref. 5), will be investigated.

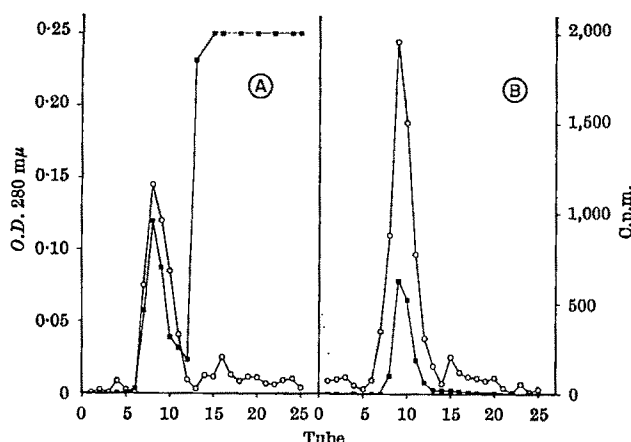


Fig. 1. Elution patterns of tritiated PMS-A from a Sephadex G-10 column (1.4 × 50 cm). The eluent was water; 1.5 ml. per fraction. A, Chromatography of PMS acetylation reaction mixture; B, chromatography of tritiated PMS-A in the presence of non-acetylated PMS. O, Optical density at 280 mμ; ■, radioactivity ( $^3$ H c.p.m./0.001 ml. of each fraction).

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### Suppressing Effects of Histamine on Lipid Synthesis in Chick Aortic Cells

Most investigations of histamine activity relative to the cardiovascular system have been concerned with haemodynamic effects. In a search for the effects of various agents on lipid synthesis in aortic cells in culture it was found that histamine interferes with lipid synthesis. Furthermore, such suppression of lipid synthesis can be prevented by the addition of an antihistamine.

Aortic cells were prepared by trypsinization from the stripped aortic intimal layer of 3 week old Rhode Island Red chicks as reported previously<sup>1</sup>. The cells were suspended 1 : 300 v/v in Eagles minimum essential medium

(MEM), plus 10 per cent foetal calf serum, planted 5 ml. in each flask and cultured until monolayer sheets formed. Secondary cell lines were prepared from the primary cells by trypsinization and comparison of metabolic rates in both generations were carried out. After 4 or 5 days of culture, histamine was added for 3 days in doses of from 0.1  $\mu$ g to 10  $\mu$ g/ml. of MEM with 5 per cent calf serum. After treatment with histamine, the aortic cells were incubated with new media containing sodium acetate labelled with carbon-14 (0.44  $\mu$ Ci/ml. of MEM) for 6 h. The exposed cells were washed three times and trypsinized. The cellular lipids were extracted twice with methanol and chloroform (1 : 2 v/v)<sup>2</sup> and washed repeatedly until the washed aqueous portion was no longer radioactive. Half of the washed lipid fraction was measured gravimetrically for total lipids after concentration and drying under a nitrogen stream. The other half was dissolved in acetone and ether (1 : 2 v/v); free sterol was precipitated as digitonide and purified by the Sperry and Webb method<sup>3</sup>. Free sterol was determined by the acetic acid-ferrous sulphate reagent<sup>4</sup>.

Preliminary experiments had shown that the amount of labelled acetate incorporated into lipids is adequate at 6 h and continues in a linear fashion for at least 12 h. The presence of histamine in the aortic cells for 3 days demonstrated its inhibitory effects on lipid synthesis in various conditions: in both primary and secondary cells; and in cells cultured in MEM with or without human lipaemic serum (total lipids 790 mg per cent and cholesterol 318 mg per cent). Examinations of the cell cultures for deleterious effects of histamine were negative. The cell monolayers were intact after 3 days of exposure to the highest

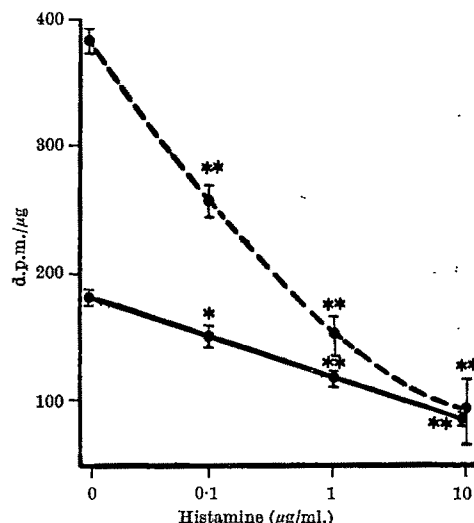


Fig. 1. Effect of the concentration of histamine on incorporation of labelled acetate into total lipids (solid line) and free sterol (broken line) in primary aortic cells. The cells were incubated with histamine at the concentration indicated for 3 days. The points and vertical lines indicate the means and standard errors. The differences in the specific activities of synthesized lipids between each level of the concentrations and of the corresponding controls are compared for statistical analysis. \*  $P < 0.01$ . \*\*  $P > 0.001$ .

concentration; cell counts and lipid weights were equivalent to control values.

Fig. 1 illustrates the effect of the quantity of histamine on lipid synthesis in the primary aortic cells. At the concentration of 0.1–10  $\mu\text{g}/\text{ml}$ . of MEM, incorporation of labelled acetate into total lipids and free sterol were significantly suppressed by histamine. The highest specific activity in the free sterol portion was related to a greater suppression of incorporation of carbon-14.

The effect of the incubation time is shown in Fig. 2, where both primary and secondary cells were incubated for the time indicated with histamine in a dose of 0.1  $\mu\text{g}$  or 1  $\mu\text{g}/\text{ml}$ . of MEM. Both generations showed similar results and the data are combined in the figure. Again, the suppression of lipid synthesis by histamine is found to be related to the incubation time and dose in both generations. In the two concentrations the rates of suppression were significant when compared with the zero levels.

The data given above clearly indicate that histamine possesses inhibitory properties toward lipid synthesis. Furthermore, the antagonistic effect of an antihistamine, diphenhydramine hydrochloride (DPH), confirmed the above observation. When this compound was added with histamine for 3 days in the primary cells, the suppression of lipid synthesis by histamine was prevented (Fig. 3). The greater concentration of DPH showed incorporation of labelled acetate in total lipids and free sterol, equivalent to the untreated control value. It

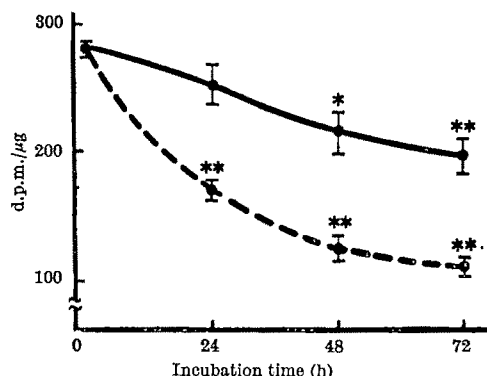


Fig. 2. Effect of the incubation time of histamine on free sterol synthesis in aortic cells. Histamine was added in a dose of 0.1  $\mu\text{g}/\text{ml}$ . of MEM (solid line) and 1  $\mu\text{g}/\text{ml}$ . of MEM (broken line) at the time indicated. Each determination was made using four to six samples. Statistical comparison of the synthesized sterol was made between the zero time and the indicated incubation time and symbolized as in Fig. 1.

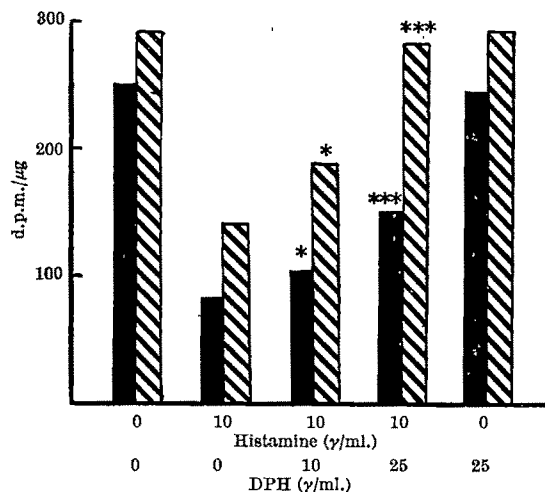


Fig. 3. Antagonistic effects of histamine and DPH on lipid synthesis in chick aortic cells. Primary aortic cells were incubated with the media containing labelled acetate (0.44  $\mu\text{C}/\text{ml}$ .) for 6 h. The black and hatched columns indicate synthesized total lipids and free sterol respectively. Statistical comparisons were made between the group treated with histamine only and the groups treated with both histamine and DPH. \*  $P < 0.05$ . \*\*\*  $P < 0.001$ .

interfered with the histamine suppressing effect. Pre-treatment of cells with histamine for 2 days followed by another 2 day interval of only fresh MEM or DPH in MEM were compared with the d.p.m./μg of total lipids of controls. The results showed, after the 4 day treatment schedules: histamine 64 per cent of control value; DPH plus histamine approximately 100 per cent.

Although these data show an effect of histamine on lipid synthesis it must be emphasized that only cells from chick aortas have been observed. The effect of histamine on cells from other organs and other species must also be investigated.

The demonstration of interference of histamine effect by DPH could be valuable in elucidating the mechanism of action of histamine in intercellular lipid synthesis. Furthermore, these data represent a simple rapid method for surveying compounds that have antihistamine potentials. The question of how these data may be related to the total organism and the development of hypersensitivity and possibly atherogenesis needs considerable attention.

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### Optical Rotatory Dispersion of Complexes of Native DNA and Helical Poly- $\alpha$ -L-glutamic Acid with Proflavine

RECENTLY, Blake and Peacocke<sup>1</sup> reported the appearance of an induced Cotton effect in the complex of deoxyribonucleic acid (DNA) with proflavine. The magnitude of the trough at 452  $m\mu$  was almost twice as large as that of the peak at 480  $m\mu$ . They considered the absorption band of the complex to be associated with a single electronic transition and made it appear that the anomalous optical rotatory dispersion (ORD) induced by the bound dye was a single rather than a multiple Cotton effect. Their interpretation of the observed Cotton effect challenges theories of optical activity which predict a single Cotton effect associated with a single circular dichroic (CD) band nearly symmetric with respect to either the centre of the CD band or the inflexion point of the ORD (refs. 2–4). We propose an alternative interpretation, which is in harmony with existing theories, of the ORD results of Blake and Peacocke.

In Fig. 1 an ORD curve of a solution of DNA plus proflavine is shown together with the absorption spectra. The experimental conditions are essentially those of Blake and Peacocke. The observed rotation is expressed in terms of the molar rotation. The general shape of the ORD resembles that of Blake and Peacocke but there are several discrepancies between the two curves with regard to the positions of the peaks and trough as indicated in Table 1a. The most important difference is found in the wavelength region below that of the trough. Going towards shorter wavelengths, the ORD curve approaches zero rotation between 420–400  $m\mu$  and then becomes increasingly negative below 400  $m\mu$ ; this detail is missing in the curve given by Blake and Peacocke.



The presence of two peaks and a trough in the region of anomalous dispersion certainly rules out, according to existing theories, the probability of there being but a single Cotton effect. The complexity of the experimental curve indicates a degree of multiplicity. In addition, the absorption spectrum of the complex shows a shoulder at 425–435 m $\mu$ .

A mixture of sodium poly- $\alpha$ ,L-glutamate (NaPLG) in a helical conformation and proflavine also exhibits extrinsic optical activity throughout the absorption band of the dye (Fig. 1). In contrast to the overt changes noted with DNA, the absorption spectrum of this NaPLG-dye complex shows no new peak or shoulder; nevertheless, the induced Cotton effect is a typical double one with opposite signs. The magnitude of the trough is more than three times that of either of the peaks.

To demonstrate the fact that the induced Cotton effects as observed are not single but multiple, the ORD curves were resolved into their components with the aid of the Kronig-Kramers transform<sup>4</sup> (Figs. 2 and 3, Table 1b).

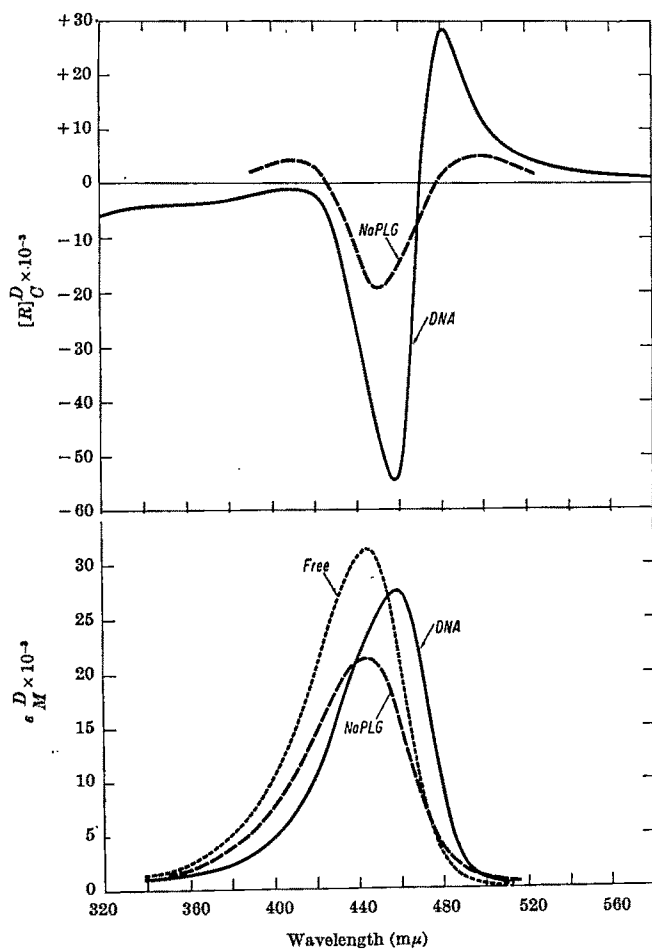


Fig. 1. Absorption spectra and ORD curves: —, DNA-proflavine, DNA phosphate  $1 \times 10^{-4}$  molar, proflavine  $2.07 \times 10^{-5}$  molar, NaCl  $1 \times 10^{-3}$  molar, pH 6.5; ---, NaPLG-proflavine, NaPLG 3.26 mg/c.c., proflavine  $2.07 \times 10^{-5}$  molar, no salt added, pH 4.5, dye/polymer  $\sim 1$ ; ····, proflavine, no salt added, pH 6.5. A 'Cary Model 60' spectropolarimeter and a 'Cary Model 14' spectrophotometer were used. Due precautions were taken to avoid complications that might arise from instrumental artefacts<sup>5</sup>. The molar rotation of the complex solution in terms of the total dye concentration,  $[R]_C^D$ , is defined as

$$[R]_C^D = \frac{10}{M_D} (\theta_C^P/l_C - \theta_P^P/l_P)$$

where  $M_D$  is the molarity of dye,  $\theta_C^P$  and  $\theta_P^P$  are net rotations in degrees and  $l_C$  and  $l_P$  is the path length in decimetres for complex and polymer solutions. The polymer concentration in both solutions is maintained at the same level.

$$[R] = \sum_k [R]_k = \frac{96N}{hc} \cdot \sum_k \frac{(R_k \lambda_k)}{\Delta_k} \left[ e^{-(\lambda - \lambda_k)^2 / \Delta_k} \cdot \int_0^{\lambda - \lambda_k / \Delta_k} e^{-x^2} dx - \frac{\Delta_k}{2(\lambda + \lambda_k)} \right] + \frac{m}{\lambda^2} \quad (1)$$

where  $[R]$  is the total molar rotation,  $N$  Avogadro's number,  $h$  Planck's constant,  $c$  the velocity of light,  $\lambda_k$  transition wavelength,  $R_k$  rotational strength,  $\Delta_k$  half-intensity band width, and  $m$  a constant. To facilitate calculation, the following assumptions were made. (a) Two partial rotations are necessary to describe fully the observed Cotton effect in the visible absorption band of the bound proflavine; and (b) the CD bands are represented by Gaussian distributions on the wavelength scale, that is,

$$\epsilon_l - \epsilon_r = (\epsilon_l - \epsilon_r)_{\max} \cdot e^{-(\lambda - \lambda_k)^2 / \Delta_k} \quad (2)$$

For the DNA-proflavine system the first component Cotton effect, with an inflexion point at 468 m $\mu$ , has a positive rotational strength. The second one centred at 442 m $\mu$  is of opposite sign and more damped than the first, as the half-intensity band widths indicate. For the NaPLG-proflavine system, the parameters of the positive component Cotton effect at 466 m $\mu$  are similar to those

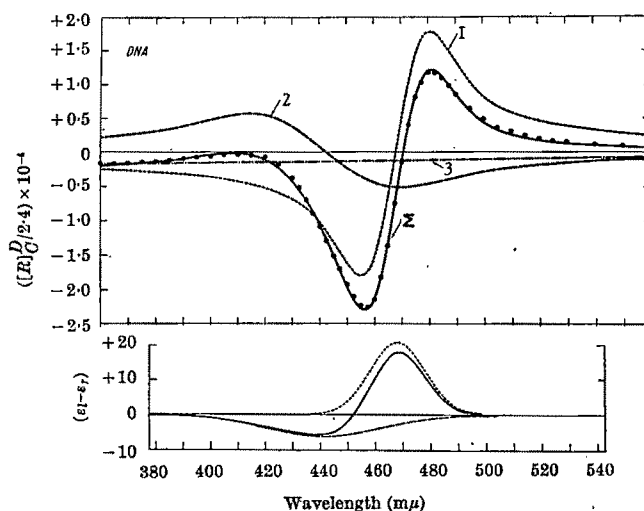


Fig. 2

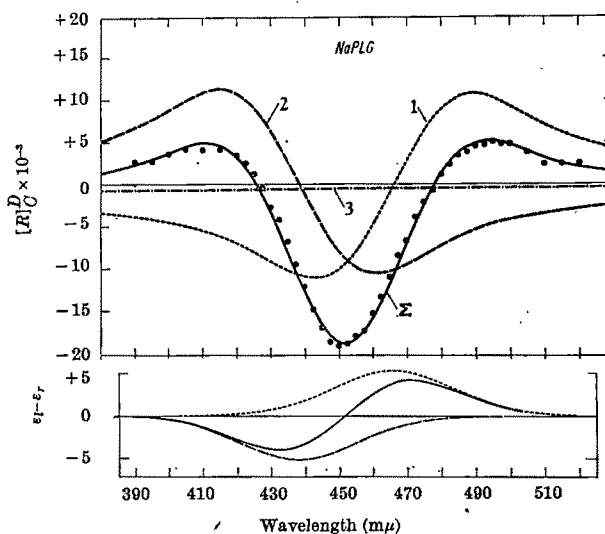


Fig. 3

Figs. 2 and 3. Resultant and component curves of ORD and CD for DNA- and NaPLG-proflavine. Filled circles are experimental points. The same symbols are used for the respective ORD and CD curves. Curves 1 and 2 are the component Cotton effects, while curve 3 is the background rotation.  $\Sigma$  is the sum of curves 1, 2 and 3.

Table 1a. RESULTS OF ORD AND ABSORPTION FOR PROFLAVINE, DNA- AND NaPLG-PROFLAVINE

Proflavine plus	Absorption		Peak (m $\mu$ )		ORD		Trough (m $\mu$ )	
	$\lambda_{\max}$	$\epsilon_{\max}$	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
pH 6.5	443.5	31,500	—	—	—	—	—	—
Itself pH 4.5	443.5	33,300	—	—	—	—	—	—
DNA (Blake and Peacocke)	460	—	480	—	460	—	452	—
DNA (present data)	457.5	27,600	481.5	481.5	471	471	457	456.3
NaPLG	443.5	21,400*	410†	410	478	478	450-451	451.5
			405-415†	410	426-427	425.7		

\* Less accurate. † Broad.

Table 1b. PARAMETERS OBTAINED FOR COMPONENT COTTON EFFECTS

Proflavine plus	$\lambda_K$ (m $\mu$ )		$\Delta K$ (m $\mu$ )		$RK \times 10^{40}$		$m^*$	$K_1^{(e_1-e_r)_{\max}}$	
	$K_1$	$K_2$	$K_1$	$K_2$	$K_1$	$K_2$		$K_1$	$K_2$
DNA	468	442	14	30	+25.9	-17.8	$-5.87 \times 10^{-8}$	+20.6	-6.3
NaPLG	466	438	25	25	+11.7	-12.5	$-7.5 \times 10^{-7}$	+5.2	-5.2

\* Deg. cm<sup>2</sup>/molarity dm.

of the negative one at 438 m $\mu$ . It is worth noting that two very diverse helical macromolecules give rise to component Cotton effects the location and rotational strength of which with regard to both sign and magnitude are closely related. Furthermore, the rotational strengths in the two systems studied are quite comparable with those obtained for helical polypeptides<sup>6</sup>. A weak background rotation expressed by the last term in equation (1) was added in each case, because a slight alteration in the conformation of the polymer may result when dye is bound; and the influence of a strong absorption band of the bound proflavine around 257 m $\mu$  should be taken into consideration. The resultant CD curves computed from the component Cotton effects are given at the bottom of Figs. 2 and 3.

The resultant Cotton effect, calculated from components, agrees surprisingly well with those measured for the two systems. The possibility exists that the second Cotton effect of DNA-proflavine may not be single, but a composite of two closely overlapping effects of like sign. This does not in any way detract from our present proposition, because it is now evident that the observed extrinsic Cotton effect consists of at least two components of opposite signs. This is the case in spite of the fact that the observed absorption spectra do not clearly indicate the electronic transitions involved.

Peacocke and Skerrett<sup>8</sup> found that values of  $r$ , the number of proflavine molecules bound per phosphate residue of DNA, determined by equilibrium dialysis, agreed with those calculated from optical density measurements in the range 430-440 m $\mu$ , but not with those based on data at 460 m $\mu$ . They concluded that the optical density at 460 m $\mu$  for DNA-proflavine must vary with values of  $r$ . This observation suggests that the absorption band of the dye in the complex is heterogeneous and that at least two optical transitions are involved. This is consistent with our results. It seems likely that a positive Cotton effect at 468 m $\mu$  may be associated with an electronic transition around 460 m $\mu$  and that a negative Cotton effect at 442 m $\mu$  may correspond to the transition between 420 and 440 m $\mu$ . The origin of these suggested optical transitions for both DNA- and NaPLG-proflavine complexes must await further studies. Detailed work on the absorption spectra of proflavine itself should precede the final assignment<sup>7</sup>.

We thank Dr. E. Charney of this laboratory for his helpful discussion.

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## New Leuco-anthocyanins in Grasses

EXAMINATION of the leaves of a series of grasses in the usual way<sup>1</sup>, which involves hydrolysing the leaves in 2 normal hydrochloric acid and extracting the hydrolysate with isoamyl alcohol, showed in four of them the presence of a component giving rise to a scarlet-coloured anthocyanidin having an  $R_f$  of 0.62 in Forestal solvent (acetic acid : concentrated hydrochloric acid : water, 30 : 3 : 10 by volume). The extract, diluted with methanol, had  $\lambda_{\max}$  between 496 and 502 nm, indicating its probable identity with luteolinidin ( $R_f$  0.62 in Forestal,  $\lambda_{\max}$  in methanol 496 nm (ref. 2)). It thus seems that the four grasses contain a leucoluteolinidin. It is unlikely that such a compound could have a flavan-3,4-diol structure, but the flavan-4-ol (4,5,7,3',4'-pentahydroxyflavan) produced by reduction of eriodictyol with sodium borohydride (unpublished results of T. Swain) and a methanolic extract of the grasses behaved identically both when boiled with 2 normal hydrochloric acid (yielding luteolinidin) and when treated with cold concentrated hydrochloric acid. In the latter case a blue colour was produced with  $\lambda_{\max}$  550 nm, the product responsible being, perhaps, the carbonium ion of the ring-opened form of the flavan<sup>3</sup>. On standing, or after dilution and heating, this was partially converted into luteolinidin. It should be noted that other flavan-4-ols show similar behaviour<sup>3</sup>, but flavan-3,4-diols (for example, leucocyanidin), although giving an intermediate blue-purple component when heated in 2 normal hydrochloric acid, do not give such a colour with cold concentrated acid.

The progenitor of luteolinidin in the grasses is not, however, a simple flavan-4-ol, because when methanolic extracts are chromatographed in either aqueous or alcoholic solvents, and chromatograms are sprayed with concentrated mineral acid (or with vanillin-sulphuric acid), the blue (or purple) coloration which develops is confined to the start-line. It seems probable that the precursor is a polymer of similar constitution to that recently proposed for the flavan-3,4-diol polymers<sup>4,5</sup>, and could, like these, have the properties of a condensed tannin.

In addition to the above compound, the hydrolysed extracts of two of the grasses had a second, fainter, reddish spot with  $R_f$  0.80 in Forestal, very near to that, 0.78, of apigeninidin and tricinidin<sup>2</sup>. That it is not the former is indicated by its reddish colour (apigeninidin being yellow) and the absence of an absorption peak lower than 496 nm (apigeninidin  $\lambda_{\max}$  476 nm). The possibility remains open, therefore, that the anthocyanidin is tricinidin.

Most of the above observations refer to *Hyparrhenia filipendula* (Hochst.) Stapf. The other species containing leucoluteolinidin were *Imperata cylindrica* (Linn.) Beauv. var. *africana* (Anderss.) Hubbard, *Themeda triandra* Forsk., and a *Bothriochloa* species, probably *B. insculpta* (Hochst. ex. A. Rich.) A. Camus, the first of these also containing the tricinidin precursor. All these genera are placed in the tribe Andropogoneae. Two other members of this tribe, *Andropogon gerardii* With. and

*Hyparrhenia hirta* Stapf, had been observed to yield anthocyanidins with  $R_f$  0.62 and 0.66, respectively, in Forestal solvent<sup>4</sup>, the only species out of more than sixty of the Gramineae examined to have such constituents. It is interesting to note that Stafford<sup>7</sup> has recently reported the occurrence of luteolinidin itself in seedlings of *Sorghum vulgare* Pers., a member of the same tribe. Blessin *et al.*<sup>8</sup> earlier showed leuco-anthocyanins to be present in the pericarp of certain varieties of sorghum; the  $R_f$  values of one of the anthocyanidins produced, tentatively identified as fisetinidin, are consistent with its being luteolinidin. Yasumatsu *et al.*<sup>9</sup>, on the other hand, reported the presence of a leuco-anthocyanin yielding pelargonidin from the seed coat of a commercial sample of sorghum (variety unspecified).

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### Potassiumless Death of *Saccharomyces cerevisiae* Cells treated with *N*-Succinyl Perimycin and the Reversal of Fungicidal Action of the Antibiotic by Potassium Ions

*N*-SUCCINYL PERIMYCIN<sup>1</sup> is a synthetic derivative of heptaene macrolide antifungal antibiotic<sup>2</sup>. The advantage of this substance is that, unlike the original polyenes, it forms readily water soluble salts, for example, the sodium salt, which retain much of the activity of perimycin itself<sup>1,3</sup>. The critical site of action of polyenes in general is the cell membrane and the lethal effect of these substances is a result of an impairment of the membrane's function<sup>4-12</sup>.

The sensitive organisms have a rather high polyene binding capacity because of the presence of a specific binding site in the membrane which is supposed to be a membrane lipid, presumably a membrane sterol<sup>9,13-15</sup>. The reorientation of the membrane lipid layer by the bound polyene<sup>14</sup> brings about the change of permeability properties. The impairment of the osmotic barrier and transport systems decreases the ability of the membrane to take up and retain a number of critical metabolites, which in consequence leads to the death of the cell.

The degree to which the membrane can be damaged depends on the structure of a given polyene, as well as on its concentration. The whole spectrum of permeability changes has been observed with various polyenes used in various concentrations. The higher the molecular weight of a polyene and the lower its concentration the more permeability changes are restricted to

fewer metabolites of decreasing molecular weight, including inorganic ions, of which the most important is the potassium ion<sup>4,6</sup>. Nevertheless, none of these antibiotics induced an effect restricted to a specific metabolite, which could be reversed and the fungicidal action annulled by the addition of this metabolite to the medium. Inhibition caused by polyenes has so far been reversed for some enzyme systems only, on the addition of metabolites which leak from the cells treated with polyene, but reversal of the fungicidal action of the antibiotic has not been achieved. Thus, for example, the addition of potassium salts to the medium caused the resumption of glycolytic activity which was inhibited by polyenes the action of which was proved to be more specific<sup>10,11,16</sup>. Less specific polyenes required the addition of potassium and a number of glycolytic cofactors for the restoration of the anaerobic breakdown of glucose<sup>4</sup>.

We have shown that the impairment of membrane permeability properties by *N*-succinyl perimycin is the most specific of all polyenes<sup>17</sup>. Conditions were found in which the damage of the osmotic barrier with respect to potassium ions was the only demonstrable effect of the antibiotic. No permeability changes were observed with respect to organic metabolites.

The specific escape of potassium from cells treated with *N*-succinyl perimycin in potassium deficient medium causes, first, inhibition of metabolic activity of potassium dependent systems. Other metabolic reactions which are not potassium dependent or which are dependent to a lesser extent are not inhibited.

The suppression of activity of potassium dependent systems, while other reactions are not inhibited, probably induces a metabolic disequilibrium, which is the proposed explanation for the subsequent death of the cells. This phenomenon has been named by us "potassiumless death". The presence of the substrate in the medium significantly enhances potassiumless death caused by the more strongly unbalanced metabolic activity of the cells. Potassiumless death is also enhanced by sodium ions, as a result of the facilitated removal of potassium from cells which results from potassium-sodium exchange.

The high specificity of action of *N*-succinyl perimycin permitted restoration of the activity of potassium dependent systems by the addition to the medium of potassium salts in concentration approximately equal to that found in cells plasma.

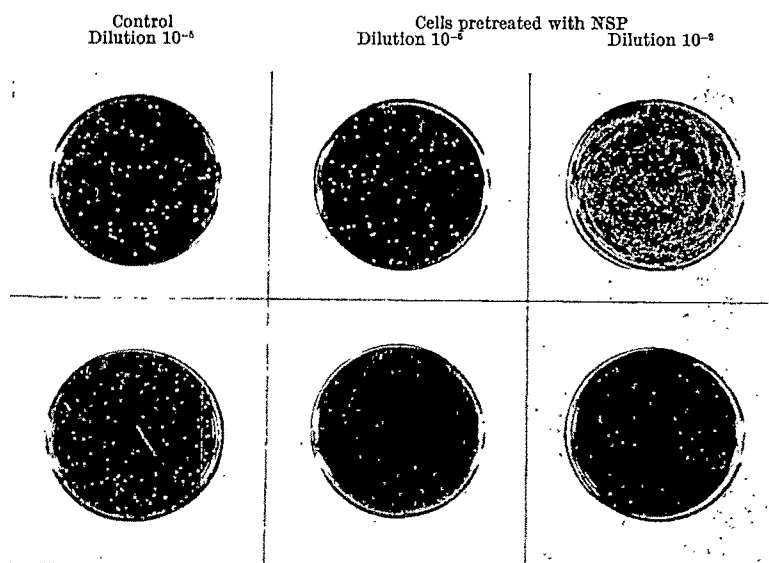


Fig. 1. Potassiumless death of *Saccharomyces cerevisiae* cells damaged by *N*-succinyl perimycin (NSP) and the regeneration of damage and restoration of viability in the presence of potassium ions. Top row, agar with 0.1 molar potassium ion. Bottom row, agar without potassium ion.

Evidence will be given for the annulment of fungicidal action of *N*-succinyl perimycin by potassium ions. *Saccharomyces cerevisiae* strain ATCC 9763 was used as the model organism. *N*-succinyl perimycin was applied in the form of water soluble sodium salt. Logarithmic cells were prepared by culturing *Saccharomyces cerevisiae* for 16 h at 30° C in a medium of the following composition: 0.3 per cent 'Bacto'-yeast extract, 0.5 per cent 'Bacto'-peptone 'Difco', 1 per cent glucose. The medium was inoculated with an agar slant culture grown on a medium of the same composition. After 16 h of incubation the culture was diluted five-fold with fresh medium and reincubated for a further 4 h. Cells were collected by centrifugation and washed three times with distilled water. A cell suspension (4 mg/ml, dry weight) was prepared in *tris*-hydrochloric acid buffer, pH 7.2, containing 0.1 molar potassium chloride, and incubated without (control) and with 10 µg/ml. sodium salt of *N*-succinyl perimycin for 2 h at 30° C with shaking. Each suspension was then divided in two parts and processed separately. One part was centrifuged, washed once with 0.1 molar potassium chloride and after making serial dilutions 0.1 ml. aliquots were plated on agar medium (1 per cent 'Bacto'-peptone, 2 per cent glucose, 0.1 molar potassium chloride). The second part was processed similarly, except that it was washed with distilled water instead of 0.1 molar potassium chloride and cells were plated on agar without added potassium salt (medium contained trace quantities of potassium ion from reagents of concentration approximately 0.0005 molar). All plates were incubated for 36 h at 30° C and colonies were counted. The results are shown in Fig. 1 and Table 1.

Table 1. REVERSAL OF FUNGICIDAL ACTION ON *N*-SUCCINYL PERIMYCIN BY POTASSIUM IONS

Medium for viable cell count	Viable cells/ml. 10 µg of sodium salt of <i>N</i> -succinyl perimycin/ml.	
	Control	
Agar with 0.1 molar potassium ion	1 × 10 <sup>8</sup>	8 × 10 <sup>7</sup>
Agar without potassium ion	1 × 10 <sup>8</sup>	2.5 × 10 <sup>4</sup>

The count of cells not treated with *N*-succinyl perimycin is identical on both agar media. Cells treated with the antibiotic did not give at dilution 10<sup>-5</sup> any colonies on agar without potassium, but about 80 per cent colonies, as compared with the control, on agar containing 0.1 molar potassium chloride. A small percentage of cells was unaffected by the antibiotic, which can be seen after plating more concentrated cell suspension on agar without potassium salt. The figures in Table 1 show that only 0.025 per cent of cells were unaffected by *N*-succinyl perimycin. This percentage could be reduced to zero after longer treatment with the antibiotic.

The cells treated with *N*-succinyl perimycin and grown on the agar with potassium salt are identical with the original strain. They grow well when transferred to agar without potassium, which proves that they are fully regenerated. No difference in sensitivity to *N*-succinyl perimycin has been observed. Inhibitory concentration of the antibiotic in streak dilution agar plates method (medium: 1 per cent 'Bacto'-peptone; 2 per cent glucose, 2 per cent agar) was identical for both strains and equal to 0.5 mcg/ml. This proves that the treated cells did not acquire the resistance.

The cells treated with *N*-succinyl perimycin and plated on agar with potassium chloride grow slowly at first in comparison with the untreated ones. This delay is probably a result of the time required for the regeneration of damage caused by the antibiotic.

Further investigations aimed at a more detailed explanation of the phenomenon of potassiumless death are in progress. The biological properties of *N*-succinyl perimycin encourages us to consider this antibiotic as a tool for investigations of the mechanism of potassium transport systems.

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## PATHOLOGY

### Circulating Cell as a Source of Myoblasts in Regenerating Injured Mammalian Skeletal Muscle

THE theory that injured skeletal muscle can regenerate by the formation of new cells although still disputed<sup>1</sup> has strong support<sup>2-4</sup>. New muscle fibres are held to develop from multinucleate "myotubes" which are formed either by the budding of damaged muscle fibres or more probably by the fusion of mononuclear cells which have been termed "myoblasts"<sup>5-8</sup>. Walker insists that myoblasts multiply solely by mitotic division and derives them from injured adult muscle cells by dedifferentiation. Later cytoplasmic differentiation occurs mainly in myotubes and in these nuclear division is no longer seen. Others have claimed that myoblasts originate in connective tissue cells in or near the site of injury. It occurred to us, however, that like leucocytes and phagocytic histiocytes<sup>9</sup> these muscle precursors could be carried in the circulation to injured areas from a central source, for example, the lymphoid tissues or bone marrow.

To test this hypothesis we injected 50 µc. of tritiated thymidine (6-T(n), specific activity 5.0 c./mmole) intraperitoneally four times successively at 6 h intervals into female adult mice. In one pair, crush lesions<sup>10</sup> were made in the right gastrocnemius 48 h after the fourth injection of radioisotope, that is, at a time when there should be, in the blood stream, no tritiated thymidine, because this material is taken up by cells preparing for mitosis and almost entirely disappears from the circulation within an hour of injection<sup>11</sup>. In six further animals, the right gastrocnemius was crushed at varying periods after the left, because it seemed possible that the regeneration process in one leg could be modified by a preceding muscle injury in the other (see Table 1). The first injection of tritiated thymidine was given to these animals 6 h after the left gastrocnemius was crushed. Intervals of 12, 24 and 48 h elapsed between the fourth injection and the crushing of the right gastrocnemius. All mice were killed 8 days after injury to the right gastrocnemius.

Table 1. TRITIATED THYMIDINE UPTAKE BY NUCLEI OF MYOTUBES IN REGENERATING MUSCLE  
Grain counts/myotube nucleus in left leg

Time between fourth injection of tritiated thymidine and injury to right leg	Median	Range	Proportion of nuclei with one grain or more (per cent)	Median	Range	Proportion of nuclei with one grain or more (per cent)
48 h*	0	0-3	11	6	0-31	86
	0	0-1	1	8	0-30	85
12 h†	5	0-21	82	6	0-22	87
24 h†	6	0-19	91	8	0-24	93
	8	0-34	87	6	0-24	93
48 h†	6	0-20	83	4	0-21	73
	8	0-31	93	7	0-31	93
	12	0-34	84	5	0-24	79

\* Left leg uninjured.

† Left leg injured 6 h before first injection of tritiated thymidine.

Note that the pattern of nuclear labelling differs little in the successively injured left and right legs of individual mice.

In autoradiographs from sections of both gastrocnemii embedded in paraffin, grain counts were made over 150 nuclei belonging either to myotubes at injury sites, or, in the first pair of mice, to normal muscle fibres. Over occasional nuclei in uninjured muscle fibres there was one grain, and over three nuclei there were two or three grains. Conversely, in injured muscle a wide variety of cells was heavily labelled. Grain counts (see Table 1) over the myotube nuclei ranged between none and thirty-four except for two nuclei where forty-two and fifty grains were seen. Medians varied between four and twelve, the percentage of nuclei with one or more granules between seventy-three and ninety-three. Myotube nuclei were thus intensely labelled while the nuclei of cells in uninjured muscle were in effect unlabelled. Grain counts made over connective tissue cells between normal muscle fibres similarly indicated that these were, with rare exceptions, unlabelled. These exceptions had five, eleven, and twenty-six grains respectively and were included in an analysis of 300 cells.

The most striking finding in the first pair of mice was the presence of intensely labelled nuclei in myotubes of the right leg. This was unexpected, for Bintliff and Walker<sup>9</sup> had maintained that tritiated thymidine, injected before injury, labelled inflammatory cells but not the precursors of regenerating muscle fibres. These workers, however, gave the thymidine three times in 12 h, whereas our injections were made four times in 24 h, a procedure which was more likely to allow for diurnal variations in mitotic activity. Because uninjured muscle was not labelled, the labelling of myotubes so long after tritiated thymidine injection strongly suggested the immigration into the lesion of an already labelled precursor.

A similar immigration of labelled cells probably accounted for the intense labelling of myotubes in the right or second injured leg. If these cells had migrated from the left or first injured leg, one would anticipate a dilution of their labelling after each subsequent cell division. This was not observed because the intensity of labelling in the right and left legs differed little, even when 12 or 24 h separated the last injection of tritiated thymidine from the time when the second leg was injured.

One possible explanation for our findings is to suppose that dividing myoblasts can reutilize labelled nucleoprotein derived, for example, from dead neutrophil leucocytes<sup>7</sup>. This concept of reutilization is difficult to reconcile with the similarity in pattern and intensity of myotube labelling in successively injured legs in our experiments, because myoblasts in the second injured leg would tend, if labelled by reutilization of thymidine, to be far less heavily labelled than those myoblasts in the first injured leg which were directly labelled by circulating recently injected thymidine.

Another explanation is that thymidine persists in tissue in a soluble form, demonstrable in frozen sections<sup>12</sup>, but largely lost from sections of tissue embedded in paraffin. This is possible, but would scarcely explain the intensity of labelling we observed in second injured legs in our experiments.

It is concluded, therefore, that many myoblasts or their precursors probably migrate into muscle lesions from the blood stream. This concept is at variance with current views on muscle regeneration. It follows that in those muscle diseases in which a disorder of regeneration has been postulated, therapy may have to be directed not so much to the affected muscles as to the body as a whole, that is, to the source of muscle cell precursors.

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### Effects of Corticoid Injection and of Adrenalectomy on *in vitro* Amino-acid Incorporation into Microsomes of P1798 Lymphosarcoma

THE involution of lymphoid tissue which follows administration of corticoids to animals could be a result of more rapid cell destruction or inhibition of an anabolic process. An increase in ribonuclease after injection of corticoid has been shown in corticoid-sensitive P1798 lymphosarcoma<sup>1</sup>, and in rat thymus<sup>2</sup>. This change is measurable 6 h or more after administration of cortisol. Enhanced ribonuclease activity could result in diminished protein synthesis.

Decreased incorporation of amino-acids into microsomes of rat thymus and mouse lymphoma ML388 3 and 12 h after treatment with cortisol has been demonstrated<sup>3,4</sup>. This inhibition could cause the involution of lymphoid tissue which follows administration of cortisol to animals. To test this possibility preliminary observations were made of the inhibition of amino-acid incorporation into microsomes of lymphosarcoma P1798. This tumour has a more uniform cell population than thymus and is available in corticoid sensitive and resistant strains<sup>5</sup>, so that the inhibition could be correlated with *in vivo* sensitivity. Steroid treatment of mice



bearing corticoid sensitive tumour gave microsomes with a marked deficit for incorporation of phenylalanine which was not relieved by the addition of polyuridylic acid (poly-U) to the incubation mixture. There was no such inhibition after treatment of mice bearing the corticoid resistant strain tumour. When glucocorticoid was administered to adrenalectomized mice bearing steroid sensitive tumour, no inhibition of incorporation into microsomes was observed.

Corticosteroid sensitive and resistant lymphosarcoma P1798 was transplanted into BALB/c mice as previously described<sup>1</sup>. 9- $\alpha$ -fluoro-prednisolone (9FP) and steroid suspending vehicles were supplied by the Cancer Chemotherapy National Service Center. Uniformly labelled phenylalanine-<sup>14</sup>C was used.

The preparation of tumour microsomes and the measurement of incorporation were adapted from the methods of Ochoa and Weinstein<sup>2</sup>. To restrict the observed hormonal effects to the microsomes and to avoid introduction of ribonuclease known to be present in the tumour supernatants, supernatant fraction from normal mouse liver was used in the incubation mixture. Microsomes were incubated for 15 min at 37° C in 0.01 molar *tris*, pH 7.4, 0.05 molar magnesium acetate, 0.06 molar potassium chloride, 0.025 mmolar GTP, 0.625 mmolar ATP, 3.12 mmolar PEP, 12  $\mu$ g of pyruvate kinase, 0.01 ml. of a mixture of twenty amino-acids excluding phenylalanine, each 0.0125 mmolar, 0.1  $\mu$ c. of phenylalanine-<sup>14</sup>C (specific activity > 300 mc./mmoles), 0.006 molar mercaptoethanol, and 0.4–0.5 mg of protein of supernatant fraction from normal mouse liver in a total volume of 0.4 ml. In these circumstances, incorporation was proportional to the amount of microsomal protein present and 0.3–0.4 mg was used in these experiments. Protein was determined by the method of Lowry *et al.*<sup>3</sup>. The concentration of magnesium ions used was optimal for both treated and untreated fractions. Poly-U, when present, was added to a final concentration of 250  $\mu$ g/ml. which was found to give maximal stimulation. Incorporation of phenylalanine was decreased 18 h after the administration of 9FP to mice bearing sensitive tumours. No such decrease was observed when resistant tumours were investigated. Table 1 also shows that the incorporation is similar in the controls from the two strains and is increased by the addition of poly-U which, however, did not prevent the inhibition after treatment.

Table 1. INCORPORATION OF PHENYLALANINE INTO TUMOUR MICROSOMES ( $\mu$ moles/mg of microsomal protein  $\pm$  S.E.M.)

Tumour	Without poly-U	With poly-U
Sensitive control	0.81 $\pm$ 0.09	5.6 $\pm$ 0.4
Sensitive treated	0.34 $\pm$ 0.08 ( $P < 0.01$ )	2.6 $\pm$ 0.2 ( $P < 0.01$ )
Resistant control	0.74 $\pm$ 0.09	5.7 $\pm$ 0.6
Resistant treated	0.89 $\pm$ 0.11 ( $P < 0.3$ )	5.1 $\pm$ 0.8 ( $P < 0.04$ )

Six separate microsomal preparations were made for each of the four tumour groups. Treatment consisted of subcutaneous injection of 0.2 ml. of suspending medium alone or containing 25 mg/kg of 9FP, 18 h before killing.

Repetition of this experiment 2, 4, 6 and 12 h after administration of corticoid failed to demonstrate inhibition, although inhibition 18 h after treatment could be demonstrated consistently.

The effect of administration of 9FP on phenylalanine incorporation into tumour microsomes was also evaluated in mice after adrenalectomy. Table 2 shows that the inhibition after corticoid administration was not found in adrenalectomized mice.

Six separate microsomal preparations were made in each group. Adrenalectomy was performed 8 days before.

Table 2. INCORPORATION OF PHENYLALANINE INTO TUMOUR MICROSOMES FROM ADRENALECTOMIZED MICE ( $\mu$ moles/mg of microsomal protein  $\pm$  S.E.M.)

Tumour	Without poly-U	With poly-U
Sensitive control	0.58 $\pm$ 0.03	4.82 $\pm$ 0.6
Sensitive treated	0.94 $\pm$ 0.09 ( $P < 0.01$ )	5.0 $\pm$ 0.2 (N.S.)
Resistant control	1.05 $\pm$ 0.07	4.4 $\pm$ 0.5
Resistant treated	1.50 $\pm$ 0.05 ( $P < 0.01$ )	6.9 $\pm$ 0.2 ( $P < 0.01$ )

Treatment and incubation conditions were identical with those in Table 1. The increased incorporation after treatment with corticoid in this experiment was not found consistently.

Lymphosarcoma P1798 is useful in investigations of the mechanism of steroid-mediated lymphocytolysis. It has far fewer non-lymphoid elements than thymus and is available in glucocorticoid sensitive and resistant strains. This latter property is a great advantage for delineation of biochemical effects relevant to breakdown mediated by steroid. The inhibition of incorporation of phenylalanine into microsomes of corticoid sensitive tumour was not observed before 18 h. This is later than the inhibition observed previously<sup>4</sup> for thymus 3 h after cortisol treatment. It is not known, however, whether lymphoid or thymic epithelial elements were involved in the inhibition. Involution of P1798 sensitive to steroid becomes just apparent about 18 h after treatment. Failure of the steroid resistant strain to demonstrate inhibition of phenylalanine incorporation following steroid treatment suggests that the decreased incorporation in the sensitive strain is either relevant to the mechanism of lymphocytolysis or secondary to that process. Even within the microsomal particle, presumably increased pools of amino-acid secondary to glucocorticoid mediated lymphoid catabolism could account for decreased incorporation. Failure of glucocorticoid treatment to result in decreased incorporation in adrenalectomized animals is not explained by our current data. Adrenalectomized animals show impressive involution of steroid sensitive tumour after glucocorticoid treatment and this suggests that inhibition of incorporation of amino-acid is not a necessary feature of glucocorticoid mediated lymphocytolysis.

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## Biochemical and Histochemical Observations on Aminopeptidase Activity in Early Wound Healing

LACK of investigations into the earliest phase of healing has supported the prevalent view that an inert lag period occurs up to the third or fifth day after wounding. Histochemically, however, the activity of several hydrolytic, transferring and oxidative enzymes has been demonstrated to increase during the very first post-operative hours<sup>1–3</sup>. An increase in aminopeptidase activity is histochemically demonstrable 2 h after the injury<sup>4</sup>. We have also investigated this enzyme biochemically during the earliest phase of wound healing.

We made an experimental biochemical investigation on rats, excising square, 1 cm<sup>2</sup> skin wounds in a shaved dorsal area. The wounds were neither sutured nor dressed. Four groups of animals were killed 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 20, 24, 32 and 48 h after wounding, and a flap of skin containing the wound was removed. The tissue surrounding a wound excised 15 min before the death of each rat served as a control for the animal. The skin flaps were frozen fresh with solid carbon dioxide. One hundred sections, 16 $\mu$  in thickness, were cut in a cryostat

at  $-20^{\circ}\text{C}$  from each edge of the quadrangular wounds. The 400 sections per one wound were ground in 3 ml. of 0.01 molar *tris* hydrochloric acid buffer (pH 7.15) at  $4^{\circ}\text{C}$  with an 'Ultra-Turrax' homogenizer for 5 sec, and the mixture was then centrifuged at 23,500g for 10 min. The supernatant was assayed for aminopeptidase activity by a colorimetric method<sup>4</sup>. For comparison, some cryostat sections were used for the histochemical<sup>5</sup> demonstration of the enzyme.

During the two first post-operative hours aminopeptidase activity decreased biochemically (Fig. 1). Thereafter, a gradual increase in activity was observed. This intensification was sharpest from 2 to 7 h after the injury. Also histochemically, an increase in aminopeptidase activity could be demonstrated 2 h after the operation, gradually increasing thereafter (Fig. 2). In the immediate vicinity of the wound edge, a central zone, 200 to 500  $\mu$  in depth, showed decreasing enzyme activity. Surrounding this, a 100 to 300  $\mu$  deep peripheral wound zone exhibited a progressive increase in aminopeptidase activity after 2 h.

The initial decrease in enzyme activity could be caused by a traumatic inhibitory effect on the enzyme or on its synthesis. The cells in the superficial (central) wound zone, being too severely damaged, are not able to show any increased enzyme activity later on<sup>2,3</sup>. On the other hand,

the trauma affecting the connective tissue cells in the peripheral wound zone seems to serve as a stimulus, activating defence forces after a short mobilization time. Thus, the initial increase in enzyme activity in this zone probably represents an adaptive defence mechanism by the local cells as a response to injury<sup>3</sup>. Active leucocytes invade the peripheral zone after 8–16 h<sup>6</sup>. The initial increase in aminopeptidase activity might be connected with the peptides that are shown by Menkin<sup>7</sup> to give rise to local hyperaemia, increase in capillary permeability, and migration of leucocytes.

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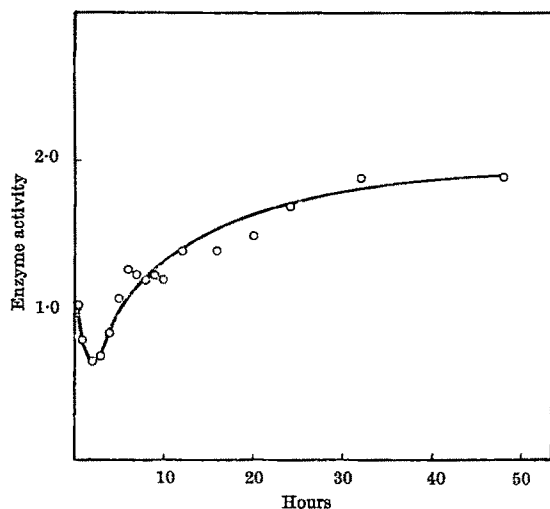


Fig. 1. Aminopeptidase activity in early wound healing, measured biochemically. The curve indicates relative activities, as compared with the control (= 1).



Fig. 2. Histochemical demonstration of aminopeptidase activity in a 7 h wound.

### Influence of Pineal Body on Melanoma of Hamsters

JORDAN<sup>1,2</sup> described the presence of melanic granules in pineal gland of sheep and del Rio-Hortega<sup>3-5</sup> found pigment granules in the pinealocytes of human pineal as well as in other mammals. Santamarina and Meyer Arndt<sup>6</sup> have more recently shown that the pigment in bovine pineal was true melanin. Ultra-violet spectral curves of bovine pineal melanin showed the same characteristics as those obtained from tyrosine melanin<sup>7,8</sup>. Pineal melanin differs slightly, however, from the melanin obtained from the choroid of the ox eye. Administration of pineal extract to tadpoles<sup>9,10</sup>, to toads<sup>10</sup> and to fishes<sup>11</sup> produces alteration in pigmentation. Thus it is possible that pineal body not only contains melanic pigment but is also capable of inducing some form of change in pigmentation. The hamster pineal body contains pigment granules in abundance (Fig. 1). On the basis of the investigations by Santamarina and Meyer Arndt, these can be assumed to be melanic granules. No data are available at present for the effects of pineal ablation or administration of pineal extract on pigmentation of hamsters. Isolated observations in other species have suggested a possible association between pineal body and pigmentation, however, and therefore a study was undertaken to observe the effect of pineal ablation on the growth and spread of a highly pigmented and spontaneously occurring neoplasm in hamsters.

Syrian hamsters of both sexes weighing between 80 and 110 g were used. Thirty-eight hamsters were pinealec- tomized according to a technique developed in this laboratory<sup>12</sup>. Thirty-six hamsters were subjected to comparative trauma and bleeding but the pineal gland and its stalk were left undamaged and intact. The remaining forty animals were used as controls. All animals were fed standard laboratory diet and water *ad libitum*. Five weeks after either surgical procedure hamsters were considered ready for tumour transplantation.

Melanotic melanoma No. 1 (M. Mel 1—kindly supplied by J. G. Fortner) was chopped into small pieces and 0.4 ml. injected through a large bore needle into the subcutaneous tissue of the dorsum of each of the experimental hamsters. After inoculation, measurements of hamsters' weight, mean tumour diameter and time of appearance of skin ulceration were recorded every 2 days, until the animals



were killed. Hamsters from the first group were killed 14 days after tumour transplantation and subsequent groups 21, 28 and 35 days after the inoculation. All animals were autopsied and the findings are recorded in Table 1. The presence of metastatic deposits were confirmed by histological examination; however, in most cases the metastases could be diagnosed on gross examination alone.

The first part (A) of Table 1 describes the observation at autopsy on the fourteenth day of tumour transplantation. The mean tumour diameter was 1.47 cm in pinealectomized, 0.7 cm in sham-operated and 0.6 cm in the control group. The increase in diameter of the tumours in pinealectomized hamsters as opposed to the other two groups is highly significant ( $P < 0.001$ ). But the difference between control and sham-operated groups was also significant ( $P < 0.02$ ). This is the only set where the tumour diameter difference was significant between control and sham-operated animals. A similar difference was found in the incidence of metastatic deposits in these three groups of animals. This trend of highly significant differences in tumour diameters ( $P \leq 0.001$ ) and incidence of metastases ( $P < 0.005$ ) between the pinealectomized hamsters and the other two groups after 3, 4 and 5 weeks of tumour transplantation is apparent in Table 1.

These results suggest that ablation of the pineal gland alters the course of pigmented melanoma in Syrian hamsters. The effect of pinealectomy on the course of melanoma seems to be more apparent during the earlier part of the growth of this tumour. The validity of these experimental results depends on the accuracy of the measurement of different parameters. All the animals in each group were transplanted and killed at the same time so the question of time interval and appearance of metastases or the size of the primary tumours could be accurately checked. Inclusion of a sham-operated group automatically enabled a further check to be made of the

Table 1. RESULTS OF AUTOPSY AT REGULAR INTERVALS

No. of hamsters in each group	Tumour diameters mean $\pm$ S.E.* (cm)	No. of ulcer- ations of primary tumour	Metastatic pattern†				Axil- lary node
			Lung	Liver	Kid- ney	Spleen	
(A) 14 days after tumour inoculation							
Control (10)	0.59 $\pm$ 0.12	1	1	0	0	0	3
Sham-operated (10)	0.72 $\pm$ 0.95	3	2	0	0	0	3
Pinealectomized (10)	1.47 $\pm$ 0.16	8	7	2	6	2	8
(B) 21 days after tumour inoculation							
Control (10)	1.63 $\pm$ 0.39	3	1	0	0	0	3
Sham-operated (10)	2.03 $\pm$ 0.27	4	1	0	0	0	4
Pinealectomized (12)	3.35 $\pm$ 0.34	11	9	4	7	2	8
(C) 28 days after tumour inoculation							
Control (10)	2.4 $\pm$ 0.27	5	3	0	0	0	5
Sham-operated (10)	2.42 $\pm$ 0.10	7	4	0	1	0	4
Pinealectomized (8)	4.0 $\pm$ 0.34	8	8	3	5	1	7
(D) 35 days after tumour inoculation							
Control (10)	3.5 $\pm$ 0.23	7	6	0	3	0	7
Sham-operated (6)	3.5 $\pm$ 0.82	5	4	0	4	0	6
Pinealectomized (8)	5.3 $\pm$ 0.13	8	8	3	7	2	8

\* Tumour diameter in pinealectomized animals as compared with those which were sham-operated was  $P < 0.001$ . (In group A only the difference in diameter between control and sham-operated animals was significant at the  $P < 0.02$  level. In the remaining groups the difference between control and sham-operated was insignificant, whereas the difference between pinealectomized animals and the sham-operated and control animals was highly significant in each group.)

† The figures shown represent the number of times each viscus has been involved. Comparison of the percentages of metastatic involvement of any sites in pinealectomized hamsters in each group was highly significant (for example, in lung  $P < 0.005$  (14 days), 21 days and 28 days, respectively).

effect of pinealectomy in these experiments. The interval of 5 weeks between any surgical trauma and tumour inoculation obviates the possible effect of trauma. The significant difference in the size of the tumours observed in group A between control and sham-operated animals remains to be explained.

The pinealectomized animals weighed more than the other two groups of hamsters at the time of killing. This could either result from pinealectomy *per se* or the increased mass of the tumour or both. From these experiments no statement can be made.

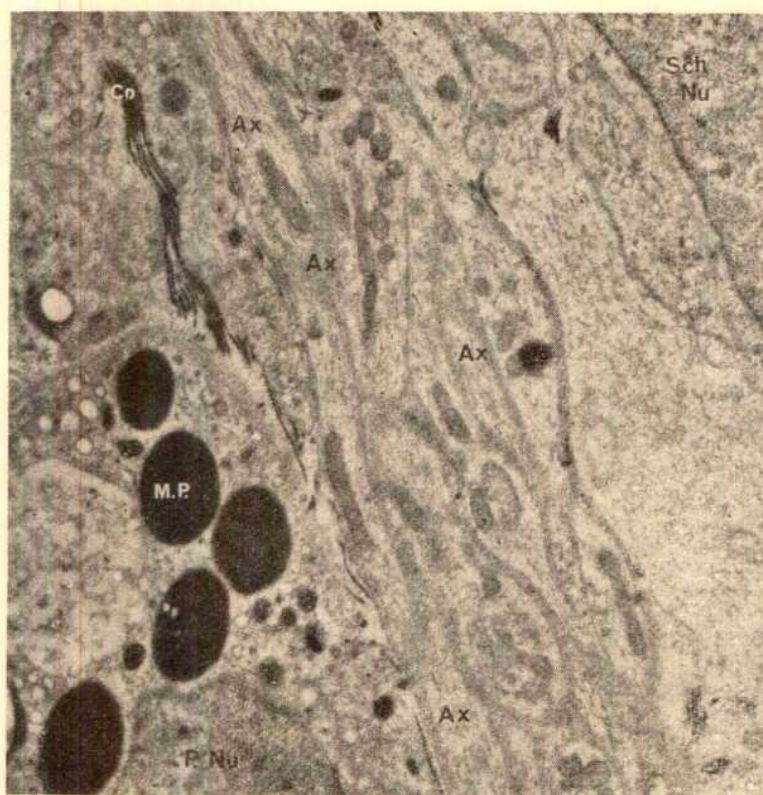


Fig. 1. Longitudinal section of a hamster pineal gland. A nucleus of a pinealocyte (P. Nu) is present at the bottom left-hand side. Melanin pigment granules (M.P.) are quite numerous in the cytoplasm. Co, Collagen fibre; Ax, unmyelinated axons. At the tip of the right hand side is a nucleus of a Schwann cell (Sch. Nu). ( $\times 9,000$ .)



None of our pinealectomized hamsters with tumours survived for more than 38 days after inoculation. It is difficult to draw any conclusions from this small group of hamsters, especially because all these tumours had ulceration, necrosis and consequent massive infection. Fortner *et al.*<sup>13</sup> have reported a host survival time varying from 34 to 82 days after inoculation of this tumour. They also reported that there was always a high incidence of lung metastases and evidence of other visceral metastases from this tumour at the time of death of these animals. But the remarkable rapidity of metastatic spread observed in this experiment after pinealectomy was not observed on any occasion by Fortner *et al.*<sup>13</sup> and was corroborated by our two sets of controls.

The data obtained from this investigation tend to support a concept that the pineal body exerts some control on the growth and spread of pigmented neoplasms in hamsters. These experiments, however, do not explain whether the control is mediated by way of a pineal hormone which acts on melanophores or whether ablation of pineal *per se* depresses host resistance to tumours and homografts. Further experiments dealing with non-pigmented tumours in albino rats are at the moment in progress in order to elaborate these points.

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### Induction of Amyloidosis by Cadmium

AMYLOIDOSIS has been produced in experimental animals by injecting a variety of substances, most of them proteinaceous<sup>1</sup>. We have found that a simple chemical, cadmium, given to rabbits as cadmium chloride in relatively small doses over a long period of time will produce a severe form of amyloidosis.

The dosage chosen was one which had been found by Kench<sup>2</sup> to produce a proteinuria in rabbits similar to that found in humans after prolonged exposure to cadmium. New Zealand outbred rabbits were injected with cadmium chloride in a dose of 1 mg/kg/day, 5 days a week. Two injection schedules were used. The first group of three animals received cadmium for an 8 week period. They were rested for 10 weeks and then reinjected for 4 weeks. They received an average total dose of 224 mg of cadmium chloride (68 mg/kg). They were killed at the end of the second injection period (22 weeks after the first injection). The second group of three animals received the cadmium for 9 weeks. One animal died of unknown causes after 6 weeks. The two that survived received an average total dose of 127 mg of cadmium chloride (47 mg/kg). They were biopsied 20 weeks after completion of the injections (30 weeks after the first injection) and were killed for complete autopsies 60 weeks after the first injection.

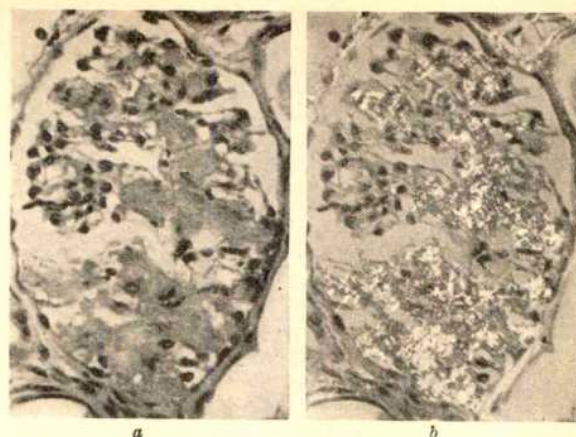


Fig. 1. *a*, Glomerulus in renal tissue obtained 30 weeks after cadmium treatment was begun, showing the amorphous amyloid occupying most of the glomerular tuft. Congo red ( $\times$  c. 260). *b*, Polarized light micrograph of the same glomerulus as in Fig. 1, showing the birefringence of amyloid stained with Congo red ( $\times$  c. 260).

The tissue obtained at biopsy or autopsy was processed both for light and for electron microscopy. Sections stained with Congo red were also examined by polarized light and fluorescence microscopy to detect the characteristic birefringence and fluorescence of amyloid stained with Congo red. Thin sections were examined in the electron microscope to detect the fibrils characteristic of amyloid in tissue<sup>1</sup>.

Histological examination revealed a small amount of amyloid in the glomeruli of animals killed at 22 weeks. The deposits involved about 10 per cent of each glomerulus. Far more amyloid was seen in the kidneys of the animals biopsied at 30 weeks, although these animals had received a smaller dose of cadmium, and autopsy of the same animals at 60 weeks revealed massive involvement of the kidney with amyloid; almost every part of each glomerulus was replaced by material which had features characteristic of amyloid. Fig. 1*a* shows a severely affected glomerulus in the tissue obtained at 30 weeks, stained by the Congo red technique. Fig. 1*b* shows the same glomerulus examined with polarized light, and showing the birefringence which is so typical of amyloid stained with Congo red. The identity of this material as amyloid was also established by electron microscopy. In addition to renal involvement, the animals killed at 60 weeks also showed extensive infiltrates in the spleen and the liver.

People who work with cadmium develop renal tubular lesions<sup>3</sup> and similar lesions have been found in rabbits<sup>4</sup> and rats<sup>5</sup> injected with cadmium. Amyloidosis has not, however, been described previously either in man or in animals with cadmium poisoning. Few of the animal studies have been carried out for a period as long as that reported here, and the failure of earlier workers to find this lesion may be due to the fact that the animals were not kept alive long enough to develop amyloidosis. The absence of amyloidosis in men who have been exposed for periods up to 15 years does, however, suggest that species differences in the susceptibility to cadmium may also be present.

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## PHYSIOLOGY

## Phagocytosis by Synovial Cells

THE ability of synovial cells to phagocytose small particulate matter has long been accepted for haemosiderin and can be readily demonstrated in such cells in ordinary histological sections from cases of chronic haemarthrosis<sup>1</sup>. Only now is the full phagocytic power of the synovial cell

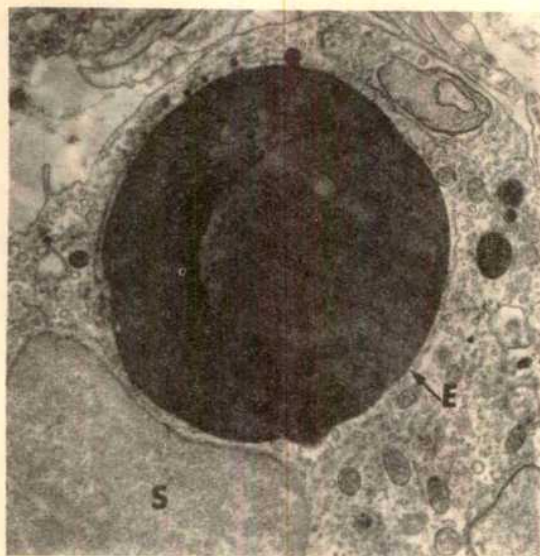


Fig. 1. Synovial cells (S) with phagocytosed erythrocyte (E) ( $\times 10,000$ ).

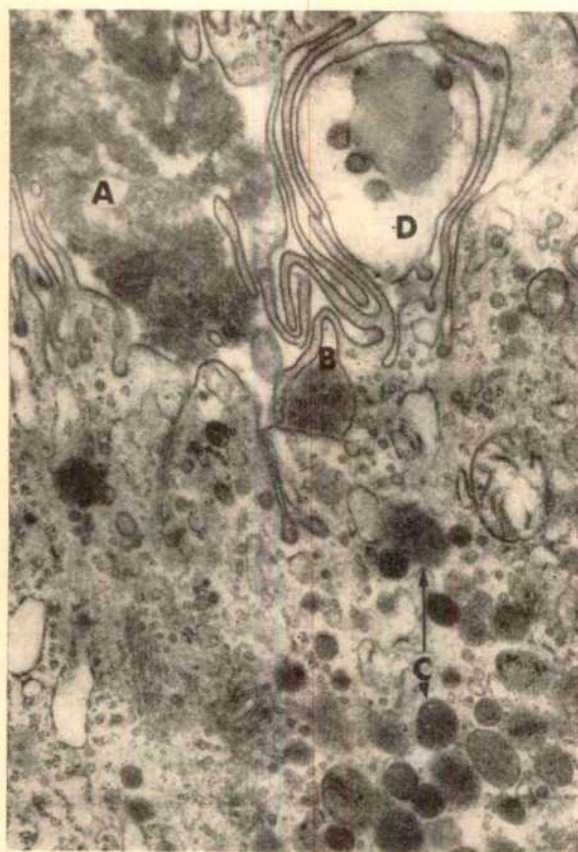


Fig. 2. Synovial cell phagocytosing "fibrinoid" material and a cell fragment. "Fibrinoid" material is seen lying free in joint space at A, and embraced by a filopodium at B. Similar material can be seen in phagosomes at C. A cell fragment surrounded by filopodia is seen at D ( $\times 19,500$ ).

beginning to be recognized. Thus, it has been shown that not only a variety of small particulate substances like ferritin<sup>2</sup>, gold<sup>3</sup>, thorotrast<sup>4</sup>, iron dextran<sup>5</sup> and carbon<sup>6</sup> when injected into the joint space are readily taken up by these cells but that entire erythrocytes<sup>7</sup> can also be ingested in this fashion (Fig. 1). This is indeed remarkable because usually erythrocytes are fragmented before phagocytosis by macrophages<sup>8</sup>.

During the course of our investigations of the ultrastructure of rheumatoid synovium we have collected further evidence about the phagocytic potentials of the synovium. In this condition cellular debris and "fibrinoid" material are frequently found in the joint space, and this is rapidly phagocytosed by the synovial cells. Fig. 2 shows some "fibrinoid" material lying in the joint space at A. This material can also be seen trapped between the cell wall and a filopodium at B. Furthermore, morphologically similar material can be demonstrated within the cell, usually in single membrane bound bodies (C). These appearances are compatible with the idea that "fibrinoid" material is being phagocytosed and incorporated into the synovial cell to form phagosomes. Phagocytosis of a cell fragment containing what appears to be a lipid droplet and four lysosomal bodies is seen at D.

Thus it is now evident that the synovial cell can phagocytose a large variety of small and large particles and that its phagocytic powers match and in some instances perhaps even excel that of the macrophage, the classical scavenger which plays a fundamental part in the removal of particulate debris in various pathological states.

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## Influence of Sleep Deprivation on Iron Metabolism

AMONG the basic factors which have a bearing on iron metabolism and its serum level certain attention has been paid during the past two decades to the influence of biological rhythms<sup>1</sup> and various stressing stimuli<sup>2</sup>. In conjunction with this we decided to make use of an experimental set-up which involves the prolonged and complete upset of the basic biological rhythm of the alternation of wakefulness and sleep—that is, sleep deprivation. This work was stimulated in particular by the finding of an extreme drop of plasma iron (revealed in preliminary experiments) during the 120th hour of vigilance in four experimental subjects<sup>3</sup>.

The level of plasma iron was investigated daily in six men and two women<sup>4</sup>, the total serum binding capacity for iron in four<sup>5</sup> and in all subjects the urinary iron excretion<sup>6</sup>. Blood specimens were collected between 7 and 8 a.m. during the control period before the experiment, then during five days of sleep deprivation and then during the recovery period. In the women the iron absorption from the digestive tract in serum was investigated after administration of six tablets of 'Feronat C'<sup>\*</sup>. Because the plasma iron level may be influenced by the administered preparation, however, the two above mentioned experi-

\* One tablet of 'Feronat C' Spofa contains 200 mg ferrum gluconicum and 20 mg ascorbic acid, that is, 1 tablet = 22 mg ferrous iron.



Table 1. CHANGES OF PLASMA IRON LEVEL IN SIX MEN

Experimental period Time of withdrawal of specimen	$\bar{X}$ $S$	$t$	First experi- mental period	Sleep deprivation						Second experimental period		
				0	24	48	72	96	120	48/II	96/II	144/II
				105 5.76	90.3 6.19	70.3 13.27	68 7.70	60.16 6.94	57.16 5.12	81.0 7.66	87.83 6.84	100.5 9.28
0:24		4.541	0.01		0:96/II	4.702	0.01	24:48	3.350	120:48/II	6.342	0.01
0:48/II		6.134	0.01		0:144/II	1.055	>0.05	48:120	2.263	96/II:144	2.649	<0.05

Table 2. CHANGES IN BINDING CAPACITY OF SERUM FOR IRON

Experimental period Time of withdrawal of specimen	$\bar{X}$ $S$	$t$	First experi- mental period	Sleep deprivation						Second experimental period		
				0	24	48	72	96	120	48/II	96/II	144/II
				326.3 8.05	320 6.32	312.50 7.58	306.00 8.94	313.33 8.33	311.6 1.31	317.6 7.47	316.25 2.49	321.50 1.05
0:48		3.494	0.01			0:120	4.414	0.01		72:48/II	2.350	<0.05
0:72		4.744	0.01			0:96/II	2.369	0.05		120:96/II	3.651	0.01
0:96		2.758	<0.05			24:48	3.042	<0.05		120:144/II	11.559	0.01

mental subjects were not included in the final evaluation, though their curves of sideraemia on fasting were on the whole similar as in the other experimental subjects. In four men the investigation was supplemented by ferrokinetics using  $^{59}\text{Fe}$ . Simultaneously, the complete haemogram was examined as well as the plasma volume and the glucose-6-phosphate dehydrogenase activity in red blood cells.

The plasma iron levels in six subjects before the experiment were balanced and within a normal range (Table 1). During sleep deprivation there was a gradual decline, the maximum drop being to half the original levels. The decline was most marked during the first 48 h of sleep deprivation, and was much slower during the subsequent intervals. The return to normal values, during the second control period after the end of the experiment, was slow and took roughly one week.

The total binding capacity of serum for iron, that is, the binding capacity of transferrin, declined much less and the maximum drop was during the seventy-second hour of vigilance (Table 2). On subsequent days it oscillated only slightly and there was no further decline. The return to normal was slow.

For the evaluation of the observed changes it is important to present the results of other examinations. First of all the participation of the renal factor can be eliminated because the urinary iron excretion does not change in the course of the experiment. Absorption does not show any detectable changes; the shape of the absorption curves on the first day and after 96 h of the experiment in the two experimental subjects is identical. Later determinations of the haemoglobin level and the number of reticulocytes ruled out anaemia caused by the withdrawal of blood specimens as the source of the observed changes. The investigation of ferrokinetics with  $^{59}\text{Fe}$  indicate that the half-time of the removal from plasma ( $T/2$ ) is in all four subjects within a normal range (normal values are 60–120 min, in our subjects 75–105 min). The absence of a more marked shortening of the half time of the clearance of  $^{59}\text{Fe}$  does not rule out the possibility that this experiment with the ferrokinetics which was started at the time of the assumed marked drop of sideraemia, that is, during the fourth-eighth hour, did not record the time of the rapid removal. The increased utilization of the removed iron by red cells is striking (100 per cent in all instances). This emphasizes the dominant position of erythropoiesis in iron metabolism. The slightly reduced iron supply is offset by enhanced utilization. The other indicators of erythropoiesis, the erythropoietic index and the relative erythropoietic activity are at the upper borderline of normal values. Assessment of activity did not reveal an enhanced deposition in tissues. The unaltered activity of glucose-6-phosphate dehydrogenase seems to confirm the view that in the investigated interval of protracted wakefulness no marked changes in erythropoiesis took place. The drop of the iron level is probably connected with increased removal into the reticulohistiocyte system.

The interpretation of mechanisms and causes of the observed changes is very complicated. Serious disorders of neurogenic and endocrine regulatory mechanisms participate in the genesis of the drop in the level of serum iron during sleep deprivation<sup>3</sup>. This is suggested in particular by the abnormal results of a number of psychological tests and the altered activity of the central sympathetic in the mecholyl test<sup>7</sup>, the increased excretion of vanillyl-mandelic acid, 17-OH steroids in urine<sup>8</sup>, the striking leucocytosis and eosinophilia.

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## A Neurosecretory Tissue in Octopus

IN view of the amount of information available about the structure of the nervous system in cephalopod molluscs, there is surprisingly little known about their neurosecretory systems. The epistellar body, associated with the stellar ganglion, was thought to be a neurosecretory organ<sup>1</sup>, but it is now known to have the structure of a photoreceptor<sup>2</sup>. Several other structures of unknown function have also been described that may have a neurosecretory function<sup>3,4</sup>. The only organ that has been shown to have an endocrine function in cephalopods is the optic gland of *Octopus*; behavioural studies show that it controls maturation of the gonads and hence sexual maturity<sup>5</sup>. A preliminary report on the fine structure of this organ gave little information of any granular content of the cells<sup>6</sup>. A group of possible neurosecretory tissues that have a similar histological structure have recently been described—the vena caval tissue, associated with the vena cava in *Eledone cirrosa*, *Octopus vulgaris* and *Sepia officinalis*<sup>7,8</sup>, and the juxta-ganglionic tissue, associated with the inferior buccal ganglia and buccal sinus in *Octopus vulgaris*<sup>9,10</sup>. The juxta-ganglionic tissue, first described by Bogoraz and Cazal<sup>9</sup>, consists of a group of small

nerve cells lying outside, but associated with, the inferior buccal ganglia (see ref. 10 for diagram of its position). These cells give rise to axons that pass down to the buccal sinus and end on its surface as a tangled mass of interweaving fibres<sup>10</sup>.

The inferior buccal ganglia of *Octopus vulgaris* together with associated juxta-ganglionic tissue were dissected out and fixed for electron microscopy with 1 per cent osmium tetroxide (buffered with veronal acetate to pH 7.3), stained with phosphotungstic acid, dehydrated in graded ethanols, and embedded in 'Araldite'<sup>11</sup>. When sections are viewed with the electron microscope the axons of the secretory cells can be seen to end by direct apposition on to the basement membrane of the sinus vessel. There are no pericytes on the outside of the basement membrane, unlike all other cephalopod vessels so far studied<sup>11</sup>. There is, however, a discontinuous layer of endothelial cells on the inside of the basement membrane. The basement membrane is particularly obvious because of the phosphotungstic acid staining. The neurons and axons are filled with membrane-bound, electron-dense granules of some 1000–2000 Å in diameter (Fig. 1). This type of granule is typical of that found in neurosecretory tissues of a variety of animals<sup>12–14</sup>. As well as these large granules some smaller vesicles that are not electron-dense are present and these have a diameter of some 500 Å. Some profiles contain mainly or solely these smaller vesicles. This variety of vesicles is reminiscent of the arrangement in the vertebrate neurohypophysis<sup>12,15,16</sup>. It seems likely that these small vesicles are associated with or derived from the larger electron-dense ones<sup>16</sup>. Other suggestions have been made<sup>15</sup>, including the possibility that they may be typical synaptic vesicles, containing acetyl choline<sup>17</sup>, and be involved with the release of the larger electron-dense granules.

The position of the axonal endings of the nerve cells and their content of numerous electron-dense granules of a typical neurosecretory sort makes it likely that the juxta-ganglionic tissue has a neurosecretory function. Studies using other techniques would be needed to confirm this. It would be interesting to examine the vena caval tissue<sup>7,8</sup>, and other similar tissue found in the brain of certain decapod cephalopods<sup>18</sup>, to see whether similar "neurosecretory granules" were present.

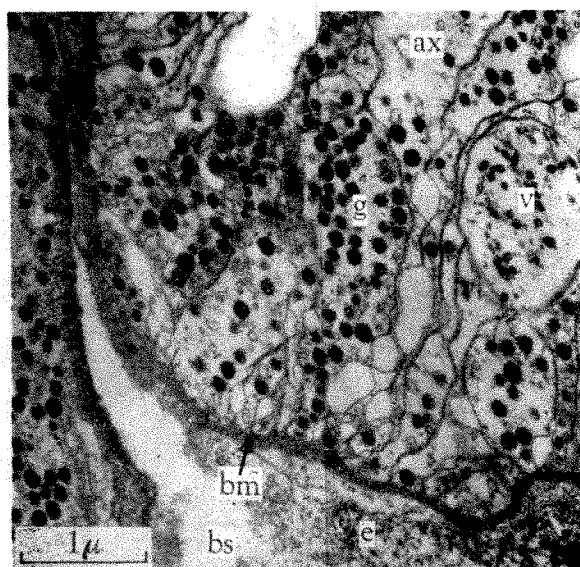


Fig. 1. An electron micrograph of axons (ax) of the juxta-ganglionic tissue ending on the basement membrane (bm) of the buccal sinus (bs). Note the electron-dense granules (g) commonly present in the axons and the less common clear vesicles (v) that are also present. e, Endothelial cell. (Fixed osmium, buffered veronal acetate, stained phosphotungstic acid.)

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### Zinc Concentrations of Fast and Slow Contracting Muscles in the Lobster

DURING an investigation of the regulation of zinc in the lobster *Homarus vulgaris* it was found that different skeletal muscles may contain very different amounts of zinc. The main flexor muscle from the abdomen contains 14.4 µg/g, but pooled samples of the extensor and flexor muscles of the coxopodite segments of the walking legs contain 60 µg/g<sup>1</sup>. Analyses of muscle samples in other decapod crustaceans have suggested that a low concentration of zinc is characteristic of muscles which can contract quickly. To confirm this I compared muscles with certain structural differences which suggest that their modes of functioning are different.

Alexandrowicz has noticed that some of the components of the dorsal musculature in crustaceans have a coarse and others a fine cross-striation<sup>2,3</sup>, and suggested that they may form two systems, one for slow and the other for fast contractions<sup>4</sup>. These muscles appeared, therefore, to possess the above requirements. The superficial and deep extensors and flexors of the abdomen and the three dorsal thoraco-abdominal muscles of the lobster were used for analysis. (For the situation and nomenclature of the latter muscles see ref. 3). The characteristics of the muscles and their concentrations of zinc are shown in Table 1.

The supposition that the coarse and fine striated muscles differ in their mode of contraction has since been confirmed by experimental demonstration of the presence of slow and fast systems in both the dorsal and ventral musculature of the decapod crustacea<sup>5,6</sup>.

Results of analyses of zinc from three different lobsters in Table 1 support the original idea that fast muscles contain less zinc than slow ones. The slow muscles contain five to seven times as much zinc as the fast muscles and twice as much zinc as any other lobster tissue (see ref. 1). Previous results have suggested that the

Muscle	Table 1		Zinc (µg/g)		Mean
	Cross striation	Mode of contraction			
First and second dorsal thoraco-abdominal	Coarse	Slow	103	100	—
Third dorsal thoraco-abdominal	Fine	Fast	21	19	—
Superficial abdominal extensor	Coarse	Slow	73	107	99
Deep abdominal extensor	Fine	Fast	15	15	15
Superficial abdominal flexor	Coarse	Slow	111	83	120
Deep (main) abdominal flexor	Fine	Fast	15	17	13
Whole blood	—	—	5.2	6.6	6.9

Table 2

Time for which zinc-65 absorbed (days)	Muscle	Muscle/Blood ratio	
		Zinc-65	Stable zinc
12	First and second dorsal thoraco-abdominal	0.62	12.1
12	Third dorsal thoraco-abdominal	0.27	2.3
30	Superficial abdominal extensor	0.89	14.4
30	Deep abdominal extensor	0.50	2.2
30	Superficial abdominal flexor	1.05	17.3
30	Deep (main) abdominal flexor	0.41	2.0

permeability of the main flexor muscle of the lobster to zinc is low, because virtually no increase in its concentration of zinc occurred when the amount of zinc in the blood was raised by a factor of six<sup>1</sup>. In the present work no specific attempt has been made to measure the permeability of these muscles to zinc. An indication of the permeabilities of slow and fast muscles, however, was obtained by measuring their concentrations of zinc-65 in lobsters which had been kept in sea water to which the isotope was added. These results are summarized in Table 2. The concentrations of zinc-65 are expressed as muscle/blood ratios and are compared with the corresponding stable zinc ratios.

Muscle/blood ratios for zinc-65 in slow muscle are roughly double those for fast muscle which suggests that slow muscle is more permeable to zinc. The stable zinc ratio for slow muscle is much higher than that for fast muscle, and so it will presumably take longer for the zinc-65 ratio for slow muscle to approach that for stable zinc.

The nature of the difference in concentration of zinc between the two types of muscle is not known, but might be related to differences in enzyme content. Enzymes such as lactic and glutamic dehydrogenase<sup>7</sup> and carbonic anhydrase<sup>8</sup> have been detected in crustacean muscles. In vertebrates these have been shown to be zinc-metallo-enzymes<sup>9,10</sup>. Other vertebrate muscle enzymes, for example ATPase, have been shown to be activated by low concentrations of zinc<sup>11</sup>. It was thought at one time that zinc takes part in the function of the relaxing factor in vertebrate muscle<sup>12</sup>, but this now seems to have been disproved<sup>11</sup>.

In the lobster, and presumably in other decapod crustaceans, the difference between the concentrations of zinc of fast and slow abdominal muscles is a feature which should make them suitable material for determining the role of zinc in crustacean muscle.

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## Calcium and Contraction of Heart and Smooth Muscle

It is well established that an increase in the intracellular concentration of ionized calcium after excitation in the membrane is the key factor for initiation of contraction of various types of muscles. In skeletal muscle the tubular structure within the muscle fibres which is in connexion with the surface membrane, the endoplasmic reticulum, has been considered by many to serve as a supply source of this ionized calcium. For heart and smooth muscles, however, in which the development of this structure has

been found to be rather poor, there is still uncertainty as to whether the calcium comes from outside the muscle. We have attempted to elucidate this point, using calcium buffers.

Experiments were carried out on the isolated taenia coli of the guinea-pig and on the ventricular strip of *Rana catesbiana*. To obtain information about mammalian heart muscle, the right ventricular strip preparation described by Feigen *et al.*<sup>1</sup> was used in some experiments. All the experiments were carried out in winter-time, from December to March. The muscle preparations were suspended in a 5 ml. organ bath, immersed in water at  $20 \pm 0.3^\circ \text{C}$  for frog and  $36 \pm 0.3^\circ \text{C}$  for mammalian muscle. The bathing solution had the composition of conventional Ringer or Ringer-Locke solution, except that sodium bicarbonate was replaced by *tris*-maleate buffer ( $\text{pH} = 7.1$ ,  $5.0 \text{ mmolar}$ ). The solution was aerated with 100 per cent oxygen. To prepare solutions with low stabilized concentrations of free ionized calcium, 5 mmoles of ethylene glycol *bis*( $\beta$ -aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) was used as a chelating agent. In the  $\text{pH}$  range  $4.0$ – $7.5$  the ligand exists mainly with two negative charges and the concentrations of ionized calcium can be calculated sufficiently accurately from the equation

$$p[\text{Ca}^{++}] = 2 \text{pH} - 7.28 + \log \left\{ \frac{[\text{EGTA}] \text{ added}}{[\text{CaCl}_2] \text{ added}} - 1 \right\}$$

As a model of the contractile response of these muscles, potassium contracture (K-contracture) was adopted, which was produced by isotonic potassium sulphate Ringer or isotonic potassium sulphate Ringer-Locke solution. The contracture tension developed was recorded on a self-balancing potentiometric recorder with the aid of a strain-gauge transducer and a d.c. amplifier. The membrane potential of taenia coli was recorded by the sucrose-gap technique.

Fig. 1 shows that K-contracture in the taenia coli consisted of the initial rapid rise of tension followed by a phase of sustained contraction, which lasted as long as

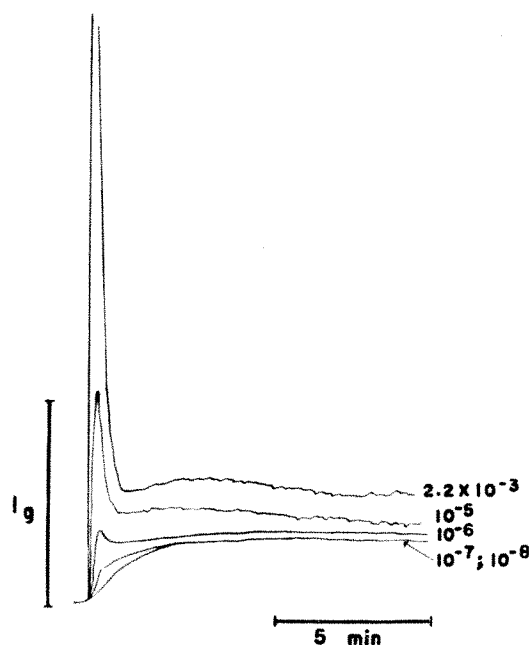


Fig. 1. Effect of the concentration of external calcium on the shape of K-contracture of guinea-pig taenia coli. Potassium contracture (K-contracture) was induced by potassium sulphate-Locke solution. Contracture tension was recorded isometrically with a strain-gauge transducer on a self-balancing potentiometric recorder. Low stabilized concentration of calcium (expressed in moles in the figure) was produced by adding 5 mmoles of EGTA. The final levels of the tonic phase in  $10^{-7}$  and  $10^{-8}$  molar calcium medium were similar and cannot be differentiated in this figure. The upper curve represents the initial phasic response in  $10^{-7}$  molar calcium and the lower that in  $10^{-8}$  molar calcium. Note the inhibition of the initial phasic portion in low calcium.

there was a large concentration of potassium in the bathing medium. In contrast, the contractile response in the frog's heart proceeded more slowly to reach a peak in more than 1 min and then declined to a phase of maintained contraction (Fig. 2). In Fig. 2 only the response of the frog's heart is depicted, for, although the response was faster, its shape in the rat heart was essentially the same. These figures show that when the concentration of the free ionized calcium in the bathing medium was reduced by addition of EGTA a diminution of the contractile response was observed both in heart and smooth muscle. In taenia coli, however, the initial quick phasic response was preferentially affected by this procedure and it finally disappeared completely when the concentration was reduced below  $10^{-7}$  molar, while the ensuing tonic portion persisted even at a concentration of  $10^{-8}$  molar or less of calcium (Fig. 1). In heart muscle of frog or rat, the contractile response to isotonic potassium sulphate was, in contrast, uniformly affected over its full time course, and at calcium concentrations below  $10^{-7}$  molar no contractile response could be elicited; the muscle actually relaxed instead (Fig. 2).

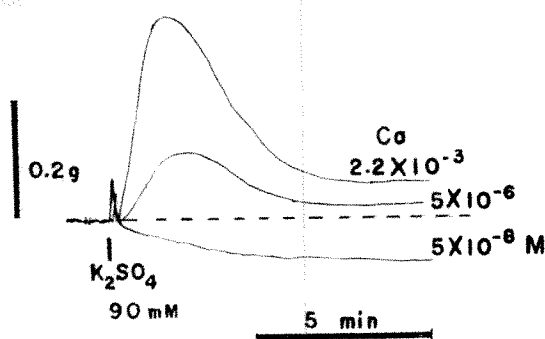


Fig. 2. Calcium and K-contracture in heart muscle of *Rana catesbiana*. Note the reversal of the response in  $5 \times 10^{-8}$  molar calcium.

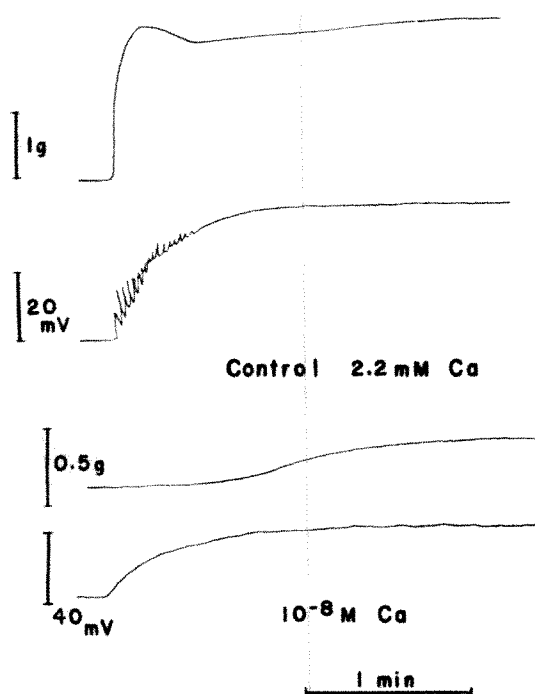


Fig. 3. Effect of concentration of external calcium on the membrane potential change during K-contracture. Guinea-pig taenia coli. Upper two curves: contracture tension and membrane potential in 2.2 mmolar calcium medium. Membrane potential was recorded using the sucrose-gap technique. Lower two curves: same as in the upper, but in  $10^{-8}$  molar calcium medium.

According to recent work<sup>2-5</sup> on rabbit myofibrils, purified actomyosin of rabbits, glycerinated skeletal and smooth muscle of the hog and the intact leg muscle of a crab the threshold concentration of free ionized calcium for the activation of these contractile systems is  $10^{-6}$ – $10^{-7}$  molar. Taking this figure into consideration, it may be concluded from the present data that contraction of heart muscle is initiated and maintained solely by a diffusion of calcium from the surrounding medium into the muscle fibres. Part of the tonic phase as well as the initial phasic portion of K-contracture of taenia coli could be viewed in the same way. But to explain the portion of the tonic phase that persisted even at a concentration of  $10^{-8}$  molar of calcium we must assume, in addition, the existence of some binding site for calcium within the muscle fibres, from which calcium is released little by little into the interior influenced by the sustained membrane depolarization, thereby producing a tonic phase of contracture. Thus, the smooth muscle fibre has two different means by which it can raise the intracellular concentration of free ionized calcium, in contrast to heart muscle, which depends entirely on the surrounding medium for supply of the necessary calcium. For comparison, we have investigated sartorius and rectus abdominis of *Rana catesbiana*; K-contracture in these muscles was often elicitable even when the extracellular calcium concentration was reduced to  $10^{-7}$  molar or less, in good agreement with the well known efficient calcium-binding capability of the vesicular relaxing factor of skeletal muscle.

The free ionized calcium entering into the muscle fibres during the initial phasic portion of the K-contracture of taenia coli could be identical with that calcium which possibly constitutes the spike potential of this muscle, for a reduction of the concentration of extracellular calcium to below  $10^{-8}$  molar invariably resulted in a complete extinction of the spike discharge seen superimposed on the rising phase of membrane depolarization of control preparations, in association with the complete abolition of the initial phasic portion of the contractile response (Fig. 3).

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## BIOPHYSICS

### Existence of Collective-excitation Energy Losses from an Electron Beam passing through Biological Materials

In radiobiology, the transfer of energy from ionizing radiation to an absorber is often expressed in terms of the mean energy transferred per primary ionization and the number of such ionizations/g of absorber. Recently, Rauth and Hutchinson<sup>1</sup>, using an electron beam spectrometer with an energy resolution of about 1 eV, have directly measured the energy losses from an electron beam passing through thin organic films ('Formvar' and polystyrene). Very few electrons were observed to have lost energy amounts of less than 10–12 eV and the most probable energy loss was about 25 eV. For electron beams of energies of between 5 and 20 keV, the mean energy lost per primary ionization was found to be  $60 \pm 10$  eV. This value is considerably less than that of 100 eV (ref. 2)

estimated from data on gases and from calculations by Bethe<sup>3</sup>.

The difference between these two values may result from the existence in solids of energy-loss processes which do not occur for gases. Collective excitation of valency electrons is one possible mechanism. Collective energy losses as observed in metals have been explained in terms of the plasma model of Bohm and Pines<sup>4</sup>, in which discrete amounts of energy, in the region of 10–20 eV, are lost in the excitation of longitudinal oscillations in the free-electron plasma. Multiple events of this type give rise to a number of peaks in the energy-loss spectrum of a metal foil. Birkhoff<sup>5</sup> has shown that for aluminium about 16 per cent of the energy dissipated goes to plasma excitation.

The plasma model is somewhat unrealistic for semi-conductors and insulators in which characteristic energy losses, thought to be due to similar collective excitations, are also observed (ref. 6 and our unpublished work). Fano has recently attempted to formulate a theoretical model in which excitation of a cubic lattice of coupled dipole oscillators is proposed as an alternative to the plasma model. Simpson<sup>7</sup> has pointed out that it is not necessary to restrict the plasma model to free electrons in metals, because the plasma frequencies are so high that the electron displacement is only about 1 Å. Collective oscillations in the valence electrons of non-metals may therefore occur.

Whatever the outcome of such theories, it is fairly clear that collective excitations are a possible mode of energy loss in organic materials and that data obtained for gases may not be directly carried over to the condensed state. It is important therefore to know if such processes occur in biological materials.

In order to test this possibility, a high-energy electron beam spectrometer (recently constructed at the University of Reading) with an angular resolution of  $10^{-4}$  radians and an energy resolution of 1 eV has been used to investigate the energy losses in thin films of calf thymus nucleic acid (supplied by British Drug Houses, Ltd.). This instrument has been described elsewhere<sup>8</sup>.

Self-supporting films approximately 2000 Å thick were produced by allowing 0.002 ml. drops of nucleic acid solution (0.05 per cent w/v sodium salt in distilled water) to dry on 3 mm diameter electron microscope specimen grids.

Energy-loss electrons scattered at small angles to a well collimated 150 keV electron beam passing through a film were analysed by a  $180^\circ$  magnetic spectrometer, using direct electronic recording techniques. Fast recording times (5–15 sec) and low beam currents ( $10^{-7}$  amp/cm<sup>2</sup>) were used to minimize radiation damage to the specimen. Electron diffraction photographs from such specimens showed a strong ring at a diameter corresponding to a spacing of 2.7 Å. This ring was absent in specimens which had suffered considerable radiation damage, in which event a more diffuse pattern similar to that obtained from carbon specimens was observed. The ring at 2.7 Å presumably arises from the existence of hydrogen bonds, with spacings in the region of 2.7 Å, in the undamaged nucleic acid specimens.

As a result of the long-range nature of the interactions which correspond to collective excitations, the transfer of momentum from the incident electron is small and electrons losing discrete amounts of energy by such interactions are scattered through very small angles. Ferrel<sup>9</sup> has given the differential scattering cross-section as approximately

$$\sigma(\theta) = \theta_E / (2\pi a_0 (\theta_E^2 + \theta^2)) \quad (1)$$

where  $a_0$  is the Bohr radius ( $5.3 \times 10^{-9}$  cm) and  $\theta_E = \Delta E / 2E$  ( $\Delta E$  is the energy loss and  $E$  the incident beam energy).

For  $E = 150$  keV and  $\Delta E = 25$  eV,  $\theta_E$  is  $0.83 \times 10^{-4}$  radians and this also corresponds to the scattering angle at which the intensity is just half the intensity at  $\theta = 0$

(from equation (1)). The majority of the scattering is therefore within a cone of semi-vertical angle  $10^{-3}$  radians. Electrons having suffered two 25 eV losses will have a broader distribution of lower intensity.

From the foregoing details it is seen that collective excitation losses are characterized by discrete peaks in the energy-loss spectra of electrons transmitted through thin specimens, and measured at angles within about  $10^{-3}$  radians to the incident beam direction. At angles of about  $10^{-4}$  radians for thin films, the single loss process is most probable, whereas for larger angles the double loss or higher multiple loss processes become relatively more probable, although at angles greater than  $10^{-3}$  radians the intensity is very low.

These characteristics are clearly seen in the energy-loss spectra from a 2000 Å thick film of nucleic acid which are reproduced in Fig. 1. The peak at 50 eV, although barely visible at  $2.5 \times 10^{-4}$  radians, becomes very prominent at a scattering angle of  $10^{-3}$  radians (note the change of vertical scale for the spectra at different scattering angles).

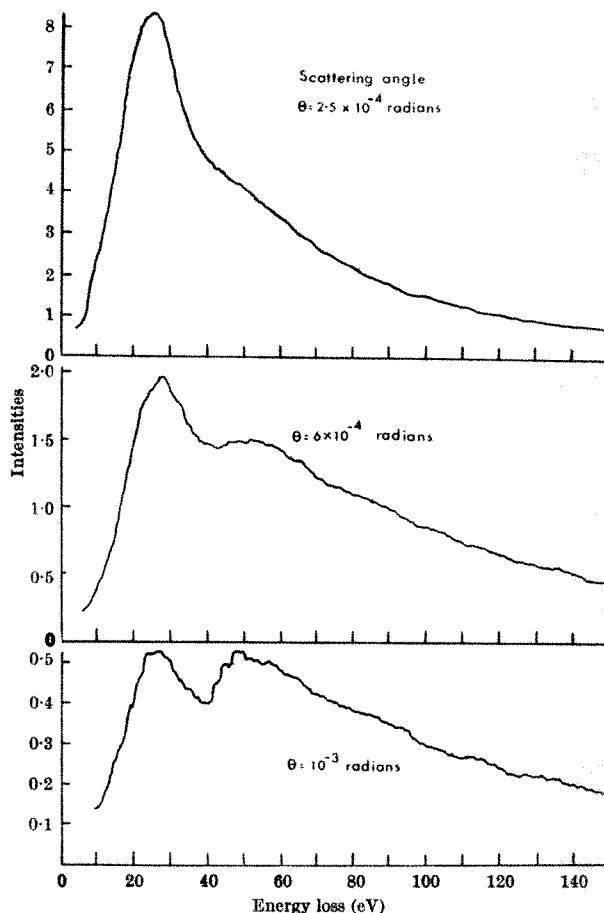


Fig. 1. Energy-loss spectra from a 150 keV beam of electrons passing through a thin film of nucleic acid.  $\theta$  is the scattering angle.

This evidence supports recent suggestions that collective losses exist and are important in radiobiology<sup>10</sup>. The lifetime of the collective excitation states is of the order of  $10^{-16}$  sec and, although the nature of the decay process is not fully understood, there is the possibility that the energy is finally dissipated in the rupture of a number of chemical bonds over a region much larger than that associated with the ion cluster. This could significantly affect determinations of radiation sensitive volumes by the associated volume method due to Lea<sup>11</sup>.

This work has formed part of an investigation of electron scattering at high energies, which is being carried out with



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## BIOLOGY

### Blood Borne Factors in Circadian Rhythms of Activity

RHYTHMS of a circadian nature in locomotor and other kinds of activity have been described in a variety of organisms. While much is known about the time course and phase relations of such rhythms, very little is known about the internal regulating mechanisms. The diurnal locomotor rhythm in the cockroach is controlled by neurosecretory cells located in the sub-oesophageal ganglion<sup>1,2</sup>. Transplantation experiments have shown the importance of the presence of the sub-oesophageal neurosecretory apparatus for the persistence of the locomotory rhythm, but no variations with the time of the day in the direct effects of the neurosecretory products on the degree of activity of the central nervous system have been demonstrated.

It was earlier demonstrated<sup>3</sup> that the isolated ventral nerve cord of the scorpion exhibits "spontaneous" electrical activity. The level of such activity was shown to vary with the time of the day, the maximum activity being noticed between about 4 p.m. and 12 midnight with peak activity around 8 p.m. From about 11 p.m. there was a decrease in activity; activity almost stopped in the early hours of the morning. The scorpion exhibits a diurnal rhythm in its locomotory activity and the time course of this locomotory rhythm is the same as that of the above-mentioned rhythm in the electrical activity of the ventral nerve cord. It therefore seems very likely that the locomotory rhythm flows from the rhythm of activity in the central nervous system. In view of this possibility it would be of particular interest to know the factor or factors that modulate the activity of the nervous system.

In view of the known effects of neurohormones on the spontaneous activity of the central nervous system<sup>4-7</sup> and the rhythm in the secretory activity of certain groups of neurosecretory cells in the sub-oesophageal ganglion of the scorpion<sup>8,9</sup>, the effects of blood and of extracts of the cephalothoracic nerve mass on the activity of the ventral nerve cord at different times of the day are of interest. Well fed scorpions, *Heterometrus fulvipes*, kept in the laboratory in conditions of normal day and night, were used in the present investigation. Blood (haemolymph) and the cephalothoracic nerve mass were taken from these scorpions at 12 noon, 5 p.m., 11 p.m. and 2 a.m. For this purpose two scorpions were taken into a cold room at the required time and first the blood was drawn from them with a hypodermic syringe at the joint of the "hand" of

the pedipalp with the proximal podomere. Blood drawn from two individuals at the same time was pooled together and immediately placed in the ice-box of the refrigerator at  $-5.0^{\circ}\text{C}$ . At all times of the day the quantity of blood that could be drawn was about the same and usually 0.4–0.5 ml. of blood was drawn from each animal. No anticoagulant was used, because clotting is not effective in this animal at such low temperatures. These scorpions were dissected immediately to remove the cephalothoracic nerve mass which is taken out quickly, from each of the two animals, into a glass homogenizer kept at  $0^{\circ}\text{C}$  in an ice-bath. A homogenate was prepared in sterilized cold ( $0^{\circ}\text{C}$ ) scorpion Ringer and this was extracted and centrifuged and the supernatant was stored at  $-5.0^{\circ}\text{C}$  until further use. No extract or blood was stored longer than 20 h before it was used.

To investigate the effects of the blood and extracts of cephalothoracic nerve mass thus prepared at different times of the day on the "spontaneous" activity of the isolated ventral nerve cord, the following procedure was carried out. The ventral nerve cord of the normal animal was exposed (always between 8 a.m. and 10 a.m. when the natural activity of the cord is minimal) from the dorsal side and was isolated by severing all the segmental nerves. A chain of four ganglia, consisting of the three mesosomatic and the first metasomatic ganglia, was isolated by severing the cord anteriorly and posteriorly, and this isolated preparation was used for recording. The recording electrodes (bipolar silver/silver chloride) were placed between the first and second mesothoracic ganglia and the electrical activity was recorded using conventional electrophysiological equipment. The photographic records were used for visual pulse height analysis.

The sequence of each set of recordings was as follows: first the spontaneous activity was recorded after prolonged (more than 15 min) bathing in the perfusion fluid (scorpion Ringer); then the Ringer solution was replaced by the required extract of cephalothoracic nerve mass or the blood and the activity was recorded at intervals of 5 min for 15 or 20 min; finally the test solution was replaced by the Ringer solution and the activity was again recorded after repeatedly washing the cord with the perfusion fluid. Thus recordings were made using blood and extracts of cephalothoracic nerve mass obtained at different times of the day indicated above. For recording, the electrodes with the nerve cord on them were lifted, just above the perfusion fluid, into the air.

The analysis of the records (Table 1) shows that there is a great increase in the spike activity when either the blood or the extract of cephalothoracic nerve mass from the 5 p.m. scorpions is applied to the isolated ventral nerve cord. This increase was not only caused by the increased frequency of the "normally" active small units but also by the recruitment of new units, especially the large "giant" units which have been reported to be active during the evening. The response is maintained as long as the nerve cord is bathed in perfusion fluid containing the active substance. On an average the activity increases by 34 per cent more than the control values obtained in scorpion Ringer. Similar increase in activity is obtained with the 8 p.m. blood and extract of cephalothoracic nerve mass. On the other hand, an opposite effect is noticed when blood or the extract obtained from either the 11 p.m. or the 2 a.m. scorpions is applied. The greatest depression in the activity of the nerve cord is noticed with the application of the 2 a.m. extract; there is a 60 per cent drop in the spike activity compared with the control values in Ringer solution. Both the blood and the extract of cephalothoracic nerve mass obtained from the 12 noon scorpions have little or no effect on the spontaneous electrical activity of the nerve cord.

From the results obtained it is clear that the effects of the blood and extract of cephalothoracic nerve mass on the spontaneous electrical activity of ventral nerve cord are not always the same. The time of the day when the

Table 1. EFFECT OF EXTRACT OF CEPHALOTHORACIC NERVE MASS AND BLOOD ON THE ELECTRICAL ACTIVITY OF THE ISOLATED VENTRAL NERVE CORD OF THE

Time of day: 5 p.m.			8 p.m.			SCORPION 11 p.m.			2 a.m.			12 noon		
Extract	Ringer	Blood	Extract	Ringer	Blood	Extract	Ringer	Blood	Extract	Ringer	Blood	Extract	Ringer	Blood
497 ± 27	365 ± 15	480 ± 40	483 ± 19	310 ± 20	451 ± 36	263 ± 15	377 ± 23	226 ± 13	125 ± 8	345 ± 18	250 ± 14	284 ± 16	310 ± 21	282 ± 26
t = 5.4		4.5	9.5		7.4	7.6		9.1	42.9		11.1	Not significantly different		

Values are spikes/10 sec. Data from six test animals are pooled. Each animal was tested with the extract and then with blood pooled from two donor animals killed at the times shown, alternating with the Ringer test. Tests on the extract and blood from all five testing times were carried out consecutively in one session of 2-2.5 h on the ventral nerve cord of test animals; therefore, ten experimental tests alternating with ten Ringer tests were carried out. Each test lasted 5-15 min during which ten to fifteen 10 sec samples were averaged. No trend was seen over the 15 min of a given test or in Ringer over the 2-5 h of a session.

blood and the extracts are obtained from the animal seems to be important. Two types of effect are seen. Extracts obtained from the 5 p.m. and 8 p.m. scorpions excite and increase the electrical activity, but those obtained at 11 p.m. and 2 a.m. inhibit and diminish the electrical activity. Both the excitatory and inhibitory effects are obtained whether the blood or the extract are used. From these results it may seem that the 5 p.m. and 8 p.m. blood as well as extract of cephalothoracic nerve mass contains some substance which excites nerve activity while a substance inhibiting nerve activity might be present in the 11 p.m. and 2 a.m. blood and extract of cephalothoracic nerve mass.

Although we do not know the nature of these excitatory and inhibitory principles, it is possible that they are of the nature of neurohormones which are produced by the neurosecretory cells in the sub-oesophageal ganglion and released into the blood. This possibility is strengthened by the recent demonstration<sup>7</sup> of the occurrence of two separate neurohormones in the brain and corpora cardiaca of the cockroach, one (neurohormone  $D_1$ ) capable of evoking hyperautorhythmia in the central nervous system and the other (neurohormone  $C_1$ ) capable of inhibiting the existing electrical activity in the ganglia. In view of the already demonstrated rhythmic variations in the activity of neurosecretory cells in the scorpion<sup>8</sup>, it is very likely that two neurohormones, one excitatory and the other inhibitory, are produced out of phase with each other by the neurosecretory cells, and released at different times into the blood. Thus, the accumulation and release of these two antagonistic neurohumoral principles can modulate the spontaneous electrical activity of the central nervous system into a circadian rhythm, with an active phase from about 4 p.m. to 11 p.m. and an inactive (or less active) period from about midnight to the next afternoon. This can be achieved if the activating neurohormone is released into the blood from about 4 p.m. till about 10 or 11 p.m. and thereafter while the release of the activating substances stops, the inhibitory neurohormone is released into the blood from about 11 p.m. onwards. Perhaps around 12 noon the two substances antagonize each other equally.

This modulation of the electrical activity in the central nervous system is of great behavioural significance because it may form the basis for the regulation of the circadian locomotory rhythm with the same time course, known to occur in this scorpion<sup>3,9</sup>.

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### Spontaneous Resistance to Chloroquine in a Strain of Rodent Malaria (*Plasmodium berghei yoelii*)

DURING an investigation of sporozoite induced infections of *Plasmodium berghei yoelii* in mice, chloroquine was given in an attempt to eliminate the erythrocytic stages of the parasite. Unexpectedly, the parasite failed to respond to the drug.

Further tests have shown that this strain is spontaneously as resistant to chloroquine as are the highly resistant strains of *P. b. berghei* which have been artificially produced in the laboratory (ref. 2 and unpublished work of Warhurst).

The early history of the strain of *P. b. yoelii* (No. 17X) has been given by Landau and Killick-Kendrick<sup>1</sup>. This is the type strain of *P. b. yoelii* which was isolated by Landau and Chabaud<sup>2</sup> in the République Centrafricaine in April 1965 from *Thammomys rutilans*. The line we have investigated was derived from mouse 38L (infected blood, preserved at -70° C for 5 months) which had been infected from *Anopheles stephensi* batch 31 L (ref. 1). After two further cyclical transmissions, with three intermediate syringe passages, a sub-line was treated with chloroquine (15 mg/kg daily oral dose) on November 8, 1965, and found to be insensitive. After another 6 week period of low-temperature preservation and fourteen more cyclical transmissions, with twenty-one intermediate syringe passages in mice and rats, another sub-line of the strain was subjected to two more syringe passages in mice, and then tested for chloroquine resistance on December 12, 1966. After the strain had been through a further three passages in mice, another drug test was carried out on January 2, 1967; the last drug test described in this note was carried out after three more mouse to mouse passages on January 16, 1967. From May 1965 to December 1966 strain 17X had been through fifteen cyclical transmissions and twenty-eight syringe passages in the laboratory. Other than when sub-lines were being tested, the strain has never been exposed to antimalarial drugs in the laboratory. The action of chloroquine on this strain was compared with the action of the drug on an old laboratory strain of *P. b. berghei* (N), and on a recently isolated mosquito-transmissible strain of *P. b. berghei* (NK65) (ref. 4). Groups of five male Parkes-strain mice were used.

In the first experiment, we compared the action of chloroquine on *P. b. berghei* (strain N), and *P. b. yoelii* (strain 17X), by a method previously described<sup>5</sup>. On December 12, 1966, mice were inoculated intravenously with infected blood, and a single dose of the drug was given intraperitoneally 2 h later. Increases in latent periods of infection were then recorded. The dose-response curves are given in Fig. 1. The prolongation of the latent period of strain N is linearly related to the logarithm of the dose of chloroquine, but this is not so for strain 17X. In strain N, a dose of 40 mg/kg caused a delay of 4.2 days, but in strain 17X a dose of 40 mg/kg caused a delay of only 1.85 days. In the second experiment, a conventional 5 day drug test<sup>6</sup> was carried out as follows. The mice were inoculated intravenously on the first day with 10<sup>7</sup> parasitized erythrocytes, and drug doses were given intraperitoneally on the day of infection, and for 3 subsequent days. Blood films were taken on the day after the last dose of the drug, and the parasitaemias were evaluated. The results are expressed in

Fig. 2 as dose-response curves showing mean parasitaemias in the experimental groups (as a percentage of the parasitaemias in the control groups) plotted against logarithm of drug dose.

The difference in dose-response of the two strains is clear. In mice infected with strain *N*, 5 mg/kg given daily for 4 days completely prevented the appearance of parasites, while in mice infected with strain 17X a dose as high as 50 mg/kg merely reduced parasitaemia to 28 per cent of undosed controls. The dose-response line of strain 17X is relatively flat, and resembles the dose-response lines of artificially produced chloroquine-resistant strains of *P. b. berghei*<sup>2</sup>.

In the comparison of the action of chloroquine on strain 17X with that on strain NK65 of *P. b. berghei* made on January 16, 1967, a conventional 5 day drug test was carried out as in the second experiment described previously. In both experiments chloroquine was given in a dose of 5 mg/kg daily for 4 days. Strain 17X developed a parasitaemia which was 40 per cent of that of undosed controls, whereas strain NK65 developed a parasitaemia which was less than 3 per cent of the control. Chloroquine-resistance is therefore a special feature of strain 17X, and not merely a characteristic of freshly isolated strains.

The response of strain 17X to other antimalarial drugs is still in the course of investigation, but preliminary observations using the 5 day test indicate that it is sensitive (possibly hypersensitive) to pyrimethamine, sulphadiazine, and proguanil. It resisted a daily oral dose of 150 mg/kg of quinine hydrochloride but not of 300 mg/kg. It also resisted a daily intraperitoneal dose of 5 mg/kg of mepacrine hydrochloride but not 10 or 100 mg/kg. It was almost completely resistant to pamaquine given orally at sub-toxic doses (20 mg/kg) but not at doses (40 mg/kg) which killed most of the mice. These results suggest that there is a slight cross-resistance to mepacrine and quinine, but this is not as marked as that found in artificially prepared chloroquine-resistant strains of *P. berghei*<sup>2,6</sup>.

The results described here show that, although it has never before been exposed to antimalarial drugs, strain 17X is as resistant as are laboratory strains of *P. berghei* which have been produced by treatment of infected animals with chloroquine. The resistance appears stable, unlike artificially produced chloroquine-resistance<sup>2,6</sup>. These observations raise several interesting points. (1) Chloroquine-resistance may be a feature of all recently isolated strains of *P. berghei*. Most drug tests on *P. berghei* have been carried out on old syringe-passaged laboratory strains, which do not necessarily behave as do recently isolated ones. It is well known that freshly isolated strains of *Trypanosoma rhodesiense* are insensitive to doses of arsenical drugs which will eliminate old laboratory strains. On the other hand, our experience with strain NK65 showed that *P. b. berghei* is not similarly

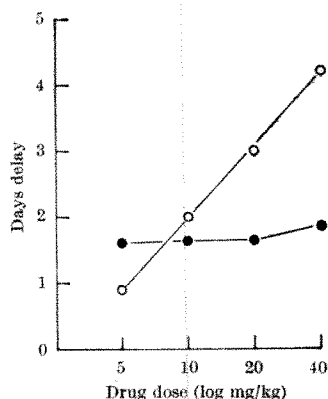


Fig. 1. Prolongation of latent period (days delay) by single doses of chloroquine in strain *N* (○) and strain 17X (●) of *P. berghei*.

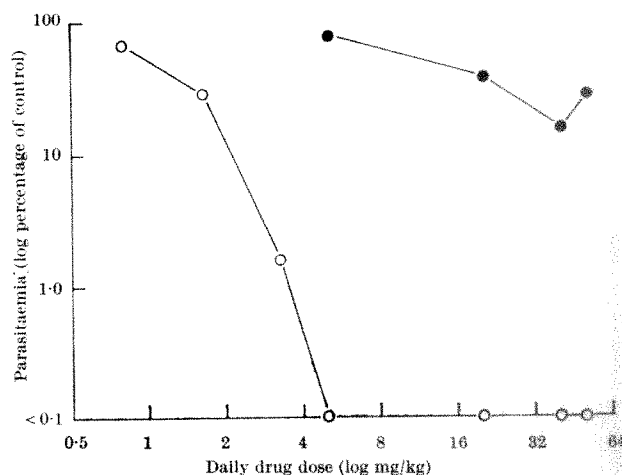


Fig. 2. Parasitaemias (as percentage of undosed control values) in mice infected with strain *N* (○) and strain 17X (●) of *P. berghei*, plotted against daily dose of chloroquine in the 5 day test.

resistant, even when freshly isolated and still infective to mosquitoes. (2) Chloroquine-resistance is perhaps restricted to the *yoelii* sub-species of *P. berghei*. We cannot be sure this is so, however, until other isolates of the sub-species have been investigated. (3) Because a spontaneously chloroquine-resistant strain of *P. berghei* has been found in nature, there seems to be no reason why the same should not apply to *P. falciparum*, which causes malignant tertian malaria of man. In South East Asia and South America, strains of *P. falciparum* have been found which are resistant to chloroquine<sup>7</sup>, while in Africa, where chloroquine has also been widely used as an antimalarial, there is no authenticated report of chloroquine-resistance. It seems likely that in *P. falciparum*, as in *P. berghei*, chloroquine-resistant strains may sometimes occur spontaneously.

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### Multiplication of *Drosophila* Hereditary Virus ( $\sigma$ Virus) in *Drosophila* Embryonic Cells cultivated in vitro

$\sigma$  VIRUS multiplies in *Drosophila* and confers to it a characteristic sensitivity to carbon dioxide<sup>1</sup>. Although many data have been collected about this virus, so far all virological and genetic investigations have been concerned with the complex system formed by the virus and the host organism. To understand better the relationships

between  $\sigma$  virus and its host at the cellular level, we have been developing techniques to cultivate *Drosophila* cells *in vitro* and infect them with  $\sigma$  virus.

We have shown<sup>2</sup> that it is possible to grow embryonic cells of *Drosophila melanogaster* in a relatively complex medium which imitates the composition of the *Drosophila* haemolymph, and is supplemented with 10 per cent foetal calf serum. These "primary" cultures have to be transferred after the sixth week. Successful sub-cultures were developed in microdrops under paraffin oil<sup>3</sup>. As long as the medium is changed frequently, the cells can be kept in good condition for many months and mitotic figures are observed often. They divide to form small continuous layers adhering to the bottom of plastic Petri dishes, on the areas corresponding to the drops. These "monolayer" sub-cultures reasonably could be expected to provide good material for investigation of the multiplication of  $\sigma$  virus.

The viral suspension we used came from *Drosophila* flies infected with a "thermo-resistant" viral mutant<sup>4</sup>. The insects were crushed with a definite quantity of our standard culture medium, and this crude extract, after centrifugation, was filtered through a 'Millipore' membrane (pore size 0.45 $\mu$ ). This sterile filtrate was used as the source of  $\sigma$  virus.

To infect the cells, the culture medium from a microdrop was removed and replaced by the viral suspension. After a 1 h adsorption period, this fluid was sucked up and fresh culture medium was substituted for it. The culture medium was changed regularly every 3 or 4 days. The infected microdrops were kept at 26°C. We systematically looked for infectious virus in the supernatant medium of the drops and determined the titre by the accurate biological method using *Drosophila* flies<sup>5,6</sup>: the viral content of every sample is assayed through the measurement of the mean incubation time of a set of about sixty inoculated *Drosophila*. The relation between the incubation time and the number of injected infectious units has been carefully established for the used viral strain, by the method of serial dilutions.

Two sub-cultures of *Drosophila* embryonic cells, transferred to microdrops under paraffin oil 2 months before, were infected with  $\sigma$  virus in the conditions which have been described. If the titre of the initial viral suspension and the volume of the drops are considered then the amount of virus which infected each of these cultures can be estimated as  $4 \times 10^3$  infectious units. From the second week after infection, the viral production became intensive and remained high and fairly constant until the end of the experiment. One of the cultures, when it was lost accidentally on the forty-fourth day after infection, had produced, if we add up the successive viral collections, about  $3 \times 10^5$  infectious units. The other culture was still in good condition on the sixty-ninth day. At this time, the cells of this culture were shaped from the bottom of the Petri dish and crushed in their last culture fluid. The total yield of this culture was found to be  $6 \times 10^5$  infectious units. No cytopathic effect was, however, observed; the cells appeared quite normal in the phase contrast microscope, and their multiplication was proceeding at the usual rate.

We can conclude that the  $\sigma$  virus multiplies in *Drosophila* embryonic cells cultivated *in vitro*. Starting with  $4 \times 10^3$  infectious units, the viral collection after 2 months amounted to approximately  $6 \times 10^5$  units. This viral production is equivalent to the maximal viral yield of one infected *Drosophila* fly. Although it is very difficult to estimate accurately the cellular content of a microdrop culture, it is undoubtedly smaller than the cellular mass of a whole fly. Furthermore, it must be noticed that the experiment was voluntarily stopped on the sixty-ninth day. Viral production had been proceeding regularly for many weeks, and the cells were still in excellent condition, so there is every reason to believe that the experiment could have been extended for a much longer time

and the total viral yield could have reached a value perhaps two or three times larger.

Thus, for the first time, it was possible to demonstrate a multiplication of  $\sigma$  virus in cultures of *Drosophila* cells. Further experiments of this type will result in a more accurate analysis of the relations between  $\sigma$  virus and the cells of its host, *Drosophila*.

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### Techniques for recording the Activity of Aquatic Invertebrates

DURING recent investigations of burrowing in worms and bivalve molluscs<sup>1-3</sup> new techniques have been developed of continuously recording activity, because conventional methods which involved the attachments of threads or levers<sup>4,5</sup> proved of little use in investigating freely burrowing animals. The techniques described here allow observations to be made with the minimum of disturbance to normal activities. They involve the adaptation of equipment commercially available and are based on the measurement of either impedance or hydrostatic pressure changes.

Using an impedance pneumograph, a small oscillatory current (2  $\mu$ amp, 25 kc/s) may be passed between two fine wire electrodes attached one to each valve of a bivalve (Fig. 1,  $E_1$ ). Any movement of the valves affected the impedance between the electrodes and a voltage proportional to the change in impedance was fed to a pen recorder by either a.c. or d.c. coupling. With a.c. coupling (time constant 2.5 sec) opening or closing of the valves

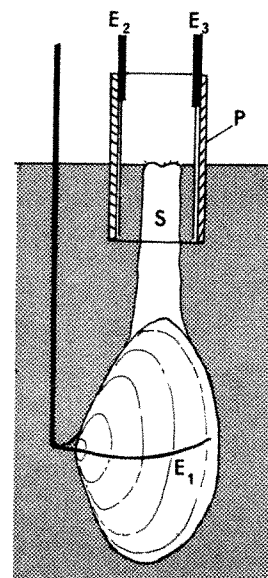


Fig. 1. Diagram of *Mya arenaria* in natural position in sand (stippled) and under water showing position of electrodes.  $E_1$ , fine wire electrode, one of a pair attached to the valves to record opening and closing;  $E_2, E_3$ , wire electrodes fixed to 'Perspex' tube (P) to record movement of siphons.

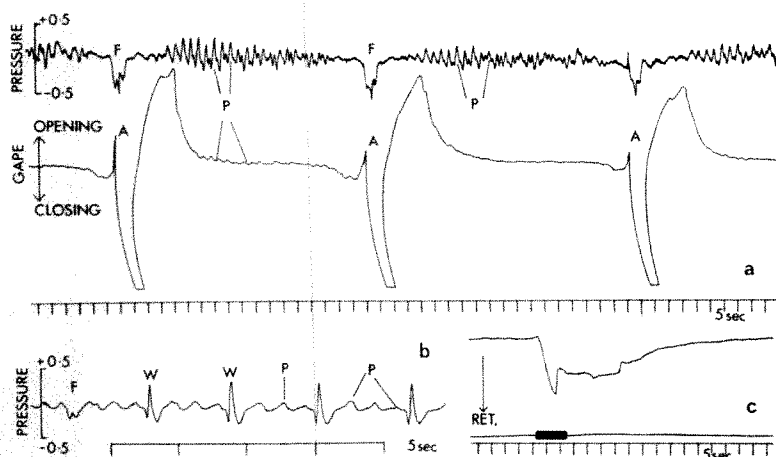


Fig. 2. Recordings of activity of bivalves. *a*, *Margaritifera margaritifera* moving across the surface of fine sand. The pressure recording (above) shows a negative swing (*F*) at adduction (*A*) which is sustained during pedal retraction. Valve movements (gape) recorded by electrodes placed on the valves (*E*<sub>1</sub>, Fig. 1) with a.c. coupling to the recording equipment. Between adductions, after the valves have reopened, oscillatory pressure waves (*P*) indicate extension and probing of the foot. *b*, *Ensis arcuatus* digging into sand. The pressure wave shows a negative swing (*F*), which corresponds to those in *a*, followed by positive peaks (*W*) caused by ejection of water from the mantle cavity at adduction into the sand as depth of burial increases. Between each digging step (*F* or *W*) the foot makes three distinct probes (*P*). *c*, Siphon movement of *Mya arenaria* recorded by impedance pneumograph attached to electrodes (*E*<sub>2+3</sub>, Fig. 1) with d.c. coupling to pen recorder. Heavy line indicates closure of siphonal apertures. Retraction (*RET*) is followed immediately by partial protraction and a slow rather intermittent recovery to the initial length. Pressure recorded in cm of water.

appeared as positive or negative deflexions, respectively, about a preset level to which the pen returned when movement ceased; d.c. coupling was suitable for more static phenomena. Although limited by the length of wire attached to the electrodes, this method allowed continuous recordings to be made of bivalves burrowing and demonstrated that adduction of the valves occurred at each downward step of the shell into the substrate (Fig. 2*a*). The same method has also been used to record the movement of the siphons of *Mya arenaria* by mounting a pair of wire electrodes on either side of a 'Perspex' tube or arch placed over the siphon (Fig. 1). Siphonal movements now changed the impedance between the stationary electrodes (*E*<sub>2+3</sub>) (Fig. 2*c*). This technique allows continuous records to be made without the animal being in direct contact with the electrodes and has been successfully used for recording the movement of sea anemones and the burrowing of *Arenicola*.

Pressure recording is the basis of another method for investigating the movements of animals through sand, by the use of a Statham pressure transducer of maximum sensitivity 0.4 cm water pressure/cm pen deflexion. The transducer was connected to pressure tubing of 3 mm bore which led a few cm below the sand and had its rigidly fixed external opening protected from entry of sand grains by a coarse nylon mesh. The satisfactory operation of the equipment was checked by recording small ripples on the surface of the water above the sand. The recordings were calibrated in cm water pressure, but the absolute values had little meaning as they were affected by the distance of the animal from the tube and the dilatant properties of the sand/water mixture<sup>6</sup>. Using fine clean sand from an open shore, a negative pressure is recorded either by a probe applied manually or by the thrust of the foot of a bivalve during digging (Fig. 2*a*, *F*). The force applied disturbs the packing of the sand/water system and the available water no longer suffices to fill the enlarged spaces so that water tends to be drawn in. As would be expected, withdrawal of the foot from the sand also gives a negative effect, but the ejection of water from the mantle cavity of a bivalve beneath the sand causes a positive peak. This is shown in Fig. 2*b*, *W*, where it should be noted that the first adduction of the valves (*F*) gives a response similar to Fig. 2*a* but as depth of burial increases the pressure (*W*)

becomes more strongly positive. Because the size of the experimental tank also affects the external pressure recordings, these must always be related to direct observations of the animal's activity. When this has been accomplished, recordings of the digging of small molluscs or worms, free of any attached electrode, may be readily obtained and interpreted even when they are completely invisible beneath the sand.

Fig. 2*a* shows some of the details of locomotion made apparent by these techniques. The external pressure (above) has been recorded simultaneously with valve movement (below) during the locomotion of the freshwater clam, *Margaritifera margaritifera*, across an aquarium. At each step the valves were rapidly adducted (*A*) and more slowly reopened. Only the foot was beneath the surface of the sand and it repeatedly retracted (*F*), immediately after adduction, pulling the shell forward about 0.5 cm each time. Between retractions, extension and probing forward of the foot produced continuous oscillations of pressure (*P*) which also had some effect on the valves. A common feature of recordings of the locomotion of this and certain other bivalves, for example, *Cardium*, at the surface of sand was the opening of the shell before adduction. This possibly represents relaxation of the 'slow' adductor muscle fibres before rapid contraction of the 'fast' fibres.

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## Studies on the Relations of Insect and Host Plant

### 1. Effects of Water Stress in Host Plants on Infestation by *Aphis fabae* Scop., *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.)

It is generally agreed that the reproduction of aphids is influenced by water stress in the host plant. The nature of this influence is, however, not so clear and both increases<sup>1</sup> and decreases<sup>2</sup> in the reproduction of aphids have been reported in connexion with such water stress. One effect of the shortage of water in plants is the hydrolysis of protein in the leaves<sup>3,4</sup>. Such enrichment of the phloem sap with soluble nitrogen would be favourable to aphids<sup>5</sup>. Another effect of water shortage in the plant is loss of cell turgor. This loss of turgor may be disadvantageous to aphids by reducing the assistance in food uptake provided by sap pressure<sup>6,7</sup>. Thus the effects on aphids of water shortage in the plant can be regarded in terms of two main interacting and largely conflicting factors: (1) the concentration of nitrogen, and (2) the pressure in the phloem (compare ref. 2).

Plants in the present greenhouse experiments were grown in vermiculite enriched with nutrients. To obtain reproducible degrees of water stress, the pots were watered to field capacity when weighing showed that a predetermined deficit (10, 50 or 90 per cent) of available water between field capacity and the permanent wilting point



had been reached. The plants were infected with a small number of aphids (ten in most experiments) and the population counted by washing the plants at the end of approximately 4 weeks. The experiments involved the following plant and aphid combinations: *Aphis fabae* Scop. on beans (*Vicia faba* L.), *A. fabae* and *Myzus persicae* (Sulz.) on marigolds (*Calendula officinalis* L.), and *M. persicae* and *Brevicoryne brassicae* (L.) on brussels sprouts (*Brassica oleracea gemmifera* Schulz). The mean number of aphids per plant at the end of each experiment is shown in Table 1.

Table 1. EFFECTS OF WATER STRESS IN HOST PLANTS ON THE REPRODUCTION OF *Aphis fabae*, *Myzus persicae* AND *Brevicoryne brassicae*

Host plant	No. of replicates	Aphid	Mean No. of aphids per plant on plants with water deficits (per cent) of:			Results
			10	50	90	
<i>Vicia faba</i>	11	<i>Aphis fabae</i>	1,304	1,160	1,263	No significant effect
	13	<i>Aphis fabae</i>	106	130	142	No significant effect
		<i>Myzus persicae</i>	273	340	171	90 per cent regime significantly lower than others ( $P < 0.001$ )
<i>Calendula officinalis</i>		<i>Aphis fabae</i>				
		<i>Myzus persicae</i>	196	488	210	50 per cent regime significantly higher than others ( $P < 0.001$ )
		<i>Brevicoryne brassicae</i>	211	186	155	Linear decrease in numbers with increasing water deficit significant ( $P < 0.05$ )

No effect of water stress could be observed with *A. fabae*, whereas the reproduction of *B. brassicae* decreased with increasing water stress. *M. persicae* showed a decreased reproduction at a water deficit of 90 per cent, and on brussels sprouts the increase in reproduction at a water deficit of 50 per cent was highly significant.

A possible interpretation of these results is that *M. persicae* gains more from an enrichment of the sap and is less dependent on phloem pressure than *B. brassicae*. The older leaves occupied by *M. persicae* are likely to show proteolysis and turgor reduction before the apex<sup>8</sup>, where *B. brassicae* normally feeds and in fact fed in this experiment. Nevertheless, the number of *B. brassicae* fell consistently with increasing water strain. In subsequent work with individual *B. brassicae* caged on the leaves, however, such a fall in reproduction with a shortage of water only occurred in old leaves, and at the apex reproduction actually increased<sup>9</sup>. With *A. fabae*, which is also mainly an apex feeder, Kennedy *et al.*<sup>2</sup> found that reproduction was significantly reduced only in their "very dry" regime, where the plants were considerably more wilted than in the 90 per cent deficit regime of the present experiments. This suggests that *A. fabae* is perhaps less dependent on phloem pressure than *B. brassicae*, but no evidence was obtained that the former aphid benefited from sap enrichment with increasing water shortage to an extent where enrichment more than compensated for a reduction in turgor pressure.

Some indirect evidence consistent with a lower dependence on pressure of *M. persicae* than of the two apex feeding aphids has been obtained by one of us (H. F. v. E.) with the artificial feeding of aphids on sucrose solutions without pressure through 'Parafilm' membranes<sup>10</sup>. Six replicate batches of ten apterous adults of each species were kept on each of twenty-five diets. These diets represented all combinations of five concentrations of sucrose (0–30 per cent) and five concentrations of sinigrin (0–2 per cent). The survival of the aphids was assessed daily, and the time at which 50 per cent of the insects had died was determined by plotting the probit of the percentage mortality against the logarithm of the time (days). As analysis showed that sinigrin had no significant effect, the survival of aphids on increasing concentrations of sucrose could be compared directly.

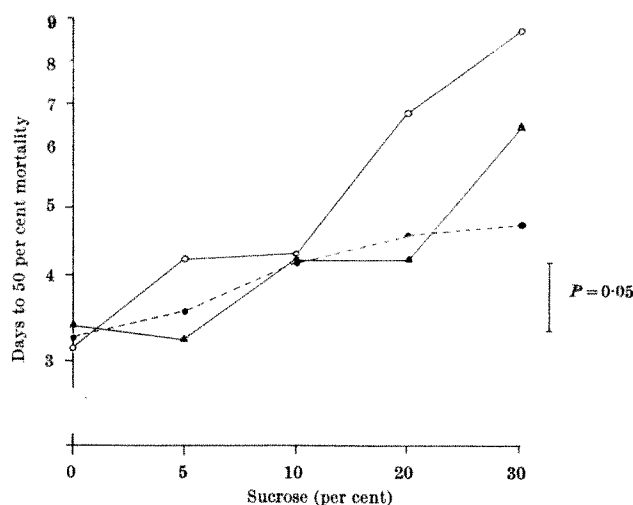


Fig. 1. The mortality of *Aphis fabae* (●—●), *Myzus persicae* (○—○) and *Brevicoryne brassicae* (▲—▲) on various sucrose concentrations fed through 'Parafilm' membranes.

The results (Fig. 1) show that *M. persicae* feeding on a pressure-less diet obtains greater benefit from an increase in the concentration of sucrose than *A. fabae* or *B. brassicae*. This is in agreement with the inference drawn here that *M. persicae* may be less dependent on a food supply under pressure than the other two aphid species studied.

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## II. Effects of Water Stress in Host Plants on the Fecundity of *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.)

IN a further investigation of the problem of the effects of water stress in host plants in pots on the reproduction of aphids, individuals of *Myzus persicae* and *Brevicoryne brassicae* have been studied on young, mature and old leaves of brussels sprouts (var. 'Jade Cross') which were growing in three water regimes<sup>1</sup>. All experiments were conducted at 20° C in a daylength of 16 h. Fourth instar nymphs in leaf cages (twelve replicates) moulted to adult and larviposited, and one of the offspring was reared to adult in each cage. Throughout the reproductive life of these adults, their progeny were removed daily and counted to measure fecundity (Fig. 1).

On young and mature leaves the fecundity of both species of aphid increased with water shortage. These increases may be related to enrichment of the phloem sap with soluble nitrogen compounds recirculated from the senescing leaves.

On old leaves the two species of aphid responded differently. The fecundity of *B. brassicae* diminished with increasing water shortage. This fall may be associated with reduced phloem turgor pressure and/or increased sap viscosity effectively reducing the quantity of sap ingested by the aphid<sup>2</sup>. Reduced fecundity was manifest at relatively little water shortage as compared with previous reports<sup>2</sup>. This suggests that *B. brassicae* is considerably dependent on turgor pressure for feeding, which is consistent with earlier work. The fall of reproduction with the shortage of water, previously reported for *B. brassicae*, occurred, however, when the aphids were feeding on the apex of the plant<sup>1</sup>. This inconsistency with the present experiments may well have resulted from too many of the aphids leaving the plants in the dryer regimes, either as alates or restless apterae. Experiments (to be published) support this belief.

On old leaves the fecundity of *M. persicae* was greater on plants in the medium regime, suggesting that the aphids were able to benefit from the enrichment of sap at this level of water shortage. The detrimental effect of loss of turgor was only evident in the dry regime where fecundity was lower. This is also consistent with earlier work.

It is clear that the response of aphids to a shortage of water in the host plant varies with the species of aphid and age of the leaf on which they are allowed to feed, as well as with the species of the host plant<sup>3</sup>. The results of the present experiments are consistent with the view that *M. persicae* is less dependent on turgor pressure for feeding than *B. brassicae*, although both aphids appear to benefit considerably from sap enrichment during host plant water shortage. These deductions are in accord

with the normal site of feeding of these two species of aphid on crucifers. *B. brassicae* most frequently feeds at the "turgid, nitrogen-rich" apex of the plant, while *M. persicae* occupies the "nitrogen-rich" senescing leaves which, during the summer months, may experience considerable and frequent fluctuations in turgidity (compare ref. 4).

In accordance with Kennedy, Lamb and Booth<sup>2</sup>, this interpretation assists in a reconciliation of the conflicting reports of the effects of host plant water shortage on aphids<sup>2,5,6</sup>. It is important to note, however, that lower phloem turgor pressure may be more important in reducing the duration and/or frequency of feeding by the aphids (increasing restlessness) than in directly reducing the rate of ingestion of sap, though the latter may be a causal mechanism of the former; in addition, other host plant factors, such as cell carbohydrates, minerals and pH, may have made an important contribution to the results.

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## PSYCHOLOGY

### Role of the Neocortex in Acquisition of Avoidance Conditioning

AFTER a series of experiments which demonstrated that an impairment of maze learning resulting from the ablation of cortical tissue was proportional to the extent of the lesion and not dependent on its location, Lashley<sup>1</sup> postulated that all areas of the neocortex have a general or non-specific function in learning. This he called the mass action effect. Recently, mass action has received convincing support from investigations with cortical spreading depression (refs. 2-4 and unpublished work of R. B. Ross and I. S. Russell, and I. S. Russell, D. Kleinman and H. C. Plotkin), where it has been repeatedly demonstrated that functional hemidecorticated rats require two to four times as many trials as normal animals to learn an escape or avoidance response. The present investigation is an attempt to extend the knowledge of the mass action effect, which, until now, has remained a descriptive phenomenon rather than an explanation of the role of the cortex in behaviour. The hypothesis is that one of the important functions of the neocortex is that it is concerned with the encoding of the information input to the central nervous system. The intertrial interval was used as a means of manipulating this information input.

Estes<sup>5</sup> has suggested that the intertrial interval determines the extent of change of the stimulus situation from trial to trial because of the random fluctuation of stimulus elements. If the animal is conceived as drawing a stimulus sample from the environment at each trial, then a short intertrial interval will result in response to the same or a similar sample on successive trials. If, however, a long intertrial interval is allowed, as a consequence of which a more substantial turnover of stimulus elements will take place, then the samples of successive trials are likely to be more varied. This would result in a broader sample of the available stimulus complex being responded to over a smaller number of trials. The response acquired will then be more rapidly adopted, because a less stereotyped response to irrelevant or non-critical stimuli will occur. An implication of the above hypothesis of cortical function

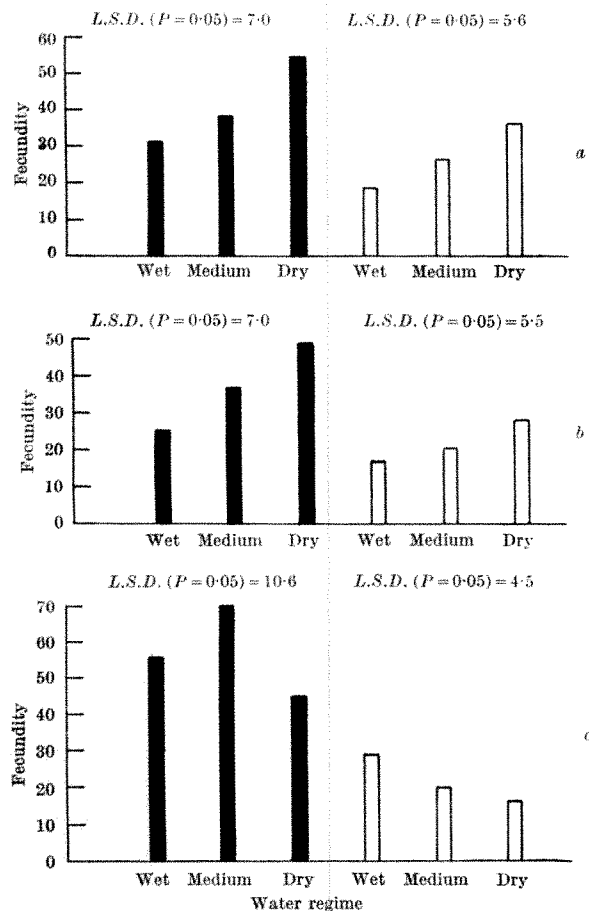


Fig. 1. Mean fecundity of apterous virginoparae of *Myzus persicae* (black columns) and *Brevicoryne brassicae* (white columns) on three leaf ages of brussels sprouts in three water regimes at 20° C. a, Young leaves; b, mature leaves; c, old leaves.

is that the sampling and fluctuation of stimulus elements are determined in part by the neocortex. Thus it seems that an interaction will obtain between the intertrial interval and the amount of functional cortex available to the animal.

A straight runway was employed for the avoidance conditioning. A delay of 5 sec followed the onset of the conditioned stimulus (buzzer) in the start box, before a current of 0.6 m.amp (unconditioned stimulus) was delivered to the grid floor of the starting box and alley. If an animal reached the safe goal box before the onset of the unconditioned stimulus, an avoidance response was recorded. Ninety-six male hooded rats were run over twenty trials each day for two consecutive days. These were divided into three groups of equal size, which comprised normal controls, unilaterals (spreading depression in one hemisphere), and bilaterals (spreading depression in both hemispheres). Each group was further sub-divided into four sub-groups, corresponding to the intertrial intervals of 15, 30, 60 and 240 sec. Spreading depression was produced by applying filter-paper pledgets soaked in 25 per cent potassium chloride solution to the dural surface which overlies the parieto-occipital cortex. Trials were only begun after all signs of placing reflexes had been abolished.

Table 1. AVERAGE VALUES OBTAINED ON DAY 1 AND DAY 2 FOR TOTAL NUMBER OF AVOIDANCES (AV) AND RESPONSE LATENCIES (LT)\*

	Day 1			Day 2	
	ITI	AV	LT	AV	LT
Normal controls	15"	9.4	61	12.7	40
	30"	15.1	42	18.0	21
	60"	14.4	40	17.6	25
	240"	15.0	37	17.5	23
Unilaterals	15"	0.9	111	5.9	73
	30"	6.5	74	12.7	44
	60"	6.7	72	11.6	45
	240"	6.5	88	13.2	41
Bilaterals	15"	0.0	157	1.3	144
	30"	0.0	258	0.5	165
	60"	0.0	215	0.9	157
	240"	0.1	255	1.9	143

\* Given in units of 0.001 min.

The results of the experiment are given in Table 1. Inspection of the avoidance totals and latencies for the normal controls reveals that the 15 sec sub-group was inferior to the other sub-groups, the latter yielding asymptotic performance from the 30 sec intertrial interval. Further, all sub-groups demonstrated improvement from day 1 to day 2. A three factor analysis of variance with repeated measures on one factor was performed on the data, and both intertrial interval and day factors were found to be highly significant.

The unilateral animals showed a similarly significant intertrial interval effect, which emerged as identical in form to that obtained by the controls; and furthermore, a very considerable improvement in performance was shown from day 1 to day 2. Table 1 shows, however, that all the unilateral sub-groups displayed a significant learning impairment during both days when compared with the controls. Counter to the prediction which had been made, no interaction was obtained between cortical and intertrial interval factors.

The bilateral animals showed no learning in forty trials in terms of avoidance responses, and so comparisons with the other groups as well as within group comparisons must be made by way of response latencies. These functionally decorticate animals, while significantly inferior in their performance to the other two groups, showed a reversed day 1 intertrial interval trend. The 15 sec sub-group showed performance superior to the other three sub-groups, the latter again asymptoting at the 30 sec intertrial interval, but at an inferior performance level. Clearly, this is the opposite to what was found for the controls and unilaterals; there is a significant interaction between cortex and intertrial interval factors when the latencies of all three groups are compared. Furthermore, while the other bilateral sub-groups yielded a considerable

saving on day 2, the 15 sec sub-group showed none at all, and the performance levels of all four sub-groups on the second day were similar.

A qualitatively parallel intertrial interval effect has been shown to hold for both the controls and unilaterals, and so core differences between the two groups cannot be directly extracted on these grounds. Nevertheless, the mass action effect was manifested in terms of a considerably greater number of superstitious responses being adopted by the unilaterals (approximately six times as many for unilaterals as for normals), as well as in the unilaterals which display persistent spontaneous regressions, the latter probably being a reflexion of a protracted early stage in the learning.

The lack of interaction between cortex and intertrial interval factors demonstrates that varying the interval in the goal box has the same effect on the performance of an animal, whether one or both cerebral hemispheres is functional. The bilateral sub-groups did not show the usual intertrial interval effect, and this suggests that the effect is mediated by the neocortex in the normal intact animal. Thus, while an interpretation of the mass action effect in terms of the function of the cortex as the primary mechanism involved in stimulus sampling and encoding has not been shown to be incorrect, this investigation has given it only indirect support.

In view of the fact that bilateral animals gave no avoidance responses in forty trials, although a decrease in latencies did occur, it would be prudent to discuss their performance, not as learning, but as an adaptation to the situation. The 15 sec sub-group (the results of which have been replicated in a second group) displayed minimal freezing and regressive responses, unlike the other bilateral animals. The degree of stereotyping was marked and is reflected in the relatively low variance of this sub-group. These apparently contradictory characteristics—the rapid (as reflected in the low latency) although limited adaptation was offset by unadaptive stereotyped responses—could tentatively be explained by the following two discrete, though related, mechanisms. The first relates the observed effects of the intertrial interval directly to the acquisition-storage aspects of the problem; the second to factors extraneous to, though intimately linked with, the neural substrate of behavioural change. (a) The bilateral animal, lacking the normal cortical encoding, and possibly holding, mechanism is partially compensated by a very short intertrial interval acting directly on a limited sub-cortical store; and (b) Brutkowski<sup>6</sup> has described, as a consequence of prefrontal ablation in dogs and monkeys, behaviour which appears to arise from the inability of the animals to inhibit tendencies resulting from training or preference and thereby continuing one response to the exclusion of others. He further reports that this condition was exacerbated in a conditioning experiment in which a 15 sec intertrial interval was employed. Spreading depression in the neocortex of the lissencephalic rat involves areas homologous with the prefrontal cortex of higher mammalian species, and so Brutkowski's findings suggest that the performance of the bilateral animal could be considered in these terms. This would only apply to the bilateral preparation, since a similar deficit in the unilateral animal would be nullified by the functional contralateral hemisphere.

I thank Dr. I. S. Russell for his help.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, March 13

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP (joint meeting with the Food Group and Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, S.W.1), at 2.30 p.m.—Meeting on "Emulsions and Emulsifiers in the Food Industry".

UNIVERSITY OF LONDON (at the School of Oriental and African Studies, London, W.C.1), at 5 p.m.—Prof. Jean-Luc Chambard (Paris): "Atlas of an Indian Village. I, The Village: Society and Economy".\*

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. Mothes (East Berlin): "On Cytokinins" (further lectures on March 14 and 15).\*

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Coachmakers Arms, 88 Marylebone Lane, London, W.1), at 6.30 p.m.—Mr. J. Barnes: "Industrial Design as Applied to Machinery".

## Monday, March 13—Friday, March 17

INSTITUTION OF CIVIL ENGINEERS, the BRITISH NUCLEAR ENERGY SOCIETY and the JOINT BRITISH COMMITTEE FOR STRESS ANALYSIS (at Church House, Great Smith Street, London, S.W.1)—Conference on "Prestressed Concrete Pressure Vessels".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2)—Conference on "Air Traffic Control".

## Tuesday, March 14

UNIVERSITY OF LONDON (at the School of Oriental and African Studies, London, W.C.1), at 5 p.m.—Prof. Jean-Luc Chambard (Paris): "Atlas of an Indian Village. II, The Village Territory: Agricultural Problems".\*

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

UNIVERSITY OF LONDON (at St. Bartholomew's Hospital Medical College, West Smithfield, London, E.C.1), at 5.15 p.m.—Dr. D. L. Revesz (Stockholm): "Medical Radiation Biology: Basic Research or Applied Science?"\*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1) at 5.30 p.m.—Mr. A. J. M. Harrison and Mr. M. W. Owen: "A New Type of Structure for Flow Measurement in Steep Streams".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "High-Power Thyristor Invertors for Standby A.C. Power Supplies" opened by Mr. R. J. Spreadbury.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Eccleston Hotel, Victoria, London, S.W.1), at 5.30 p.m.—Mr. C. E. Worrell: "Practical Organization of a Modern Millroom"; Mr. L. Phillips: "Polymer Requirements for Extreme Service Applications".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. J. R. D. Francis: "Of Shoes—and Ships—and Sealing Wax..." (Inaugural Lecture).\*

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. Passow (University of Saarland): "Passive Ion Permeability of the Erythrocyte Membrane" (further lecture on March 16).\*

## Wednesday, March 15

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 2.15 p.m.—Meeting on "Sea-Ice and Marine Navigation".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. P. M. Hamilton: "Vertical Profiles of Total Precipitation in Shower Situations"; Mr. J. C. Drake, and Mr. M. J. Mason: "The Melting of Small Ice Spheres and Cones"; Mr. P. L. Kamburova and Mr. F. H. Ludlam: "Rainfall Evaporation in Thunderstorm Downdrafts".

GEOLOGICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. J. M. Hancock and Mr. W. J. Kennedy: "Photographs of Hard and Soft Chalks taken with a Scanning Electron Microscope" (Demonstration); Dr. R. Goldring: "The Shallow Marine and Deltaic Facies of the Baggy Beds (Upper Devonian) at Baggy, North Devon" (Memoir).

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Prof. J. Neyman (University of California): "Experimentation with Weather Control".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 6 p.m.—Mr. B. Shackel: "Ergonomics in Electronic Equipment and Systems Design".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Robert Cockburn, K.B.E., C.B.: "Science, Defence and Society" (Trueman Wood Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Croydon Technical College, Fairfield, Croydon, Surrey), at 6.30 p.m.—Mr. J. Moir: "Hi-Fi Quality Sound Reproduction".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.2), at 6.30 p.m.—Discussion Meeting on "The Determination of Hetero Elements (other than C.H. and N.) in Sub-Milligram Quantities", introduced by Miss J. P. Dixon.

UNIVERSITY OF LONDON (at the Institute of Orthopaedics, Royal National Orthopaedic Hospital, Great Portland Street, London, W.1), at 8.15 p.m.—Prof. Ernst Gutmann (Prague): "Fast and Slow Muscles".\*

## Thursday, March 16

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 1 p.m.—Mr. O. G. W. Stallybrass: "How Faraday 'Created' Living Animals: the Story of a Myth".

CHEMICAL SOCIETY (at Queen Mary College, Mile End Road, London, E.1), at 2 p.m.—Half-day Symposium on "Fast Reactions".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the I.E.R.E. Medical and Biological Group, at Savoy Place, London, W.C.2), at 2.30 p.m.—Discussion Meeting on "Automatic Processing in Nucleonic Isotope Studies".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. J. G. Sheals, Dr. P. H. A. Sneath and Mr. L. Watson: "Numerical Taxonomy".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. P. J. Hilton: "The Homotopy Type of Compact Polyhedra".

UNIVERSITY OF LONDON (at the School of Oriental and African Studies, London, W.C.1), at 5 p.m.—Prof. Jean-Luc Chambard (Paris): "Atlas of an Indian Village. III, The Village Territory: Agrarian Problems".\*

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, W.C.1), at 5.30 p.m.—Prof. Ernst Gutmann (Prague): "The Motor Unit in Old Age".\*

BRITISH INSTITUTE OF RADIOLOGY (in the Reid-Knox Hall, 32 Welbeck Street, London, W.1), at 6 p.m. Discussion Group meeting on "Dose-Time Factors in Radiotherapy". 8 p.m.—Ordinary meeting. Dr. H. F. Cook: "Training in Radiological Physics".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. W. B. Curtis, Mr. L. Tasker and Mr. J. R. Taylor: "The Theoretical and Practical Considerations of the Use of Water Soluble Resins in the Electrodeposition of Paint".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Twickenham College of Technology, Edgerton Road, Twickenham), at 6.30 p.m.—Mr. P. R. Styles: "Medical Electronics".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 7 p.m.—Madame Nicole Leger (Paris): "Aspects of the Pharmacology of Anthelmintic Drugs".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the Royal Army Medical College, Millbank, London, S.W.1), at 7.30 p.m.—Laboratory Meeting. Chairman: Prof. G. Macdonald.

## Friday, March 17

BRITISH INSTITUTE OF RADIOLOGY (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 10 a.m.—British Radiological Protection Association Symposium on "The Radio-Iodines in Man".

NUTRITION SOCIETY (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 10.30 a.m.—Symposium on "Food Habits and Nutritional Status of Minority Groups in the United Kingdom".

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W.1), at 1 p.m.—Dr. E. Vander Donckt: "pK of Excited States".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting of the I.E.E. History of Technology Group and the Newcomen Society, at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. R. L. Smith-Rose, C.B.E.: "Early Days in Radio Research".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the AUTOMATIC CONTROL GROUP OF THE I.MECH.E., at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "The Present State of Stability Theory" opened by Dr. A. G. Dewey, Prof. G. D. S. MacLellan, Mr. F. C. Parks and Prof. C. Storey.

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. M. Nicholson: "The Resolution of Conflict" (First Caradog Jones Lecture).

UNIVERSITY COLLEGE LONDON (in the Anatomy Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. Gutmann (Prague): "Neurotrophic Relation between Nerve and Muscle Cells".\*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. G. E. Bacon: "Looking at Solids with Neutrons".

## Saturday, March 18

ASSOCIATION FOR SCIENCE EDUCATION (at Parliament Hill School, London, N.W.3), at 2.30 p.m.—Symposium on "The Teaching of Science for the C.S.E.".

## Monday, March 20

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Royal Society and the I.E.R.E. Computer Group, at Savoy Place, London, W.C.2), at 2.30 p.m.—Colloquium on "Automated Cartography: Scientific Needs and Engineering Possibilities".

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Prof. P. G. Moore: "Operational Research in Business".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5 p.m.—Dr. P. S. Hewlett: "Synergism and Potentiation in Insecticides".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Rendez-vous du Diable" (colour film).

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. F. Bennett: "Monomer Approach to Flame Retardancy".

## Monday, March 20—Tuesday, March 21

GRIMSBY COLLEGE OF TECHNOLOGY (at Nuns Corner, Grimsby)—Symposium on "Modern Developments in Food Preservation".

## Monday, March 20—Wednesday, March 22

BIOCHEMICAL SOCIETY (at Trinity College, Dublin)—489th Meeting. Included in the programme will be an Ordinary Meeting and a Discussion Forum on "Metabolic Role of Vitamin A".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER (preferably with interest in ecological and population genetics) in ZOOLOGY—The Registrar, University of Manchester, Manchester 13, quoting Ref. 17/07/Na (March 17).

ASSISTANT LECTURER (preferably with research interests and experience in botany and archaeology and with an appreciation of modern physical techniques) in QUATERNARY PALAEOECOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (March 18).

SENIOR RESEARCH ASSISTANT (with an honours degree in metallurgy together with postgraduate research or industrial experience) in the DEPARTMENT of METALLURGY for an investigation of the structure and properties of cast and wrought austenitic steels—The Personnel Officer, Brunel University, Kingston Lane, Uxbridge, Middlesex (March 18).

ASSISTANT LECTURER in GENETICS—The Registrar, University College of Swansea, Singleton Park, Swansea (March 20).

LECTURER in ENGINEERING MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (March 20).

TUTORS (2) in the DEPARTMENT of GEOGRAPHY to assist with tutorial groups involving small groups of students and to provide assistance in practical map classes—The Registrar, University College of Swansea, Singleton Park, Swansea (March 23).

BIOLOGIST (with an interest in crop physiology) in the SYSTEMS SYNTHESIS SECTION of the DEPARTMENT of BIOLOGY, to work on the theoretical aspects of plant and crop growth—The Secretary, The Grassland Research Institute, Hurley, Maidenhead, Berkshire (March 24).

LECTURER (physicist or engineer with active interests in the field of thermodynamics and fluid mechanics) in MECHANICAL ENGINEERING—The Secretary of the University Court, The University, Glasgow (March 27).

ASSISTANT LECTURER or LECTURER in the DEPARTMENT of APPLIED MATHEMATICS and MATHEMATICAL PHYSICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (March 28).

LECTURERS or ASSISTANTS (2) (with a good honours degree and preferably some postgraduate experience) in CELL BIOLOGY—The Secretary of the University Court, The University, Glasgow (March 29).

LECTURER in DEPARTMENT of ZOOLOGY, University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 31).

LECTURER (with specialized experience in general pharmacology especially in the field of physical pharmacy) in PHARMACEUTICS—The Registrar, The University, Nottingham (March 31).

TURNER AND NEWALL RESEARCH FELLOWS in ENGINEERING, INORGANIC CHEMISTRY, PHYSICS and allied subjects—The Registrar, The University, Manchester, 13 (March 31).

PROFESSOR of MICROBIOLOGY—The Registrar (Room 30, O.R.B.), The University, Reading (April 3).

LECTURER (with experience of teacher training and special interests in the philosophy of education and related theoretical bases) in the PHILOSOPHY AND THEORY OF EDUCATION in the School of Education, University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 10).

POSTDOCTORAL RESEARCH ASSISTANTS in the DEPARTMENT of CHEMISTRY for studies on organic compounds of beryllium and aluminium in collaboration with Prof. G. E. Coates and Dr. K. Wade—The Registrar, University of Durham, Old Shire Hall, Durham (April 10).

ASSISTANT LECTURER (interested in experimental psychology) in PSYCHOLOGY—The Registrar, The University, Liverpool, 3, quoting Ref. RP/412 (April 11).

LECTURER (with a special interest in the fields of structural geology and petrology) in GEOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 15).

SECOND CHAIR OF PURE MATHEMATICS—The Registrar, The University, Sheffield (April 15).

LECTURER in the DEPARTMENT of ORGANIC CHEMISTRY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 30).

LECTURER in the DEPARTMENT of ORGANIC CHEMISTRY, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 20).

LECTURER or ASSISTANT LECTURER (with a medical or scientific qualification) in VIROLOGY in the DEPARTMENT of BACTERIOLOGY and VIROLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 25/67.

LECTURER or SENIOR LECTURER in PLANT PHYSIOLOGY in the DEPARTMENT of ARTS, SCIENCE and EDUCATION—The Registrar, University of London Goldsmiths' College, London, S.E.14.

READER (with high qualifications and research experience in a branch of science related to vision) in OPHTHALMIC or PHYSIOLOGICAL OPTICS—The Academic Registrar, The City University, St. John Street, London, E.C.1.

SENIOR LECTURER and LECTURERS (2) (with a good degree in mathematics or statistics with special interests in statistics or electro-magnetic theory or a branch of pure mathematics) in the DEPARTMENT of MATHEMATICS—The Clerk to the Governing Body, Northern Polytechnic, London, N.7.

Science Museum. Chemistry 2: Chemical Laboratories and Apparatus from 1850. By Frank Greenaway. (A Science Museum Illustrated Booklet.) Pp. 50. (London: H.M. Stationery Office, 1966.) 5s. net.

Ambassade de France, Service de Presse et d'Information. France's Commercial Seaports. Pp. 21. (London: Ambassade de France, Service de Presse et d'Information, 1966.)

Anti-Locust Research Centre. Anti-Locust Memoir No. 8: The Upsurges and Recessions of the Desert Locust Plague—an Historical Survey. By Z. Waloff. Pp. 111. (London: Anti-Locust Research Centre, 1966.) 25s.

The Royal Society. International Relations: a Progress Report by the Foreign Secretary of the Royal Society. Pp. 14. (London: The Royal Society, 1966.)

The Royal Observatory, Edinburgh. Publications, Vol. 5, No. 11: Observations of Interstellar Reddening. 3: Results for Region in Cassiopeia. By K. Nandy. Pp. 233-243. (Edinburgh and London: H.M. Stationery Office, 1966.) 5s. net.

University of Oxford. Nuffield Committee for the Advancement of Medicine—Annual Report 1965. Pp. 43. (Supplement No. 2 to the *University Gazette*, Vol. 97, November 1966.) (Oxford: The University, 1966.) 2s. 6d.

The Development of Mathematical Activity in Children: The Place of the Problem in this Development. (A report prepared for the Sub-Committee on Mathematical Instruction of the British National Committee for Mathematics by the Research and Development Panel of the Association of Teachers of Mathematics.) Pp. 80. (Nelson: The Association of Teachers of Mathematics, 1966.) 5s.

## Other Countries

Proceedings of the California Academy of Sciences. Vol. 32, No. 15 (October 24, 1966): The Larval Development of Chitons (Amphineura). By Allyn G. Smith. Pp. 433-466. Vol. 32, No. 16 (October 24, 1966): Revision of the Nearctic Species of *Sitts* (Cantharidae: Coleoptera). By John Wagener Green. Pp. 447-513. Vol. 34, No. 2 (September 19, 1966): *Efferia* Coquillett in America North of Mexico (Diptera: Asilidae). By J. Wilcox. Pp. 85-234.

Vol. 34, No. 3 (October 21, 1966): New Species of Anthomyiidae and Muscidae from California (Diptera). By H. C. Huckett. Pp. 235-306. Vol. 34, No. 4 (October 21, 1966): The Taxonomic Status of Bornean Snakes of the Genus *Pseudorhabdion* Jan and of the Nominal Genus *Idiopholis* Mocquard. By Robert F. Inger and Alan E. Leviton. Pp. 307-314. Vol. 34, No. 5 (October 21, 1966): Tardigrada from the Galapagos and Cocos Islands. By Robert O. Schuster and Albert A. Grigarick. Pp. 315-328. Vol. 34, No. 6 (October 21, 1966): The Marine Iguana of the Galapagos Islands, Its Behaviour and Ecology. By Charles C. Carpenter. Pp. 329-376. (San Francisco: California Academy of Sciences, 1966.)

Transactions of the American Philosophical Society. New Series, Vol. 58, Part 6: Roland de la Platière: a Public Servant in the Eighteenth Century. By Charles A. Le Guin. Pp. 128. (Philadelphia: The American Philosophical Society, 1966.) \$2.50.

Harvard University. Program on Technology and Society. Second Annual Report of the Executive Director, July 1, 1965 to June 30, 1966. Pp. iii + 59. (Cambridge, Mass.: Harvard University, 1966.)

Indian Forest Bulletin, No. 250 (New Series): Forest Pathology. Studies on Indian Telephoraceae. 3: The Genus *Stereum*. By P. S. Rehill and B. K. Bakshi. Pp. 19 + 3 plates. (Delhi: Manager of Publications, 1966.)

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Fisheries and Oceanography, 1965-66. Pp. 54. (Cronulla, N.S.W.: Commonwealth Scientific and Industrial Research Organization, 1966.)

United States Department of Agriculture: Agricultural Research Service. General Catalogue of the Homoptera. Fascicle VI: Cicadelloidea. Part 15: Iassidae. By Z. P. Metcalf. Pp. 229. (Washington, D.C.: Government Printing Office, 1966.) \$0.65.

Tropical Fish Culture Research Institute, Malacca. Report for 1965. Pp. 42. (Batu Berendam, Malacca, Malaysia: Tropical Fish Culture Research Institute, 1966.)

Commonwealth of Australia. Department of National Development: Bureau of Mineral Resources, Geology and Geophysics. Bulletin No. 81: Australian Mineral Industry: Production and Trade, 1842-1964. Compiled and edited by Z. Kalix, L. M. Fraser and R. I. Rawson. Pp. ix + 473. Report No. 100: Geology of the Southern Half of the Bowen 1:250,000 Sheet Area, Queensland. By E. J. Malone, A. R. Jensen, C. M. Gregory and V. R. Forbes. Pp. 87 + 6 plates. Report No. 110: Magnetic Mean Hourly Values from Watheeroo Observatory, Western Australia, 1948-1949-1950. By P. M. McGregor. Pp. v + 172. (Canberra, A.C.T.: Bureau of Mineral Resources, Geology and Geophysics, 1966.)

Commonwealth of Australia. Third Report of the Australian Universities Commission—Australian Universities, 1964-1969. Pp. x + 185. (Canberra: Australian Universities Commission, 1966.)

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### Great Britain and Ireland

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Vol. XXV, No. 4: Experiments in the Large-Scale Culture of the Larvae of *Ostrea edulis* L. By P. R. Walne. Pp. iii + 53 + 2 plates. (London: H.M. Stationery Office, 1966.) 22s. 6d. net.

Royal Observatory Bulletins. No. 123: Time and Latitude Service 1965, October-December. Pp. B57-B70. (London: H.M. Stationery Office, 1966.) 2s. 6d. net.

Natural Environment Research Council. Institute of Geological Sciences—Incorporating the Geological Survey of Great Britain, the Museum of Practical Geology and Overseas Geological Surveys. Annual Report for 1965. Part 1: Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology. Pp. vii + 114 + 8 plates. (London: H.M. Stationery Office, 1966.) 11s. net.



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#### UNIVERSITY OF OTTAWA DEPARTMENT OF GEOLOGY OTTAWA CANADA

Applications are invited for two staff positions in the fields of PLEISTOCENE GEOLOGY and processes and Stratigraphic Palaeontology. Applicants should be prepared to initiate and carry out research programmes and to conduct courses in these fields. Rank and salary depend upon qualifications and experience.

Requests for further information, and applications should be sent to the Chairman, Department of Geology, University of Ottawa. Closing date May 10. (1054)

#### UNIVERSITY COLLEGE OF NORTH WALES BANGOR

##### DEPARTMENT OF AGRICULTURE

Applications are invited for the post of RESEARCH ASSISTANT in this Department for work in connection with the effects of shelter on physical environment, animal performance and behaviour. Applicants should be Honours graduates in agriculture, agricultural science, or geography. The Research Assistant will be expected to register for a higher degree. Salary in the range £600 to £800.

Further particulars are obtainable from, and applications should be made by March 31, 1967, to the Professor of Agriculture, University College of North Wales, Memorial Buildings, Bangor. (1187)

#### HERIOT-WATT UNIVERSITY EDINBURGH

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Mr. R. F. Turnbull, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House Kingsway London, W.C.2,

to whom applications (quoting Appointment No.: 301/30) should be addressed by the 14th April, 1967. (1172)

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Applications should be sent to: The South African Science Office, Chichester House, 278 High Holborn, London, W.C.1, Not later than 28th March, 1967. (1174)

## AMATEURS AND PROFESSIONALS

THE report of the Sutherland Committee on liaison between the British universities and government establishments is a mouse of a document—a good mouse as far as it goes, but without a proper sense of its own importance. What the committee has done is to compile a catalogue of ways in which universities and establishments can derive more benefit from their mutual existence without radically affecting the principles on which their relationship is based. The committee has been marvellously thorough in spelling out the ways in which one university or another establishment is willing to consider the transfer of people, so that the report will be a godsend to young graduates looking for a cosy berth where they can earn a living and work for a Ph.D. at the same time, as well as to vice-chancellors turned recruiting sergeants. But this is unfortunately only a part of the story, valuable but small. What the circumstances require is a much more radical examination of the ways in which the relationship between the universities and the establishments might be transformed not for their separate or even joint benefit, but so as to make fuller use of the resources they both employ.

That said, it is obviously sensible that the universities and the establishments should work more closely together whenever this is possible. This is where the committee is on sure ground. Its enquiries have apparently revealed that the establishments are eager for closer collaboration, which is something to be grateful for. It is particularly welcome that the committee has joined the growing clamour that the movement of people between the civil service and the universities should not be needlessly impeded by administrative difficulties about pensions and removal expenses. It will also be valuable if the committee can win support for the notion that new research establishments should wherever possible be sited near universities. The research councils, particularly the MRC and the ARC, are already aware of this need, but other public corporations and government departments are woefully ignorant of the way in which a functional laboratory and a university can stimulate each other. And, of course, there are great opportunities for more joint appointments between research establishments and universities, more part-time lecturers, and more collaborative research projects.

The Sutherland Committee is on much less certain ground in its advocacy of more widespread delegation of responsibility for postgraduate teaching from universities to the establishments. The committee would like to see more postgraduate students farmed out to the establishments, and more of those who work in the establishments entitled to submit a description of the research they do as raw material for a Ph.D. It is understandable that the establishments

should be keen on these arrangements—at the very least they help with recruitment. But the committee is surely far too casual in its conclusion that the anxieties of the universities are not “justifiable”. It is hardly fair to say that the universities fear they will be “denuded” of postgraduate students. To the extent that the loss of people is in any sense important, what the universities worry about is the possibility that they will lose the brightest of their students. But the real cause for worrying is concerned much more directly with the character of postgraduate teaching. British universities are already awakening to the need that the traditional Ph.D. dissertation on original research should be supported by more deliberate teaching in related fields. It is only natural that they should ask awkward questions about the capacity of government establishments to provide a sufficiently rounded further education for postgraduates. The Sutherland Committee is right in believing that the establishments and the universities could profitably work more closely together, but a more sensitive discussion of the obvious real problems would have made its report much more convincing.

These, however, are niggling points. A much more obvious defect of the committee's work is that it has ignored the most serious set of problems in the interaction between the universities and the establishments—the balance of resources between the two kinds of institutions. It is, of course, entirely proper that there should be some overlap between them. It is understandable, for example, that the establishments should seek to round out programmes of directed research by undertaking more long-term projects. The result is that some of the research at the establishments is essentially academic. Indeed, the establishments are often better equipped than the universities for academic work, perhaps because their programmes as a whole derive a sense of urgency from some military or industrial connexion. For the establishments, of course, these circumstances are entirely welcome, and there is no doubt that they add to what is called morale. But are they also in the interests of the universities? And of the country as a whole? On the face of things, at least, resources might be better employed if more of the work of the establishments were farmed out to the universities, on a contract basis or by simple grant, with no strings attached. There are difficulties on both sides, but they are not insuperable. The trouble is that it is impossible to know just where the balance should be struck without careful study. It is a pity that the Sutherland Committee did not tackle this useful job. As things are, the evidence the committee has compiled of the willingness of the establishments to help with teaching can be interpreted not only as a proof of goodwill but also as a

sign that the establishments have been endowed with resources which might have been better spent in universities.

## WHIMPER, NO BANG

THE dispute between the British universities and the Government about the proposal that students from overseas should pay higher fees has ended in an anti-climax. The statement by the vice-chancellors (see page 1059) bristles with discontent, but Mr. Crosland will have his way, and fees will be increased in the autumn by a factor of roughly three. The fact that the Government has agreed to set up a hardship fund for students who will be badly hit by the new regulations will repair some of the damage, although it would obviously have been better for the reputation of the Government if this proposal had not been wrung from it by a storm of protest. There remain two separate kinds of issues to be resolved.

The level of fees which the universities should charge is in itself an important issue. The protest about the raising of fees for students from overseas has been levelled at the discriminatory character of this proposal, and there is good reason to fear some of its repercussions. But there is a good case for a general increase in the tuition fees for all students at British universities. The Robbins Commission was only voicing a view that is widely held when it recommended a general increase, largely on the grounds that the universities would then be less directly dependent on a single source of funds—the University Grants Committee. A more realistic scale of charges would also help to remind the universities of the real costs of their operations, and might therefore be a spur to a greater concern for efficiency. If the Government is now prepared to have serious discussions on these problems, the vice-chancellors will have something to be pleased about. They would, however, be mistaken if they thought that they could establish a higher scale of fees without making a spontaneous and convincing demonstration that they are prepared to operate in a thoroughly modern way. In the long run, they will also have to reconcile themselves to a much greater variation from one university to another.

The dispute about overseas fees has also questioned in an acutely disturbing way the character of the relationship between the universities, the University Grants Committee and the Government. Theoretically, the universities are autonomous, and are protected by the UGC from direct interference by the Government. On this occasion, it now seems plain, the UGC was not consulted about the specific proposal that fees for overseas students should be increased, while the universities as a whole have had to act as the Government decreed. To be sure, some universities agreed with the Government's proposal, and others had no stomach for a fight, but even so, this sequence of events

lends support to those who think that the UGC is no longer an effective buffer. Changing circumstances, and particularly the growing scale of public support for the universities, have made it inevitable that there should be important changes in the past two decades. Unfortunately the universities have been slow to recognize what has been going on. The result is that they have no collective means of resisting pressure from outside—or of applying it when necessary. In the discussions which lie ahead, the Committee of Vice-Chancellors must work not merely for more realistic fees but for recognition as the proper negotiating body on all matters of this kind.

## STARTING FROM SCRATCH

THE Natural Environment Research Council has produced its first report (see page 1059), and this is something to be grateful for even if it is later than it should have been. Now it should at least be possible to make more reasonably informed guesses about the way in which the new council will tackle the exceedingly difficult job of supporting research in a field which is necessarily ill-defined, and which is almost certain to remain like that. To judge from what the first report has to say, planning has been conditioned by the character of the laboratories which have fallen into the council's hands—the Institute of Oceanography, the various institutes concerned with the geological sciences and now tidily grouped together, and a number of marine biology laboratories, for example. The Nature Conservancy is also an inner force to be reckoned with, especially in its occasional role as the professional counterpart of the Council for the Preservation of Rural England. Nobody will be surprised that oceanography and marine biology get the fullest treatment in the report.

If, however, the immediate future is filled with sensible projects, the more distant future is probably as much of a puzzle for the research council as for those who may work with it. The council seems to have pinned its faith in a utilitarian concept of its influence. In places the first report reads like a précis of some of the things said in the name of the International Biological Programme. There is talk of making the Atlantic more productive of fish, and of the way in which a better understanding of wave motion may in the long run help both with the design of ships and the disposal of coastal effluents. But can the council hope to hold to this line without also undertaking the technology of exploitation? Will it be found drilling in the North Sea one day? And what will be its relations with the universities? These are some of the questions which the committee of the National Academy of Sciences on oceanography has been trying to answer (see *Nature*, 213, 937; 1967). It is to be hoped that by a year from now, the Natural Environment Research Council will have answers of its own.

## NEWS AND VIEWS

### More for British Science

THE Civil Estimates for 1967-68, issued earlier this week (see page 1066), suggest that the total expenditure from public sources on research and development will increase by roughly 4 per cent in the financial year ahead. Although details are as yet available for only 90 per cent of the British Government's expenditure in this field, the total is likely to increase from £433 million in 1966-67 to between £450 and £460 million in 1967-68. These figures do not, of course, include the expenditure of private industry and the foundations on the support of research and development. Although the rate of growth in the public sector has evidently slackened off, the increase in the coming year will outstrip the GNP, which is not expected to increase at all.

Table 1. RESEARCH COUNCIL SPENDING (£ MILLION)

	1966-67	1967-68
SRC	33.9	36.6
NERC	6.2	7.7
MRC	11.9	14.2
ARC	10.3	11.9
	<u>62.3</u>	<u>70.4</u>

Now that the full details of the estimates for the year ahead are available, it is clear that the rate of growth of support for basic research will be even greater, and more like 14 per cent over the year than the increase of 9 per cent suggested by the figures in the Vote on Account published a few weeks ago. The budgets of the four research councils concerned with the natural sciences will increase from £62.3 million in 1966-67 to £70.4 million in the financial year immediately ahead, as shown in the Table. The expenditure of the Social Sciences Research Council will grow even more rapidly, as befits a new organization. The Department of Education and Science will also be spending more in its direct grants to scientific causes. Thus expenditure on the Office of Scientific Information will grow from £290,000 to £370,000.

### Beating a Retreat

THE Committee of Vice-Chancellors and Principals has now agreed to recommend to universities that they should charge the increased fees for students from overseas suggested by the Department of Education and Science at the end of last year. In a statement earlier this week, the committee said that it had been mollified by an undertaking from the Secretary of State for Education and Science that there should be consultation on the long-term problem of university fees, and that the hardship fund intended to help overseas students with special problems would in reality be generous. The fund, whose existence was announced at the height of the clamour against discriminatory fees some weeks ago, is intended to help students already embarked on courses (*Nature*, 213, 853; 1967).

Although the vice-chancellors have for all practical

purposes conceded what the government has been asking for, they make a great show of their displeasure. The making of a public statement is in itself a sign of this. They also repeat their complaint that a discriminatory fee structure is bound to have unfortunate repercussions within the university system and without. They complain that they were not consulted before the original statement on fees for students from overseas, and that the University Grants Committee "appears to have been bypassed". The Committee of Vice-Chancellors even puts on record its disagreement with the official view that the University Grants Committee was consulted by saying that it understands the UGC was "consulted some six months ago and not consulted on the specific decision which the government announced on December 21".

In the immediate future, the Committee of Vice-Chancellors is likely to press for a general increase of university fees from something like £70 a year at present to nearly twice that amount at the end of the decade.

### Research into Environment

THE first report of the Natural Environment Research Council (H.M.S.O., 8s.) covers the first 10 months of the existence of the council, up to March 31, 1966. This was a period of some administrative chaos, which explains why there is little solid information in the report and why it has taken so long to appear. The council took over the Nature Conservancy, the National Institute of Oceanography, the Institute of Geological Sciences and the Hydrological Research Unit, and assumed responsibility for fisheries research, marine and freshwater biology, seismology and geomagnetism, long-term forestry research, and the awarding of grants and postgraduate training awards to universities. These activities are now run by five main committees, soon to be joined by a sixth when the council takes over responsibility for the British Antarctic Survey on April 1. Sir Graham Sutton is the chairman of the council, and the chairmen of the main committees are Lord Howick (Nature Conservancy), Professor J. C. Mitcheson (Geology and Geophysics), Professor M. J. Lighthill (Oceanography and Fisheries), Mr. N. A. F. Rowntree (Hydrology) and Professor M. V. Laurie (Forestry and Woodlands).

The budget of the council for the period of the report was almost £3.8 million. Just over £1 million went to the Institute of Geological Sciences, £853,000 to the Nature Conservancy, £440,000 in grants to universities, and £288,000 for training awards and fellowships. Since the period of the report, the Geological Survey has been amalgamated with the Overseas Geological Survey, and a start has been made in creating a centre of geochemical research in London. The plans for this centre are complete, although it has yet to be given a name. It will be in the Gray's Inn Road premises previously occupied by the overseas division of the Geological Survey, and will bring together the two mass spectroscopy sections at present in Oxford and the survey's premises in Young Street, London. When complete, the centre will have a staff of 80-90 people.

The British Antarctic Survey, previously the responsibility of the Commonwealth Office, moves to the council at the beginning of April. Whether there will be any change in the management of the survey has



not yet been decided. The only hint of a change in the work of the survey is the intention of expanding the biological side, on advice from the Council for Scientific Policy. The budget of the survey, however, is unlikely to be increased, unless the magic word "oceanography" is muttered sufficiently often. The council has two oceanographic enthusiasms; one is to study the North Atlantic, for some people are afraid that it is getting significantly colder, which could have catastrophic effects on the stock of fish; the other is to investigate ways of extracting uranium, gold, and silver from sea water by ion exchange. The process is not commercial yet, but might be if uranium prices increased, or if the French view on the price of gold prevailed. The Nature Conservancy, bloody but unbowed after its unavailing struggle with Imperial Chemical Industries, Ltd., over the building of a reservoir in Teesdale, has not yet decided whether to take ICI up on its offer of £100,000 for a crash course of research into the botany of Teesdale. It should make up its mind quickly, before ICI forgets it ever made the offer.

## Technology Gap

LAST week (March 6) a one-day meeting at the Institution of Electrical Engineers under the title "Making the Bosworth Report Work" discussed the means of attracting more able graduates into industry. It has been a constant theme of the Ministry of Technology, which sponsored the conference, that industry and the universities should draw closer together, but it is not yet clear whether its pious pleas are having any effect.

In some respects university departments are showing themselves more responsive than industrial firms—apart, of course, from those traditionally employing graduates. At the beginning of the year, four of the physics staff at University College, London, opened a consultative practice (as a limited company, New Industrial Concepts, Ltd.) to advise firms with process control and inspection problems. Their experience with manufacturers has shown that small British companies in particular seldom know how even well-established electronic techniques can help them; really advanced methods are a closed book. "There are still an astonishing number of standard jobs done by hand and measurements made by eye . . ."

The group—Dr. R. Stebbings, Dr. D. G. Davis, Dr. A. Boksenberg and Dr. A. C. H. Smith—have already nearly completed their first commission—to solve a control problem (including fabricating a unit) in the manufacture of specialized electric light bulbs—and have a satisfied client. They have undertaken that their commercial excursion shall not interfere with their lecturing and research commitments at University College, but apart from this the college has made no conditions and has been encouraging. So has the Ministry of Technology, which has put the group in touch with its regional liaison officers. The group have not asked for a grant so they have not got one.

Rather different is the scheme headed by Dr. Kenneth Ball at the Department of Mechanical Engineering at the University of Liverpool, now in its fourth year and supported by a grant of £32,000 from the Science Research Council. In this case the academic team has sought opportunities to get into the shop

and design specific pieces of plant for actual use. "Industrial jobs provide us with a laboratory where we can create a design, and then look backwards at it," says Dr. Ball. "Not until a real job is tackled can one identify either the central problems of systems design or of teaching systems designing . . ." The most substantial job tackled so far has been in association with Ruston and Hornsby, Ltd. (matching a freshly designed turbo-charger to its diesel engine), and evolving computer software to project further engine development.

## Best Buy in Space

AN agreement signed on March 8 between the European Space Research Organization (ESRO) and the U.S. National Aeronautics and Space Administration may mean that British taxpayers will have to contribute more towards ESRO launchings than towards other means of putting their experiments into satellites. The new agreement lays down that ESRO will in future pay for satellite experiments launched by the United States. In practice, however, well-established research groups in Britain have hitherto been able to include their experimental equipment in American satellites without payment of any kind except that which is invested in hardware. Groups from University College, London, the Culham Laboratory of the Atomic Energy Authority, and the R.A.F. Institute of Aviation Medicine are among those who have been able to find space in American satellites.

From an exclusively British point of view, ESRO launchings are expensive, and now increasingly so, because the British contribution towards the running costs of the organization, including the cost of launching rockets, is roughly 25 per cent of the total budget. This contribution is payable whether or not British experiments are included in ESRO satellites although the costs of salaries and equipment for particular experiments come out of separate national budgets. The first ESRO launch under the agreement with the United States will be the satellite due to be launched into a highly elliptical orbit in late 1968 to record events in the magnetosphere at around the next solar maximum. The contract with NASA is for just under \$4 million and the cost of the satellite and its equipment will be roughly the same again. Later American launchings of ESRO satellites will be relatively cheaper because of the comparative sophistication of their payloads costing roughly £8 million for the two.

In the circumstances, the British national space programme, much maligned, may yet be the second best buy, at least on the surface. The *Black Arrow* satellite payloads now in the offing should be comparatively cheap to launch if only because a large part of the cost will be hidden in the defence budget.

## Another Exhortation

THE United States National Committee for the International Biological Programme has now published a more detailed outline of the kind of research it is anxious to encourage when the second phase of the operation begins in July this year. The document *U.S. Participation in the International Biological Programme* is the second of two reports to have been produced by the committee, and is to be had from the

National Academy of Sciences. Dr. Roger Revelle, chairman of the committee, lists in his general introduction the "three related objectives—human welfare, scientific advance and international scientific co-operation". He says that biologists can "contribute uniquely to human welfare only by advancing scientific understanding, and the basic premise of the International Biological Programme is that the growth of understanding will be accelerated by international co-operation among the world's biologists". Dr. Revelle explains that in the United States the U.S. National Committee will work closely with the Interagency Coordinating Committee responsible for the efforts of federal agencies in this field.

So far, the work planned for the International Biological Programme, which will last for five years or thereabouts, consists chiefly of conferences. The Committee on Environmental Physiology has plans for six conferences on specific topics during 1967. The Committee on the Productivity of Marine Communities is planning five conferences on a regional basis. Others of the seven sections of the IBP have plans for various regional conferences and of others organized around specific subjects. From the outline programme, it is clear that the organizing committees are at this stage keeping a prudently open mind about the kind of work which can most usefully co-ordinate the work of individual laboratories in the years ahead. At the same time, the organizers are anxious that working scientists should formulate proposals for research in the areas of science considered to be most in line with the objectives of the IBP. Although the IBP is not itself a grant-giving agency, it does intend to scrutinize research proposals to see whether they accord with the aims of the IBP. Evidently the U.S. National Committee is hoping, however, that its certificate will carry weight. Time will no doubt tell.

## Out of the Record

*The Zoological Record* seems to have allowed its centenary to slip by with uncommon lack of ceremony. The *Record* was established in 1864 to keep zoologists informed of the most recent researches in their subject and has led a very chequered career. It started as the private undertaking of the publisher Mr. Van Voorst, under the editorship of Dr. Albert Günther, passed to the Zoological Society of London, thence to the Royal Society and finally back again to the Zoological Society, which now both compiles and publishes it. Early in its career when private enterprise failed, a Zoological Record Association had to be established to keep the publication solvent, and for several years the British Association provided an annual grant of £100 in its support. Expenses are now met in part by donations from institutions and individuals in the British Commonwealth and the United States, from the International Union of Biological Sciences and the Naples Zoological Station.

The *Record* is an annual compilation from more than 4,000 journals and is divided into twenty sections. Eighteen of these deal with a class or family of animals, and the other two with "Comprehensive Zoology" and a "List of New Genera". The information is presented in the form of three indexes. There is a bibliographical index arranged alphabetically under authors, with titles of research papers given in French, German or

English. The subject index is arranged under broad headings, and in the latest volume for "Pisces" the economics section includes, for example:

"Fisheries research.—Aspects of marine fisheries research, Lucas; tropical fisheries research, McConnell; freshwater fisheries research, Pentelow."

"Effects on fishes of industrial effluents.—Effect of heated effluents, Alabaster; effects of sewage effluents, Lloyd; Young; . . ."

A systematic index provides information relevant to a particular species or to groups of the phylum concerned together with details of new taxa.

## Molluscan Structure

THE growing number of scientists engaged on research on the Mollusca, and the suitability of these animals as experimental material, was plain at the international symposium held last week under the auspices of the Zoological and Malacological Societies. There were contributions on such varied topics as the molluscan fauna of the rain forests of the Solomon Islands, the fine structure of muscle and the bivalved gastropods. The gaps in knowledge of excretion in this phylum were dealt with by another speaker, and workers from the Free University of Amsterdam and the University of Southampton emphasized the neurosecretory activities of gastropods. Professor G. A. Kerkut and his associates from Southampton described aspects of the fine structure of the brain of *Helix* and the pharmacology of certain of its neurones, while speakers from Professor J. Lever's Department in Amsterdam gave accounts of studies of the nervous system of *Lymnaea*. Investigations of neurosecretion in the Mollusca are, as yet, in their preliminary stages, but they represent an important new field in which neurosecretion may be more closely related to the activity of the whole animal.

A new theory to account for the mechanism of calcification of the molluscan shell was put forward in a stimulating paper by P. S. B. Digby. Observations on the mussel *Mytilus edulis* have indicated that at the growing margin of the valves, the outer surface of the periostracum is acid and the inner is alkaline. Measurements of potential across this superficial membrane, which consists of quinone tanned proteins, showed that the outer side becomes increasingly negative and the inner positive as the mantle edge exerts suction on the permeable periostracum and outer layer of the valve around the shell margin. The membranous periostracum acts as a semi-conductor and electronic charge is considered to flow through the material. Calcification is attributed to electrode action at the inner side of this membrane producing alkalinity which precipitates lime. The process of calcification of the molluscan shell was thus thought to be comparable with that of the crustacean cuticle and of mammalian bone, in that they all represent electrochemical processes.

In a contribution on the burrowing activities of bivalves, E. R. Trueman pointed out how the shell of these animals operates as the basis of a hydraulic system by means of which the strength of the adductor muscles is used in digging by the production of high hydrostatic pressures. He suggested that the bivalved form of the shell may be an adaptation for active burrowing into or over soft substrates.

## Immunochemical NMR

by a Correspondent in Molecular Biology

NUCLEAR magnetic resonance spectroscopy has not so far been much used in the study of proteins or indeed of nucleic acids. Its great potential in this field is only now being recognized more widely, and with improvements in instrumentation and the appearance during the past year or so of a number of strikingly successful applications, an increasing number of problems will be found to lend themselves particularly well to this approach.

The unique advantage of NMR for the study of polymers lies in its sensitivity to freedom of motion. Sharp signals are obtained only when the motion of the nuclei under observation (most commonly protons) is very rapid: this situation will obtain for small molecules in solution or for flexible polymers with rapid segmental motion—a denatured protein, for example. When the polymer becomes rigid, as in a globular protein or an  $\alpha$ -helical polypeptide, the signal broadens, sometimes to a degree at which it effectively disappears. The possibility exists, moreover, of identifying sharp signals with a particular class of protons, as, for example, those of particular amino-acid side chains in proteins. The possibilities for the study of conformational changes, with perhaps isolated observation of particular groups, will commend themselves for many purposes.

The NMR technique also offers a method for the observation of ligand-binding to macromolecules, because signals from the ligand nuclei will be broadened when the molecule is immobilized by binding to the macromolecule. A good example of the potential of the method in the protein field is given by Haugland *et al.* in the current issue of *Biochemistry* (6, 498; 1967). They have studied the interaction of an antibody with a hapten but with a more refined procedure which permits the use of minimal concentrations. Rather than proton resonance, they observe that of the chlorine ( $^{35}\text{Cl}$ ) nucleus, which is present in the hapten. The hapten is a mercurial, 2,4-dinitro-4'-(chloromercuri)diphenylamine. The experiments are carried out in sodium chloride solution, and the existence of free chloride ions, which can exchange with the mercury-bound chlorine in the hapten, leads (as shown in an earlier article by Stengle and Baldeschwieler) to an amplified line-broadening effect. Because the hapten is added to the antibody, so a broadening of the  $^{35}\text{Cl}$  resonance is observed, and it is possible to perform a titration by this means to give the stoichiometry of the association at antibody concentrations down to  $6 \times 10^{-7}$  M. When dinitrophenyllysine is added to the complex, the line-width again decreases, showing that the mercurial hapten is displaced.

The observed broadening of the signal when the mercurial hapten is bound defines a limiting rate of exchange of chlorine between hapten and solvent, and demonstrates that the hapten is fully exposed. The line-width will also reflect the rotational freedom of the chlorine in the hapten—that is to say, in effect the flexibility of the binding region. It is interesting to note that unbound hapten does not interfere with the measurements, and that weak interactions can therefore be studied; the authors state that a 1 per cent level of binding can be detected.

## Nuclear Hyperfine Spectroscopy

PROFESSOR J. F. DUNCAN writes: The majority of papers delivered at the International Conference on Nuclear Hyperfine Spectroscopy held at Victoria University of Wellington, New Zealand, from October 17–21, 1966, were concerned with Mössbauer spectroscopy, but there were also contributions on nuclear magnetic resonance and electron spin resonance. The conference was attended by visiting scientists from Australia, Brazil, the Congo, Germany, India, Israel, Japan, New Zealand, United Kingdom, U.S.A. and U.S.S.R., and consisted of four principal review papers and about forty short research papers.

The first of the review papers was given by Professor H. Frauenfelder, who described the use of Mössbauer spectroscopy in physics. New techniques, such as populating the excited state by coulombic excitation, or using scattering techniques which enable higher energy states to be more readily detected, were described. The second review paper by D. P. Craig outlined problems in calculating the interaction energies within a crystalline matrix. E. Fluck reviewed the current state of nuclear magnetic resonance spectroscopy of heavy nuclei, such as phosphorus-31, fluorine-19 and cobalt-59. The last review paper by V. J. Goldanskii was a most informative contribution on recent advances of Mössbauer spectroscopy in chemistry. It outlined the rapidly increasing applications in the field of structural determination, and discussed work carried out in Russia on investigating some biologically important compounds, including several components of RNA.

The research papers covered a wide range of topics—theoretical work, magnetic fields, chemical structure problems, solid state problems, instrumentation, general effects—and concluded with a most stimulating paper by P. Hillman on Mössbauer studies on hearing.

The range of topics discussed in the research papers may be judged from the following brief summary. N. N. Greenwood assessed NMR techniques for measuring the stability of complexes; there are two methods for determining formation constants: the first can be applied where there is rapid exchange between free and complexed ligand, the second when there is also dissociation as well as exchange. Hyperfine interaction constants from NMR spectra of low spin ferric compounds was discussed by Golding and by Kurland. M. A. Collins discussed the measurement of nuclear hyperfine coupling constants in complex spectra. The calculation of  $g$  and hyperfine tensors for small molecules in double quantum transitions in ESR spectra was described by J. E. Wertz, and B. G. Wybourne discussed the use of pseudo-interactions in calculating spectroscopic properties and the effect of ionic contributions to  $S$  state rare-earth ions. A. J. F. Boyle spoke on relaxation and the Mössbauer effect in small magnetic particles, and gave a theoretical description of the effect of electron spin relaxation on the energy spectrum when there is an axis of quantization which remains fixed despite the time averaging introduced by the relaxation. J. J. Spijkerman and F. C. Ruegg have developed instrumentation for routine and high precision Mössbauer spectroscopy. R. E. Bailey and J. F. Duncan have shown that the three compounds  $\text{FeP}$ ,  $\text{Fe}_2\text{P}$  and  $\text{Fe}_3\text{P}$  fall into a regular array, and that the magnetic field at the iron atom is determined by

the number of nearest neighbour phosphorus atoms.

The week's discussion showed that many of the problems in this field require a detailed knowledge, not only of the physics of the system, but also of the chemistry of the molecules under investigation. The need to cross fertilize between the two disciplines was reflected in the composition of the conference, which was attended by roughly equal numbers of physicists and chemists.

## Select Committee

THE Select Committee of the House of Commons on Science and Technology continued to hear evidence from Sir William Penney, Chairman of the U.K. Atomic Energy Authority, when it resumed discussion of the British reactor programme on March 9. The costs of the OECD high temperature reactor (*Dragon*) were carried largely by the United Kingdom and Euratom, Sir William said. Each paid about 45 per cent, and another £4.5 million would be needed to carry the project on until 1970; the total cost would then be £31 million. Britain was prepared to go on, but because of political difficulties Euratom was unable to make up its mind. If Euratom refused, he said, the project might well come to an end; it would certainly be expensive to continue without Euratom, and the possibility had not yet been given serious thought.

Sir William said that Britain was not developing a small reactor, although the authority was keeping an eye on developments elsewhere. There seemed no point, he said, since nuclear propelled ships had so far shown no chance of being more economical than conventional ones, and there was no other obvious use for small reactors. For fast reactors, the authority had calculated capital costs of £50 per kW installed, and running costs of 0.3d. per kWh. So far as he was aware, no other source of power could compete with this—natural gas might be able to, but he had seen no figures. Mr. Eric Lubbock said that in the United States figures of \$100 per kW installed were already being quoted, but Sir William replied that comparisons were hard to make, and that running costs were just as important as capital costs.

Diversification was an essential part of the authority's programme, Sir William said. Despite this, and the fact that the total staff of the authority had been allowed to decline from 40,000 in 1962 to 32,000 in 1966, there were still too many people on the payroll. Reduction of staff would be largely by wastage, and 20 per cent of the staff would move to non-nuclear work in the next five years. The chief item in the diversification programme was desalination, but the best market for this, Sir William said, might turn out to be with a conventional power source rather than with nuclear power. Under the Ministry of Technology umbrella there was an enormous amount of materials research—at Harwell, Farnborough, and the National Physical Laboratory. If this could be integrated—although not geographically concentrated—considerable improvements ought to be possible. Asked how easy it is for people to leave the authority, Sir William said that pension rules were liberally interpreted, but it was hopeless expecting men from Harwell to go into schoolteaching—there simply was not enough money in it.

## Parliament in Britain

IN a written answer in the House of Commons on March 6, Mr. Roberts stated that Science Research Council awards are provided for research training or an approved course of postgraduate instruction but not primarily for obtaining a higher degree. In 1962–63 there were 1,623 qualified applications and 1,610 awards were made: for 1963–64 the corresponding figures were 1,968 and 1,932; for 1964–65, 2,567 and 2,507; for 1965–66, 2,928 and 2,785; and for 1966–67, 3,096 and 2,775. Mr. Roberts declined to consider lowering the standard of qualification for awards and added that in 1966 the number of research and advanced studentships awarded by the Science Research Council was about 18 per cent of the number of students graduating in that year and provided support for about half the total number of graduate students in science and technology. In 1962–63 the Science Research Council awarded 48 fellowships tenable in the United Kingdom; 28 NATO and Council fellowships tenable abroad; and 16 fellowships to British scientists in North America to enable them to return to the United Kingdom. For 1963–64, the corresponding figures are 44, 18 and 20; for 1964–65, 52, 27 and 32; for 1965–66, 83, 25, 41; and for 1966–67, 54, 26 and 41. A proposal by the Science Research Council to increase the value of fellowship awards is being considered in relation to relevant aspects of incomes policy.

REPLYING for the Government in a debate in the House of Lords on Overseas Information Services on March 8, the Minister of State for Foreign Affairs, Lord Chalfont, admitted the need for careful examination and planning of such services and also that the development of modern communication systems had made such effort more important. He saw no reason for any further co-ordinating process than that which normally took place through the Cabinet and its committees: each Minister was responsible for co-ordinating the information activities of his department. Lord Chalfont maintained that reductions so far made and planned in United Kingdom based staff were fully in accordance with the recommendations of the Plowden Committee. He also mentioned that special campaigns had been instituted on such subjects as Britain's achievements in desalination and in the development of nuclear energy. Sir Thomas Rapp's report reviewing the work of the B.B.C. External Services had been received in August 1965 with important recommendations for capital expenditure to improve the audibility of B.B.C. programmes, and the government had now asked Sir Harold Beeley to review the overseas information services with a view to reconciling their effectiveness with the containment of public expenditure.

IN a written answer in the House of Commons on March 7, the Joint Parliamentary Secretary, Ministry of Technology, Dr. J. Bray, stated that booklets describing methods of determining eight toxic substances in factory atmospheres, based on research carried out in the Laboratory of the Government Chemist for the Ministry of Labour, were published by H.M.S.O. in 1966. Booklets on chromic acid mist, phosgene, nitrous fumes and mercury were in the press and methods for hydrogen fluoride, ozone and organic di-isocyanates are being investigated.

## University News:

## London

PROFESSOR A. O. MACK, professor of dental prosthetics in the University of Newcastle upon Tyne, has been appointed professor of dental prosthetics at the Institute of Dental Surgery from September 1, 1967. Professor P. D. Wall, professor of physiology at the Massachusetts Institute of Technology, has been appointed professor of anatomy at University College from August 1, 1967.

## Loughborough

PROFESSOR E. J. RICHARDS, dean of the Engineering Faculty, professor of applied acoustics, and director of the Institution of Sound and Vibration Research in the University of Southampton, has been appointed vice-chancellor, in succession to Dr. H. L. Haslegrave. At Vickers Armstrong, Ltd., from 1945-50, he was aerodynamic designer of the Viscount and Valiant.

## Nottingham

PROFESSOR R. E. COUPLAND, professor of anatomy at Queen's College, Dundee, has been appointed to the Foundation chair of human morphology in the new Medical School, and Dr. J. R. A. Mitchell, first assistant to the Regius professor of medicine in the University of

Oxford, has been appointed to the chair of medicine in the Medical School.

## Appointments

DR. J. A. HALL, at present assistant general manager for international activities of the United States Atomic Energy Commission in Washington, has been appointed deputy director general in charge of the Department of Administration of the International Atomic Energy Agency. Mr. A. A. Wells, the current deputy director general, is returning to Washington to become chairman of the USAEC Atomic Safety and Licensing Board Panel.

PROFESSOR R. R. WILSON, professor of physics and director of the Laboratory of Nuclear Studies at Cornell University, has been appointed director of a proposed National Accelerator Laboratory.

## Announcements

THE Corday-Morgan medal and prize have been awarded by the Chemical Society to Professor J. I. G. Cadogan, Purdie professor of chemistry in the University of St. Andrews, and Professor R. Mason, professor of inorganic chemistry in the University of Sheffield. Professor Cadogan received his award in recognition of his contribu-

## North Atlantic Treaty Organization Study Institutes

Director	Subject	At	Duration	Director	Subject	At	Duration
Prof. S. Amelinckx, Laboratoire du CEN à Mol Douk, Belgique	Radiation effects on solids as a measure of radiation dose	Brussels, Belgium	Pending	Prof. R. J. Gillespie, Dept. of Chemistry, McMaster University, Hamilton, Ontario, Canada	Non-aqueous solvent chemistry	Hamilton, Canada	June (14 days)
Prof. R. Mason, Dept. of Chemistry, The University, Sheffield 10, U.K., and Prof. J. Lewis, Dept. of Chemistry, University of Manchester, Manchester, U.K.	Inorganic chemistry	Stratford- on-Avon, U.K.	July (14 days)	Prof. A. Zichichi, CERN, Geneva, Switzerland	Advances in particle physics	Erice (Trapani), Sicily	June-July (14 days)
Prof. S. Pontremoli, Istituto di Chimica Biologica, Università di Ferrara, Ferrara, Italy	Molecular aspects of enzyme syn- thesis and its control	Pisa, Italy	July (14 days)	Dr. K. van Gehlen, Mineralogisches Institut, Universität, Schlossgarten 5a, 852 Erlangen, Germany	Quantitative methods in reflected light microscopy	Frankfurt, Germany	August 21-27 (7 days)
Prof. R. Mason, Dept. of Chemistry, The University, Sheffield 10, U.K., and Dr. G. O. Phillips, University College, Cardiff, U.K.	Energetics and mechanisms in radiation biology	Port Merion, Wales, U.K.	April 1-15	Prof. Dr. M. Anastasiadis, The National Observatory of Athens, Thessalon, Athens 306, Greece	Solar eclipses and the ionosphere	Greek Islands	May 20- June 12
Prof. J. Roche, Collège de France, Biochimie Générale et Comparée, Place Marcellin-Berthelot, Paris, France	Biochemistry and evolution; homologous enzymes	Paris, France	March 28-30	Prof. G. Reverberi, Istituto Zoologica, Università di Palermo, Via Archirafi 18, Palermo, Italy	Nuclear potentia- tion and the activation of genes	Palermo, Italy	September (three weeks)
Dr. M. L. Williams, Merrill Engineering Building, University District, Salt Lake City, Utah 84112, U.S.A.	Engineering analysis of viscoelastic media	Pending	August (14 days)	Prof. Dr. Chr. Bruusgaard, Ullevål Hospital, Avt. II, Oslo, Norway	The physiology of gastric secretion	Lysebu, Oslo, Norway	May 1-11
Prof. Dr. Ing. M. Federici, USEA, S. Terenzo (La Spezia), Italy	Stochastic prob- lems in under- water sound propagation	La Spezia, Italy	September 18-23	Dr. C. Moser, and Dr. R. Lefebvre, Centre de Mécanique Ondulatoire Appliquée, 23 rue du Maroc, Paris 19, France	Calculation of correlation effects in atomic and molecular physics	Frascati, Italy	July 3-16
Dr. Finn Lied, Norwegian Defence Research Establishment, P.O. Box 26, Kjeller, Norway	Radio communication at high latitudes	Tromsø, Norway	Pending	Prof. Cecile DeWitt, Dept. of Physics, University of North Carolina, Chapel Hill, N. Carolina, U.S.A.	Many body physics	Les Houches, France	July 3- August 26
Prof. W. N. Everitt, Department of Mathematics, Queen's College, Dundee, U.K.	Instructional conference on differential equations	Edinburgh, U.K.	August 28- Septem- ber 9	Dr. P. Löwdin, Quantum Theory Project, Nuclear Sciences Bldg., University of Florida, Gainesville, Florida 32601, U.S.A.	Quantum chemistry, solid state physics and quantum biology	Gainesville, Florida, U.S.A.	December 5, 1966- January 21, 1967
Dr. P. Dohrn, Stazione Zoologica, Naples, Italy, and Prof. J. Z. Young, University College London, Department of Anatomy, Gower Street, London, W.C.1, U.K.	Information pro- cessing and storage by the central nervous system	Naples, Italy	July (10 days)	Prof. A. O. Barut Department of Physics, University of Colorado, Boulder, Colorado, U.S.A.	Scattering theory	Istanbul, Turkey	July 24- August 4
				Prof. F. Vasco Costa, Faculdade de Ciências, 58, Rua da Escola Politécnica, Lisbon 2, Portugal	Engineering applications of statistical extremes	Lisbon, Portugal	September 18-27

The North Atlantic Treaty Organization sponsors each year a number of international meetings and courses at which scientific topics are discussed at an advanced level. The scientific programme of each meeting is decided by the director, who also arranges for selection of the participants, and publicity. Interested scientists should therefore write to the appropriate director for further details and to check the place and time of the meetings.



tions to the organic chemistry of trivalent phosphorus compounds and Professor Mason received his award for his contributions to the structural chemistry of inorganic and biologically important compounds.

THE Institution of Structural Engineers, in collaboration with the Aluminium Federation, is offering a research scholarship of two years duration, worth £700 per annum, plus fees of about £80 per annum. The winner will carry out research in the use of light alloys in structural engineering, and should be British and under the age of 30 on October 1, 1967.

CHANGES are announced by the Royal Aeronautical Society in the rules of the Kremer Competition. The main prize of £5,000, endowed by Mr. Henry Kremer, for a man-powered flight around a figure-of-eight course with two turning points not less than half a mile apart has been increased to £10,000. The competition will now be open to all nations, instead of being limited to the British Commonwealth. In addition there will be another competition, open initially to members of the Commonwealth, over an easier course half a mile long in the form of a slalom. The prizes for this easier test will be £2,500, £1,500 and £1,000 for the first three competitors to complete the course; if the prizes are not won entirely before December 31, 1968, the rules may be revised. Rules for the £10,000 competition may be revised if the money is not won before December 31, 1973.

A CONFERENCE on the "Rheology of Building and Road Materials", organized by the British Society of Rheology, is to be held in Sheffield during April 4-5. Further information can be obtained from Dr. G. H. Tattersall, Department of Building Science, University of Sheffield (Materials Laboratory), Shearwood Road, Sheffield 10.

AN international symposium on "Discrimination Learning", organized by the Experimental Analysis of Behaviour Group, is to be held at the University of Sussex on April 5-6. Further information can be obtained from Dr. R. M. Gilbert, Department of Psychology, University of Aberdeen, Old Aberdeen.

A ONE day symposium on "Lubrication of Textile Machinery" will be held at the Manchester Institute of Science and Technology on April 13. Further information can be obtained from the Conference Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

A SHORT course on "Recent Advances in Chromatography" will be held in the Lanchester College of Technology, Coventry, during April 13-14. Further information can be obtained from Dr. C. W. Went, Lanchester College of Technology, Priory Street, Coventry.

A SYMPOSIUM on "Aspects of Nitrogen Metabolism and Utilization in Plants", sponsored by the Agricultural Committee of the University of Bristol, will be held at Long Ashton Research Station during April 18-19. Further information can be obtained from the Scientific Liaison Officer, Long Ashton Research Station, Long Ashton, Bristol.

THE 1967 spring informal symposium of the Gas Chromatography Discussion Group of the Institute of Petroleum will be held at the Royal Institution, 21 Albemarle Street, London, W.1, on April 28. Further information can be obtained from Miss J. Healey, Institute of Petroleum, 31 New Cavendish Street, London, W.1.

A DISCUSSION on "Helicoidal Macromolecules in Solution" is to be held by the Société de Chimie Physique during their sixteenth annual meeting in Paris during May 2-6. Further information can be obtained from Professor G. Emschwiller, Société de Chimie Physique, 10 rue Vauquelin, Paris 5<sup>e</sup>.

A SHORT course entitled "Copolymers—Their Preparation and Properties" will be held at the Lanchester College of Technology, Coventry, during May 3-4. Further information can be obtained from Dr. A. F. Johnson, Department of Chemistry and Metallurgy, Lanchester College of Technology, Coventry.

THE fourth annual national colloquium on "Information Retrieval" will be held in Philadelphia during May 3-4. Further information can be obtained from Mrs. Regina M. Hildreth, Fourth Annual National Colloquium on Information Retrieval, Radio Corporation of America, Electronic Data Processing, Camden, New Jersey, U.S.A.

A CONFERENCE on three growth prospects of the chemical industry: agricultural chemicals, pharmaceuticals and plastics, is to be held by the Chemical Group of Aslib at Attingham Park, near Shrewsbury, during May 15-17. Further information can be obtained from Mr. J. R. Clew, 42 Bedford Road, Horsham, Sussex.

AN international conference on "Essential Oils Production in Developing Countries", organized by the Tropical Products Institute of the Ministry of Overseas Development, will be held in London during May 15-19. Further information can be obtained from the Director, Tropical Products Institute, 56-62 Gray's Inn Road, London, W.C.1.

ERRATUM. In the article "Canine 'Field' in Sexual Dimorphism of Tooth Size" by S. M. Garn *et al.* (*Nature*, 212, 1501; 1966), two subscripts were inadvertently transposed in the sentence at the bottom of page 1501 which should read: "The same tendency holds in the mandible, though less completely, with  $I_2$  exceeding  $I_1$  in percentage sexual dimorphism and  $P_1$  exceeding  $P_2$  in the same respect".

CORRIGENDUM. In the article entitled "Resistance to Water Transport in Plants" by O. V. S. Heath (*Nature*, 213, 741; 1967) line 16 of the second column should read "ratio was 19:1".

## CORRESPONDENCE

### Information Exchange Groups

SIR,—This is to correct a complete misinterpretation in the statement by the secretary of the Commission of Editors of Biochemical Journals of the "primary original purpose" of the IEGs as unqualifiedly stated in "Biological Journals and Exchange Groups" (*Nature*, 213, 547; 1967). The actual purpose was to provide, on a test basis, a means for quick communication within the total pool of research manpower at work in some sharply focused research area. What they communicated was theirs to decide, as much as what scientists communicate in their private professional correspondence—which can, indeed, be regarded as the prototype of the IEG. Behind this purpose was the belief that quick communication of research findings, from an originating laboratory to all other laboratories in which there was significant probability of need for the information, accelerated scientific discovery in the research area. The reader will appreciate how untenable the notion is as presented in the article—namely, that the original purpose of the IEGs was limited to "rapid exchange of informal suggestions, comments, queries and general discussion".

Yours, etc.,

ERRETT C. ALBRITTON

National Institutes of Health,  
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# Estimates for Science

The Civil Estimates, published this week, give notice of what the British Government intends to spend in the coming financial year on non-military operations. The estimates for the Ministry of Technology are complicated by the recent takeover of the Ministry of Aviation, but show some significant changes in emphasis. Those for the Department of Education and Science show a steady advance in educational and scientific spending, despite the credit squeeze.

THE Civil Estimates for the Ministry of Technology for 1967-8 show the sort of increases that might be anticipated. Administration charges have shot up to £53 million from £24.4 million with the takeover of the costs of the Ministry of Aviation. Costs of the ministry research establishments have advanced, but unevenly; the National Physical Laboratory and the National Engineering Laboratory are among those which show increases, to £2.5 million and £1 million respectively, but some of the laboratories—the Fire Research Station, Torry Research Station, and the Water Pollution Research Station—show marginal reductions associated with reductions in staff.

The industrial services of the ministry show a considerable increase, from £15.3 million to £24.2 million. The bulk of the increase falls under two headings: research and development contracts, and assistance to the shipbuilding industry, which now represent £6.4 million and £1.9 million respectively. (The shipbuilding figure is part of the Shipbuilding Industry Bill now before Parliament.) Other industrial services, such as the Production Engineering Advisory Service and the Industrial Liaison Service, amount to a further £1.2 million, and purchases of machine tools and similar measures will cost £0.9 million. The grant to the National Research Development Corporation is down, from £7.4 million to £5.5 million, but this is because of a reduced grant for interest relief. The National Computing Centre gets £800,000, against £290,000 last year.

The aerospace section of the ministry shows some changes. Research and development work by industry is down from £190 million to £177.6 million, and the contribution to ELDO falls by £3.3 million to £9.5 million. The cost for the production and development of *Concord* is up by £10 million to £42.2 million, and a further £7 million is needed for the development of other aircraft. Acting as procurement agency for the services, the ministry will spend £366.6 million on aircraft. The Atomic Energy

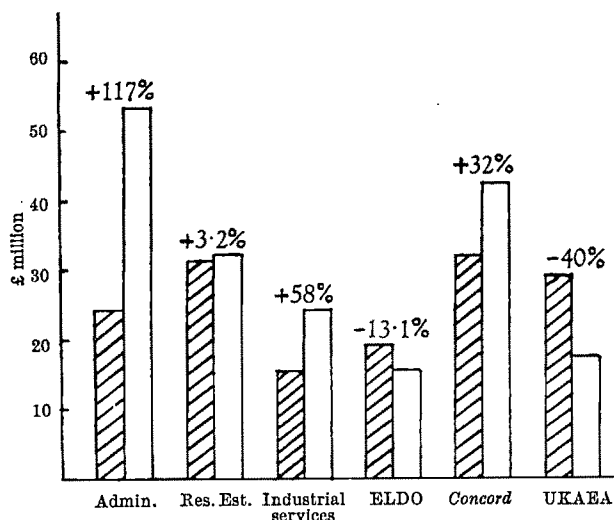


Fig. 1. Expenditure, Ministry of Technology. Shaded areas, 1966-7; open areas, 1967-8. Sharp increase in administration charges is caused by absorption of the Ministry of Aviation.

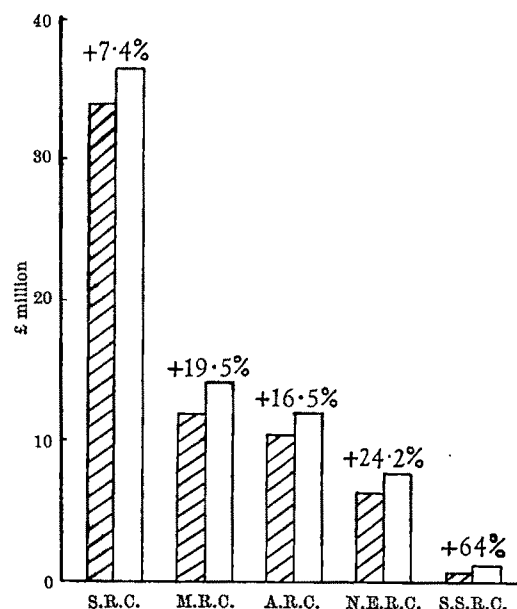


Fig. 2. Expenditure by Department of Education and Science on the research councils. Shaded areas, 1966-7; open areas, 1967-8.

Authority shows a marked decrease, from £29 million to £17.5 million. Current expenditure of the authority is down by £7 million to £58.4 million, through substantial reductions in the salary bill and in stores, materials, and services. Income to the authority through sales has increased from £9 million to £14 million.

The Department of Education and Science spends money on research and development directly, as through the research councils, and indirectly in the sense that the grant to the University Grants Committee includes more than £40 million a year which is eventually spent in university laboratories. The department is directly responsible for the British Museum (Natural History) and will spend £0.96 million there in the coming year. The grant to the Royal Society will be £657,000, and spending on what is called scientific and technical documentation £0.37 million. There are also substantial expenditures on the National Lending Library (£681,000), the National Reference Library (£700,000) and the Office of Scientific and Technical Information (£150,000).

The estimate for the University Grants Committee is £236 million, an increase of almost £16 million over last year. Grants for recurrent expenditure are up by about £17 million to £151.4 million, but capital expenditure shows a fall from £77.4 million to £74.9 million. The grants towards the cost of new accommodation in teaching hospitals increase sharply from £2.5 million to £4.1 million. The research councils all show increases in their grants (Fig. 2), and the smaller ones are advancing faster, as would be expected. The Science Research Council, with a budget of £36.5 million, shows only a 7.4 per cent increase, while the Social Science Research Council more than doubles its income to almost £1.2 million.

# British Universities and Government Establishments

Two years ago the Council for Scientific Policy appointed a working party to consider ways of achieving closer collaboration between universities and government establishments. The committee, under Sir Gordon Sutherland, has found the establishments to be more willing partners than the universities.

THE view that government research establishments would welcome a much closer relationship with universities is one of the principal conclusions of the Sutherland committee on liaison between universities and government establishments. The committee, however, is less certain that the universities are ready for a closer relationship. "Opinion is not quite so definite in the universities . . . and at present only a minority of the universities appear to us to be willing to take a strong initiative in this matter." The committee says, by way of oil on potentially troubled waters, that the diffidence of the universities is understandable because the advantages of collaboration are usually more obvious to the government establishments. The committee says that some universities are afraid that their research work would be "weakened" by some of the proposals that it has been discussing.

The Sutherland committee was set up by the Council for Scientific Policy in April 1965. Its members are Sir Gordon Sutherland, Dr. A. H. Cottrell, Professor K. C. Dunham, Professor E. B. Chain, and Professor R. V. Jones. Its scope allowed it to consider practices in all government research establishments, including those of the research councils and the Atomic Energy Authority. The working party set out deliberately to collect information about the degree of collaboration, and in particular circulated a questionnaire to universities, with the collaboration of the University Grants Committee which undertook to solicit replies and analyse them.

## Opportunities

The underlying theme of the report is that there are at present many neglected opportunities for collaboration between government laboratories and universities. It singles out five principal ways in which more might be done—closer staff relationships, co-operative research projects, formal association of research establishments with universities, recognition of establishment research for higher degrees, and easier mobility in transfer for people. Evidently much of what the committee has to say will bear on discussions such as those by which the Fulton Commission will eventually recommend what should be done about the Civil Service.

The committee says that "a considerable majority" of government establishments would like to see some members of their staffs given university status and university titles so that they could spend some of their time lecturing, supervising students and "taking some part in the discussion of university policy on research and advanced teaching". Something along these lines is at present being done at twenty universities, although most existing arrangements are concerned with the staff of research units established by the Agricultural and Medical Research Councils. The committee commends the way in which the universities of Strathclyde and Birmingham have set up close collaboration with the National Engineering Laboratory and the Royal Radar Establishment respectively, says that academic status seems to be a harbinger

of closer collaboration in research, recommends that both universities and government establishments should consider ways of strengthening collaboration, and argues that when new government research establishments are created they should be sited near a suitable university.

On co-operative research projects, the report says that the establishments at present subsidize by means of extramural contracts roughly £1.9 million of research a year in universities. Its enquiries have, however, revealed that universities are usually unwilling to accept these contracts unless they are for work which fits well with research already under way. The committee thinks that there is more that can be done and has apparently been particularly attracted by the way in which the Swedish Government is able to initiate research work at universities in fields of national importance by creating research groups which are in due course taken over by the universities. The committee thinks that government departments must take the initiative in this field and it urges them to make specific proposals for co-operative research projects with appropriate universities.

## Collaboration

A more radical vehicle for collaboration is the formal association of a research establishment with a university. It is particularly attracted by the way in which the United States government has set up research centres near universities and has delegated to the universities responsibility for their operations. The Sutherland committee argues that this arrangement gives university staff and research students a valuable insight into research of national importance without leading to government control of academic research because "participation in the work of the establishment is voluntary". But the Sutherland committee is clearly aware that this is a thorny issue and it recommends that arrangements like these should be studied in greater depth by the Committee of Vice-Chancellors and Principals and by government departments to see whether Britain can make use of precedents established elsewhere. On the awarding of higher degrees, the Sutherland committee points out that there is at present no uniformity of policy among the universities and "even within a single university towards different establishments". It is, however, full of admiration for the way in which the University of London has designed new academic regulations which make it possible for people to read for Ph.D. degrees while working at government establishments. Under the new arrangements, the university is responsible for seeing that supervision is satisfactory, while candidates for higher degrees are given a "real contact" with the university. The committee rejects the fear that an extension of these arrangements to other universities would mean that the universities would be denuded of research students. The argument is that if a student can earn a full-time salary as a member of a research establishment and still read for his degree, he is unlikely to be satisfied with the usual

student's grants. The committee's reason for thinking otherwise is that only a small part of the work at an establishment can lend itself to Ph.D. work, so that the student turned civil servant will nearly always have other things to do. But then there is the problem of writing a thesis in private time, the fact that "the more academically inclined will always prefer to spend all their time in a university", and the fact that government research establishments have a limited capacity to absorb students. Altogether, the committee thinks that the government establishments would not be able to absorb more than 150 potential Ph.D. candidates a year, or less than 7 per cent of those at present graduating with Ph.D.s in science, engineering and technology from British universities.

### Establishments

The argument that scientific people should be able to move more easily from and into government establishments has been a steady chorus for some years now, and the Sutherland committee has lent its own support. It says that pension arrangements are often quite different in the universities and the research establishments, which means that easy movement is discouraged. The committee points out that, elsewhere than in Britain, pension difficulties are a less serious obstacle and it expresses the hope that something will soon be done. The committee also complains that the Civil Service establishments are usually unable to help with removal expenses.

In practice, it seems that the migration of scientists from some parts of the government research machine in Britain is comparatively high. Thus in the five years 1961-65 inclusive, 159 members of the MRC staff, or about 19 per cent of the total, left for university employment. Elsewhere than in the MRC, migration seems to be much less frequent. The government establishments also appear to be able to recruit from the universities, at senior as well as junior grades, scientists whose numbers "almost exactly equal the losses". In the period 1961-65, 346 losses from government establishments to universities were almost exactly cancelled out by 342 recruitments. In practice, however, the MRC appears to be in an especially favoured position and recruited 29 per cent of its total staff from the universities in this period. This implies, of course, that other kinds of public research establishments were less able to recruit from the universities and the committee estimates that only 2.4 per cent of the research staff of other laboratories was obtained by recruitment from the universities in the period of five years.

The enthusiasm of the government departments for closer collaboration is plainly moderated, at least in part, by an awareness of the administrative difficulties thereby entailed. The Ministry of Technology, in its evidence to the Sutherland committee which necessarily refers to the period before amalgamation with the Ministry of Aviation, points out that the ten research stations on its strength would welcome closer association with the universities. Directors of establishments can receive research students without charging the universities for them. The National Physical Laboratory is one example of a laboratory which has undertaken to help neighbouring universities with teaching and which has found the courses provided to be valuable and stimulating for the staff. The United Kingdom Atomic Energy Authority is more free than most public laboratories to make special arrangements with universities and is in the habit of making special facilities such as reactors, accelerators and computers available to university scientists. The Authority says that during the year 1964-65 ten university scientists worked at each of Harwell and Aldermaston on reactors, and that more than 80 scientists from universities were attached to Atomic Energy Authority laboratories for work on accelerators and computers. Altogether the Authority has appointed 140 consultants from 30 universities and has made a number of joint appointments with universities.

The research councils differ not so much in their willingness to collaborate with the universities as in their policy on supporting research. The Medical Research Council, with its device of creating research units which frequently entail joint appointments with the universities, is understandably the most closely embedded in the university system. But the Sutherland committee points out that five of the units and institutes of the Agricultural Research Council do not have arrangements with universities. At present it seems that something like 94 staff members and 135 research students at Agricultural Research Council laboratories are working for Ph.D. degrees.

The service departments are chiefly engaged with the universities by means of research contracts. The Royal Navy does, however, employ 70 university consultants in various ways and the loan of naval survey ships to university oceanographers is one example of collaboration. The Army boasts of relationships with university consultants and students even at the Chemical Defence Experimental Establishment. The Royal Air Force, of course, has the Meteorological Office under its wing and close links with the universities on matters such as meteorological research. The service departments said in their evidence to the Sutherland committee that security need not inhibit relationships with universities in "quite large sectors" of their research activity.

### Universities

The Sutherland committee records somewhat laconically the conflict among the universities on the desirability of training Ph.D. students at government laboratories. Most universities, it appears, have some links with government establishments although geography is frequently against them. Opinion was divided on the desirability of part-time lecturers from government establishments, and three universities (Bristol, Brunel and Wales) say they would prefer full-time teachers to part-time. Altogether it seems that 21 universities are using staff from government establishments for research purposes, while a similar number uses them for teaching as well. Another nine universities use staff from government establishments to provide occasional lecture courses. It seems that all universities allow registered research students to conduct some research at government establishments, but that arrangements vary from place to place and that some residence is usually thought to be necessary. The universities of Birmingham, Bristol and Manchester argued to the committee that candidates reading for a Ph.D. degree should be regarded as trainees in research who require a continuous period of close supervision and help. Manchester emphasized the importance of links between students and fellow research workers, while the Vice-Chancellor of the University of Birmingham (Sir Robert Aitken) was particularly anxious that government establishments might be in a position to "offer the recruitment bait of Ph.D. in addition to the drawing power of a substantial salary and attract potential university students thus prejudicing the future of research within universities".

As a sub-committee of the Council for Scientific Policy, the Sutherland committee cannot have the force of law. Its recommendations will in fact only acquire their full force when a number of other committees have reported on matters such as the mobility of scientific personnel and the loss of trained people from Britain to the United States. Moreover, it is clear that the universities will have to work out some machinery for taking a view on issues such as the proposal that the arrangements in the University of London for Ph.D. students at government establishments should be extended elsewhere. Another issue, not raised by the Sutherland committee but recently made topical by Professor P. M. S. Blackett (*Nature*, 213, 755; 1967), is that of whether the scientific work at present carried out in government establishments should remain there in perpetuity.

# The School Mathematics Project

by  
BRYAN THWAITES

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The School Mathematics Project is one of several organizations for the improvement and renewal of parts of the school curriculum in Britain. It is now concerned with problems of application as well as of innovation.

THE School Mathematics Project is now in its sixth year of effort aimed at reforming mathematics in secondary schools. It is the largest of the English reform groups working on secondary mathematics; it is the only one which has already set examinations for the General Certificate of Education at both the Ordinary and Advanced levels according to a radically new course covering, in a continuous fashion, the whole age range of 11–18 years; and it is exerting considerable influence in overseas countries, notably in East Africa where there is an EASMP and in the United States. It is therefore of interest (i) to discuss to what extent the project has been able to fulfil its original objectives, (ii) to examine whether the influence of the project is, on the whole, beneficial both to the course of mathematics itself and to the boys and girls who will have undergone its rigours, and (iii) to have some inkling of the future activities of the project. I would like, in this brief article, to consider these three points in turn and to draw some conclusions.

First, then, to what extent are the original objectives being fulfilled? These objectives were fairly clearly stated in 1961 when the five founders of the project met to plan a co-operative experiment between a number of schools. They were to draw up a mathematical syllabus for grammar schools, and to organize appropriate O- and A-level examinations, so as to reflect, more truly than do the traditional syllabuses, the modern character and usages of mathematics. It was felt at the time, and events have fully justified it, that the most effective way of ensuring that new material is introduced into schools is to arrange for the corresponding examinations. The project has therefore claimed as one of its major achievements the agreement which exists between all the GCE Examining Boards that they should make available to their own schools, and through their own respective organizations, the special SMP examinations. The co-ordinator of this scheme is Mr. A. E. E. McKenzie of the Oxford and Cambridge Schools Examination Board, Trumpington Street, Cambridge (to whom all formal queries about the examination arrangements should be addressed).

## Growth

The effectiveness of this approach can be judged from the following figures for the candidates for the SMP O-level examination:

1964	1965	1966	1967 (est.)	1970 (est.)
919	1,548	3,526	7,000	60,000

These may be compared with the national total of O-level mathematics candidates which, in 1966, was 204,000.

As to the content of the GCE syllabuses, the evangelistic role is no longer necessary for any of the reform groups, because the need for a change from the traditional syllabuses is now generally accepted both in the United Kingdom and abroad. At O-level, there is a broad consensus of opinion in this country as to the desirable

directions to take. Thus, for example, the O-level course proposed by the Midlands Mathematics Experiment (directed by Mr. Cyril S. Hope of the Worcester Training College) contains much the same material as the SMP's course (except for its notable inclusion of some elementary calculus treated in a rather traditional way) as does also the Scottish Mathematics Experiment organized by the Scottish Education Department.

## Content

All these O-level syllabuses emphasize the nature of number, and the fundamental properties of the ordinary arithmetic operations on the real numbers; and for this and other purposes, they introduce the notion of sets and the operations on sets, with simple applications of these outside the field of real numbers. Geometry is treated in a more intuitive fashion than is traditional and, although the usual Euclidean theorems are recognized as containing much useful general knowledge, boys and girls are not trained nowadays to produce proofs of theorems or riders which are rigorous according to conventional Euclidean methods. The SMP has paid special attention to geometry and has produced "elementary-geometry from an advanced standpoint" by studying plane geometry principally through the medium of the plane transformations. This approach to geometry has, in the SMP's view, some decisive mathematical advantages, not least of which is the consequence that much of algebra and geometry can then be unified in such a way as to teach the underlying structure and wide applicability of certain mathematical systems.

If these exemplify some of the major innovations introduced into the SMP syllabus, there are many more which ensure that the syllabus represents a major step away from the traditional, as very many parents, in trying to help their children with homework, have found to their cost. At O-level, therefore, the organizers of the project think that it is certainly well on towards reaching its original objectives.

A-level, however, is rather a different proposition. For one thing, there are many more external constraints on a sixth-form course than on an O-level course. One major constraint arises from the use made of the A-level examination as a university entrance examination rather than its proper use as a school-leaving examination. This means that university departments of all kinds—ranging nowadays from departments of mathematics to those of biology or economics—can impose their demands (however implicitly or unconsciously) on the A-level syllabus. Again, pupils themselves, in their half-role of consumer, are unwilling to accept without misgiving a sixth-form course which lays greater stress on the study of a subject for its own sake than on its usefulness as a qualification in their later careers. Then there is the very difficult problem of the time allowed for mathematics in the sixth form; the amount of mathematics which can be done—and especially of "new" mathematics, because there is at this level a basic amount of manipulative mathematics



which is almost bound to be included—obviously depends on whether seven, ten or fourteen teaching periods a week are set aside for mathematics. And the final major problem concerns the character of mathematics at the sixth-form level; in particular, should mathematics remain the kind of unity which is accepted and indeed urged at O-level, or should it be split up into identifiable parts such as pure, applied, statistics, computation, mechanics, and so on?

### Time

The School Mathematics Project has, it is reckoned, devoted some 10 man-years, all told, to these problems of the sixth form in an intensive effort to solve them in a practical form by the production of syllabuses, texts and examinations which, it was hoped, would meet with general approval. In this effort, there have been very wide consultations within the world of mathematics; in particular, very specific consultations took place with university departments of mathematics, as a result of which a lengthy report was published as an appendix to the 1963/64 *Director's Report*\*.

All this effort has been a failure in the sense that not everyone is equally enthusiastic about the A-level solution produced by the SMP. Broadly, this solution is a "core" syllabus (leading to a single subject A-level) which is a unified treatment of what is considered to be absolutely necessary for sixth form mathematics; then, in addition, there is an optional "Further Mathematics" syllabus (again regarded as a unified body of mathematics, and also a single subject A-level) for those sixth formers who, for any reason, may wish to devote more of their time to mathematics. Members of the SMP are, of course, convinced of the soundness of their solution, and they particularly welcome the movement which the Schools Council seems to be taking towards one major subject in mathematics together with a number of optional minor subjects, because they feel that the SMP course is ideally suited to just such a development.

Readers may be interested to judge for themselves the worth of some of the "new" type of questions at A-level which the SMP is now producing. The following examples are taken from the July 1966 examination:

Show that the set of matrices of the form

$$\begin{pmatrix} \cos \theta & -\sin \theta \\ \sin \theta & \cos \theta \end{pmatrix}$$

is closed under the operation of matrix multiplication.

This question combines experience of matrices as tools with an appreciation of structure of mathematical systems.

$$"x \text{ real} \Rightarrow (x^2 > 1 \Rightarrow x > 1)."$$

Is this assertion true or false? If it is true, prove it; if it is false, give a counter example.

This places great emphasis on precision of thought.

Use a step-by-step method, with 0.2 as the step-length, to estimate the value of  $y(1)$ , given that

$$dy/dx = 1 + y^2, \text{ and } y(0) = 0.$$

Lay your work out in tabular form after the pattern:

$x$	$y$	$y^2$	$1 + y^2$	$\Delta y$
0	0	0	1	0.2
0.2				

Compare your value of  $y(1)$  with the exact value.

This example illustrates the importance which the SMP attaches to computers and their potentialities, and to the ideas of approximation. Flow diagrams form an inherent part of the SMP teaching.

Early experimenters on the flow of fluids through capillary tubes proposed a formula of the form

$$Q \propto \frac{1}{\mu} \left( \frac{dp}{dx} \right) r^n$$

where  $Q$  is the volume flow per unit time

$\mu$  is the coefficient of viscosity (of dimensions  $ML^{-1}T^{-1}$ )

$dp/dx$  is the pressure gradient along the tube

$r$  is the radius of the tube, and

$n$  is a numerical index to be found.

Suggest a suitable value for  $n$ .

This is a question which illustrates the attitude of the SMP to mechanics, namely that a qualitative appreciation of a physical situation, and the ability to formulate a mathematical model of it, are more valuable assets to a pupil than the capability of manipulating the standard forms of Newton's second law for highly idealized situations.

### Future

These four examples will give readers an inkling of the objectives of the SMP; the *Report* for 1965/66 reprints the 1966 A-level examinations *in toto* and will repay further study.

We must now return to the two remaining aspects of the SMP's work mentioned in the first paragraph of this article. As to the influence, so far, of the SMP's work on schools, one can only judge from the subjective reports which flow in from schools and also sometimes from pupils. It seems beyond question that, over the past five years, something of a revolution has taken place in our schools as to the atmosphere and morale in the mathematical classroom and as to the teaching techniques now exploited by mathematics teachers. The SMP can claim only a very small part of the credit for this, for it is an effect wrought by many individuals and organizations. The Nuffield work (directed by Dr. G. Matthews, Nuffield Lodge, Regent's Park, London, N.W.1) at the primary level should be mentioned together with that of people such as Miss E. Biggs of the Department of Education and Science and of organizations such as the Mathematical Association. But the SMP has played a particularly vital part at the secondary level by providing teachers with both the texts to get their teeth into and the teaching conferences for in-service training aimed directly and the most helpfully at a specific course.

As to the future of this particular project, it is now clear that the SMP has set itself on a road to which there is no end. Its English team now consists of some sixty mathematicians who work in an entirely voluntary association with an immense and untiring enthusiasm. Their work has spread across the world and has inspired other voluntary teams to form overseas under the same banner. So long as there is vitality and enthusiasm, so long will the SMP continue to push on in the vanguard of mathematical reform. The first wave of reform, started possibly by American groups in the early nineteen-fifties, is now nearly complete. The work done during this first wave now needs consolidating in the many hundreds of secondary schools which are now teaching "new" material of one kind or another; and this consolidation is best left to the long established organizations, such as the university institutes of education, which have deep experience of such work. The time has now come, however, for the second wave to be initiated, and it is to this task that the energy and resources of the SMP will soon be directed.

\* These comprehensive *Reports* can be obtained, annually, from the SMP Office at Westfield College, University of London, London, N.W.3.

## BOOK REVIEWS

## ATOMS FOR STUARTS

Atomism in England from Harriot to Newton

By Robert Hugh Kargon. Pp. viii+168. (Oxford: Clarendon Press; London: Oxford University Press, 1966.) 42s. net.

SINCE Kurd Lasswitz's pioneering study of the *Geschichte der Atomistik* appeared in 1890 our knowledge of atomic theories in the post-Renaissance period has grown rapidly. The corpuscularian ideas of Descartes and Gassend in France and Hobbes, Boyle and Newton in England, in particular, have been the subject of intensive research. Most scholars have followed Lasswitz's internalist approach, treating the development of atomism as essentially the interplay of explanatory ideas put forward by a few great scientists, wholly divorced from the social, political and theological realities of their age. By ruthless chopping of contrary fact a simple picture of the growth of atomism in the seventeenth century can be drawn: this sees the scientists of the period, in reaction against the stilted, imprecise, categorized qualities of mediaeval aristotelianism, as irresistibly attracted to the vivid, quantitative, open-ended explanations afforded by models of small, hard, "massy" bodies in motion. In Kargon's present view such an idealized portrait is both ahistorical and inadequate: to gain a balanced insight into the historical part played by atomist theories of matter in England in the Stuart period it is necessary not only to take account of the many secondary figures responsible for the acceptance and dissemination of the ideas proposed by the gifted few, but also to assess the cultural milieu in which they lived.

In his book Kargon has not always succeeded in integrating these opposed internalist and externalist attitudes, and too often it is difficult to seize the essence of a particular scientist's atomic point of view through a blurring fog of historical detail. Certainly, his close-knit narrative communicates an immense amount of unfamiliar information. In step with the recent revival of interest in this long underestimated Elizabethan giant, we are urged to look on Thomas Harriot as the father of English atomism even though his influence was virtually restricted to his friends Torporley and Warner. Thereafter in quick succession we are given a summary of Bacon's early atomistic views and a condensed account of the Gassendist revival of Epicurean atomism (introduced into England by Hobbes, Digby, Cavendish and especially Walter Charleton), while the concurrent impact of Cartesian mechanical philosophy is traced in the writings of Robert Boyle, Isaac Barrow and Newton. It is less easy to identify general conclusions in this mass of narrowly documented if not wholly pertinent detail, but he asserts sensibly that, because Greek atomistic ideas were too closely involved in philosophical dispute over the existence of the "indivisibly" small to be a viable basis for quantitative explanation, not till a kinematics (and indeed dynamics) of the motion and impact of bodies had been formulated was it possible to have a physically useful atomic model. Disappointingly there is not a single diagram in the book—he gives no examples of how individual atomists differed

in their technical accounts of such intractable phenomena as the simultaneous reflexion and refraction of light at an interface, the shapes of crystals or gravity. The vulnerability of atomism in the early century to the popular charge of being intrinsically pagan and so atheistic is stressed, while Bacon and Barrow are provocatively cited as the sources for Newton's concept of hypothetical method, but many readers will find that this book's real value is in its excellent bibliography.

D. T. WHITESIDE

## EDITORS WANTED

## Chymia

Annual Studies in the History of Chemistry, Vol. II. Edited by Henry M. Leicester. Pp. 208. (Philadelphia: University of Pennsylvania Press; London: Oxford University Press, 1966.) 40s. net.

THE most interesting paper in this volume is that by J. B. Phillips, *Liebig and Kolbe, Critical Editors*. This is the sort of historical essay which has something useful to say to any practising chemist of today; in this case, about the assessment and communication of research. It is doubly interesting because it makes one ask: how critical are the editors of *Chymia*?

The other twelve papers vary remarkably in merit. The extended view is taken by N. A. Figourovskii, who does his fellow historians of other countries a substantial service by a detailed study of *Chemistry in Early Russia*. In contrast, a single instructive episode is described, with his usual lucidity, by W. A. Smeaton (*Macquer on the Composition of Metals*). V. M. Schelar, in a well organized account of the origin of the third law of thermodynamics, faces up bravely to a topic which the average historian of chemistry passes fearfully by.

One would rightly expect any editor to welcome these papers as they stand, but not that of W. D. Miles on the European travels of J. C. Booth, the American consulting analyst. This pedestrian selection of extracts from a manuscript diary ends with a quotation from Edgar Fahs Smith: "[Booth's] idea was that the laboratory should be a miniature factory and the factory a mammoth laboratory". Had this quotation come first and been used as a guiding light for the interpretation of the diary extracts, Miles could have produced a paper of far greater value. It is an editor's duty to try to draw out of a writer the best that is in him, to be constructively critical, which could have been done in this case.

The same failure of constructive editorship is to be seen in the paper by N. L. Jain on *Chemical Theories of the Jains*, which contains, for example, confused references to "electrical properties of substances" at a period apparently (it is not at all clear) around the first century A.D. when there were no electrical concepts of the sort used in the context. Again: "The atoms have Brownian movement. This makes the atoms dynamic rather than static. This movement may be due to natural or internal causes or to external forces. Sometimes this may be so vigorous that the atoms traverse the whole universe in a moment". What justification is there for calling a random motion of this vague sort a "Brownian movement"? The matter theories of other periods or cultures can be very interesting, but no service is done to their study when insufficient effort is made to protect the reader from confusion with modern concepts or terminology.

If the editors of *Chymia* expect its papers to have style and substance, they could surely, with their great experience, guide their contributors into tidier writing and clearer thinking. Liebig and Kolbe may have overstepped the mark at times, but it would be better for the editors of *Chymia* to take this risk rather than that of allowing *Chymia's* good reputation to decline.

F. GREENAWAY

## RADAR CONTINUED

### Radar Techniques for Detection, Tracking and Navigation

Edited by W. T. Blackband. (Proceedings of the 8th Symposium of the AGARD Avionics Panel, London, September 21-25, 1964.) Pp. viii+608. (New York and London: Gordon and Breach, 1966. Distributed in the U.K. by Blackie and Sons, Ltd.) \$37.50.

ONE of the useful organizations which has stemmed from NATO has been the Advisory Group on Aeronautical Research and Development (AGARD). This organization covers the usual fields of aeronautical research including aerodynamics and propulsion, and also includes in its activities that broad field of ancillary devices which is loosely termed "Avionics". AGARD takes the realistic view that electronic devices are essential elements of any airborne vehicle whether it is to be used for military or civil purposes.

Over the years AGARD has held a number of symposia which have been devoted to various aspects of the avionic field, and the present volume covers such a meeting which was held in September 1964 and which was devoted to the themes of radar techniques for detection, tracking and navigation. This volume consists of thirty-one papers which have been written by engineers and scientists active in the field of radar who are working in government, university or industrial research laboratories located in the various NATO countries.

Clutter is still the principal enemy of radar system designers and a number of papers in the present volume are concerned with the modulation problem and of how radar systems can be devised which will provide methods of enhancing the probability of detecting moving targets in the presence of clutter. The technique of pulse compression is described; frequency modulation is employed within the pulse together with a dispersive line in the receiver system which provides time delay varying with the frequency. In this way the effective pulse length of the radar can be compressed to 1/1000th of the transmitted pulse and resolution improved accordingly.

A number of the authors recognize that the target itself can materially affect the nature of the reflected signal. This interaction may prejudice radar detection on the one hand, but it can also provide information about the target which can be extracted by appropriate signal processing. This kind of study has been applied to the examination of the radar echoes from the planets, designated somewhat quaintly as "unco-operative targets", the non-co-operation arising from the remoteness of these bodies and the nature of their reflecting surfaces. It is very impressive to read that the power density of the radar echo from Venus at its apogee is  $3 \times 10^{-25}$  W. Radar echoes from extended scatterers have also been considered by a number of authors, and these papers include careful examination of the nature of ground returns, Doppler navigation, ground mapping and also the applications of radar to meteorology.

Although a number of the papers deal with the advanced theory of radar systems and the nature of radar information, the techniques of radar are not neglected, and improvements in transmitters, frequency and phase scanning arrays are dealt with.

It is probably true to say that even during the war years the main difficulty was not so much the collection of radar information as the ability to analyse and present the information contained in the echo signals. Some of the papers show very clearly that the emergence of a new electronics, based on solid state devices, is providing the means for the storage and analysis of radar information on a scale, and with a wealth of detail, which greatly transcends anything that was possible during the war. The application of computers operated in the real time mode permits executive commands to be extracted from

this body of information and used for the purpose of aircraft control in both the civil and military case.

It is clear from this volume that radar research and development is still very active indeed, and that methods of radar monitoring the air space have become most subtle. Although the papers in a collection such as this are bound to be somewhat uneven, they never fail to be exciting and interesting, and the book may be taken as a good presentation of radar developments which have taken place during the past ten years. The complexity of radar equipment is now so great, and the time scale of development so long, that although two years have passed since the AGARD conference that prompted these papers the book is still up to date and will be of great value to radar research men for some years to come. E. EASTWOOD

## SINGULAR WAVES

### The Plane Wave Spectrum Representation of Electromagnetic Fields

By P. C. Clemmow. (International Series of Monographs in Electromagnetic Waves, Vol. 12.) Pp. vii 184. (Oxford, London and New York: Pergamon Press, Ltd., 1966.) 50s. net.

IN 1919 Weyl published the formula

$$2\pi \frac{\exp\{ikR\}}{ikR} = \int_0^{2\pi} d\varphi \int_L \exp\{ik(\alpha x + \beta y + \gamma z)\} \sin\theta d\theta$$

valid for  $z > 0$ ,  $\varphi, \theta$  being the polar angles of the unit vector  $(\alpha, \beta, \gamma)$  referred to the  $z$ -axis,  $R^2 = x^2 + y^2 + z^2$ , and  $L$  a certain path of integration in the complex  $\theta$ -plane. He thereby obtained the plane wave representation of a singular solution of the wave equation in the presence of a point source at the co-ordinate origin. The superposition involves inhomogeneous plane waves or alternatively plane waves travelling along imaginary directions, and Weyl's step is crucial in obtaining an expansion or representation theorem involving plane waves for general electromagnetic fields generated by sources or bounded by surfaces. This theorem received little attention—it is not mentioned in the older textbooks—until soon after the Second World War when Brooker and Clemmow took up the idea. In two classic papers they demonstrated the application of the angular spectrum of plane waves to radio propagation over flat surfaces, to certain aerial problems and to diffraction theory. Soon afterwards, Clemmow obtained the exact solution of a wide class of two-dimensional diffraction problems and there have been other applications since then.

Dr. Clemmow has now written a connected introduction to the subject. It is a most beautiful book and will surely become a classic in its field. Maxwell's equations in M.K.S. units and the idea of induced electric and magnetic surface current densities are of course assumed to be familiar, and the reader must be at home in the complex plane, but that is all that is required. A self-contained and careful exposition establishes, for example, the plane wave representation, its relation to other representations and the way to separate off the field in the radiation zone. Then the applications are considered. These cover diffraction by a plane screen, propagation over plane surfaces, the fields of point particles, both synchrotron radiation and the Čerenkov radiation and finally sources in anisotropic media. There is a very satisfying demonstration of the optical theorem and Babinet's principle.

The text is completely self-contained; there is not a single reference to other material or authors, and Dr. Clemmow is too modest to mention his own contributions to the subject. He clearly enjoyed writing the book, and I enjoyed reading it. It can be most warmly recommended, not only to students of electromagnetic theory but to everybody concerned with scattering problems. S. ZIENAU

## INTEGRALS AND SYMMETRY

### Homology and Feynman Integrals

By Rudolph C. Hwa and Vigdor L. Teplitz. (Mathematical Physics Monograph Series.) Pp. xi+331. (New York and Amsterdam: W. A. Benjamin, Inc., 1966.) n.p.

### Unitary Symmetries and their Application to High Energy Physics

By M. Gourdin. Pp. xi+303. (Amsterdam: North-Holland Publishing Company, 1967.) 86s.

(1) ANALYTIC properties of collision amplitudes play a central part in the study of strong interactions of elementary particles. Some of these properties can be derived rigorously from quantum field theory and some can be obtained from an axiomatic approach to  $S$  matrix theory. By far the largest amount of information about analyticity has been obtained, however, from the study of Feynman integrals in perturbation series for collision amplitudes. The basis of the approach is due to Cauchy, whose theorem on complex integration provides a method for the analytic continuation of Feynman integrals as functions of the relativistically invariant energies that describe the collision. This method fails when singularities of the integrand cannot be avoided by the contour of integration, and this may be a singularity of the Feynman integral. When there are several complex variables of integration, it is difficult to ascertain whether singularities of the integrand actually meet the (several dimensional) contour of integration so as to make the Feynman integral singular. This difficulty provided the motivation for developing and applying homology to Feynman integrals.

In this book the authors first describe the classical methods of studying the problem that are based on what a physicist would regard as the obvious extensions of Cauchy's theorem. They then outline homology theory and show how it converts the problem of the geometry of Feynman integrals into an algebraic problem. Their account of the theory is a valuable addition to the reprints of some of the main papers on this subject that are included in the second half of the book.

The book is recommended for graduate students in theoretical physics who have strong mathematical interests and abilities.

(2) The importance of the unitary group  $SU(3)$  for describing the symmetries of elementary particles was recognized very soon after the classic papers written in 1961 by Gell-Mann and by Ne'eman. Subsequent generalizations have encountered difficulties in formulating symmetries in a fully relativistic manner, but various procedures have been established, based on an algebra of currents that incorporate both angular momentum symmetries and  $SU(3)$  symmetries.

The first two-thirds of *Unitary Symmetries and their Application to High Energy Physics* presents a mathematical description of the physics of symmetries. It begins with the essential features of Lie groups and the classification of the strongly interacting particles using the group  $SU(3)$ . This is followed by applications to strong, electromagnetic and weak interactions. The group  $SU(6)$  is then introduced and some of its consequences are investigated. There is a chapter on current algebra, but this covers only a small fraction of the very extensive literature on the subject that has appeared in the past eighteen months.

The last third of the book describes the mathematical formulation of Lie groups and Lie algebras. It is likely that an increasingly important part in the theory of elementary particles will be played by Lie groups. This book is a good introduction to the subject of Lie groups and elementary particles at a level that is suitable for graduate students in theoretical physics.

R. J. EDEN

## MORE GAS FOR BURNING

### Natural Gas

A Study. By E. N. Tiratsoo with an Introduction by Sir Harold Hartley. Pp. xvi+386. (London: Scientific Press, Ltd., 1967.) 70s.

THE publication of this excellent and comprehensive study on natural gas is greatly to be welcomed. The author is a geologist, but he has successfully "tried to widen the scope and interest of the book for the non-geological reader by including chapters on utilization and transportation, as well as an historical background". In fact, there is hardly an aspect of this great field of human endeavour on which the author has failed to provide more complete and significant information than has hitherto been conveniently available. The British reader, thrilled with the discovery of important natural gas reserves in the North Sea Continental Shelf, will perhaps be particularly grateful for chapters eleven to sixteen which deal with the methods of locating, measuring and exploiting gas reserves; the transportation and storage of gas; and the uses of natural gas and the economic considerations involved, with particular reference to the problem of converting the existing distribution system and burning appliances to the new fuel. "The problems of conversion for 12-13 million households are obviously formidable, and much depends on the composition of the natural gas to be used." As these problems are described by the author, it becomes very clear that the amount of natural gas likely to be used in Britain in, say, 1970-36 months from now—will depend primarily on the speed at which conversions can be carried out and new uses developed, and only secondarily on the quantity of gas reserves found. The Canvey Island conversions, an experimental enterprise on a small scale in a community conveniently isolated from other gas users and close to the methane terminal, have shown that it is not an easy matter to get the thing right, so that the consumer is really satisfied, and the idea that conversions at the rate of 600,000 to 1,200,000 per annum could be achieved in the near future will have to be treated with some caution.

The author's discussion of the problems of leakage, pipe failures and gas-borne dust, which arise when natural gas at high pressures is sent through old low-pressure coal gas distribution systems, is extraordinarily interesting and of the greatest practical importance, and so is his analysis of the storage and transportation problems. No one concerned with national fuel policy matters in Britain or abroad will fail to be grateful to Dr. Tiratsoo for placing his encyclopaedic knowledge at their disposal.

E. F. SCHUMACHER

## VOLCANIC ROCK IN RUSSIA

### Tufflavas and Ignimbrites

A Survey of Soviet Studies. Edited by Earl F. Cook. Translated from the Russian by Miriam Carty. Pp. xii+212. (New York: American Elsevier Publishing Company, Inc.; London: Elsevier Publishing Company, Ltd., 1966.) 60s.

IGNIMBRITES include the most impressive rock-bodies that volcanoes are capable of producing. The largest are the consequence of gigantic eruptions during which, in the space perhaps of hours or days, tens or hundreds of cubic kilometres of rock-melt are converted to small fragments which flow as a turbulent suspension in gas away from the volcano. The flow incinerates, and the resulting deposit buries, everything over an area that can be measured in thousands of square kilometres, and in

the deposit the fragments are often welded together to form a rock as tough as a lava flow. Ignimbrites are widely distributed, and it is clear from this book that they are not lacking in the Soviet Union.

*Tufflavas and Ignimbrites* is a collection of thirty papers by Soviet geologists, presented at a symposium on the subject in Moscow in 1961. The first part, devoted to general problems of tufflavas and ignimbrites including their genesis, begins with a review, specially revised and brought up to date by the Soviet volcanologist, V. I. Vlodavetz. Tufflavas are described as resembling ignimbrites in that they contain rock-fragments, but they differ from them in having a homogeneous lava base and a somewhat different origin. The papers in the second part of the book describe many different occurrences, ranging in age from Cambrian to Pleistocene and in geographic position across the continent of Asia (a map at the front of the book locates the occurrences). The third part discusses aspects of the economic geology of ignimbrites, such as their use as building stones.

This is a book for the specialist rather than the general reader. It is a useful addition to the literature on the subject, and will give both an insight into the development of ideas in the Soviet Union and an introduction to new occurrences from the Caucasus and Kazakhstan to Kamchatka.

G. P. L. WALKER

## GEOLOGY IN THE SOUTH-EAST

### Geology of the Country around Canterbury and Folkestone

By J. G. O. Smart, G. Bisson and B. C. Worssam, with Chapters on Palaeontology of the Gault by R. Casey, and on Palaeontology of the Chalk by R. V. Melville, and other contributions by P. A. Sabine, M. Mitchell, G. P. Jones and H. A. Hope Macdonald. (Memoirs of the Geological Survey of Great Britain—England and Wales.) Pp. x + 337 + 6 plates. (London: H.M.S.O., 1966.) 70s. net.

ONE tends to think of the Victorian geologist as a person who, filled with awe at the mysteries of nature, carefully recorded the details of rocks before him without showing any intellectual curiosity towards them. This approach is still followed by the Geological Survey, and is manifested in its publications. The memoir on the country around Canterbury and Folkestone is no exception. As a report it embodies the worst features of reports: dullness, reiteration, and lengthy lists of irrelevant or trivial information. There are several reviews of the literature which only duplicate the information available in the bibliography, while many topics have an introduction, a general account, and then a detailed account. These accounts are always in text form although much of the information could be better presented diagrammatically.

Because the memoir has many authors it is inevitably patchy—thus the section on the Coal Measures is better than those on the Old Red Sandstone and the Lower Carboniferous because it takes account of modern sedimentological work. Given that this memoir is in the style of previous memoirs the major criticism that can be levelled at it is that it deals insufficiently with sedimentology and overmuch with the palaeontology in the general text. The only serious petrologic investigations reported are those on brick-earths and clay with flints—both in the Recent section. The work on the Recent section is good and is one redeeming feature of the book.

Considering the major contribution that Percy Allen has made to our understanding of the geology of the Weald, much more prominence than one short paragraph should have been given to his work. Casey's work is better represented (possibly because Casey is one of the occasional authors) and his chapter on the palaeontology of the

Gault contrasts in liveliness with Melville's chapter on the palaeontology of the chalk, which is little more than a list of fossils and a few words on the problems of zonation.

If the memoir functions largely as a local geology book then at 70s. it is too expensive. It is true that the detailed descriptions are very much better than the more general passages in this work, but this is only because the general accounts are so bad. The Survey could easily reduce the memoir to a half or two-thirds its present length by re-writing the general sections and replacing much of the detail by diagrams. Let us hope the new director of the Survey will see the reformation of the memoirs as one of his first duties.

A. M. MARSHALL

## BIOLOGY OF SKIN CANCER

### Carcinogenesis

Edited by William Montagna and Richard L. Dobson. (Proceedings of a Symposium on the Biology of Skin held at the University of Oregon Medical School, 1965.) (Advances in Biology of Skin, Vol. 7.) Pp. xiii + 358. (Oxford, London and New York: Pergamon Press, Ltd., 1966.) 110s. net.

THE seventh volume in the series *Advances in Biology of Skin*, edited by W. Montagna and R. Dobson, contains a series of papers on skin carcinogenesis presented at a symposium in April 1965. The book begins with three chapters on tissue homeostasis. Although cancer constitutes a failure in homeostasis, only in recent years, with the work on gene control<sup>1</sup> and on histones<sup>2</sup>, has much attention been turned to the study of normal control mechanisms. W. S. Bullough and E. B. Laurence's contribution in this field concerns the combined role of tissue-specific chalones (mitotic inhibitors synthesized by cells) and adrenal stress hormones in the control of epidermal cell mitosis<sup>3</sup>. O. H. Iversen, who recently confirmed the existence of epidermo-specific chalones<sup>4</sup>, discusses the kinetics of the reaction of mouse epidermis to chemical carcinogens and other irritants. Whereas other workers (particularly those anxious to find a quick-screening test for carcinogens) have stressed the dissimilarities between the response of the skin to carcinogenic and non-carcinogenic hyperplasia-producing irritants, Iversen concludes that the dynamics of the responses to single applications of the two are similar. This similarity, however, does not apply to the repeated application of carcinogens. In the latter case, cell loss becomes progressively less after each application, which suggests the acquisition by cells of an increasing capacity to resist damage from the carcinogen. T. S. Argyris continues the same general theme by discussing the induction of proliferation in normal tissues by substances derived from tumours, and the relationship between such substances and embryonic inducers<sup>5,6</sup>. The best example of such a substance is the nerve growth factor (NGF) of Cohen and Levi-Montalcini<sup>7</sup>, a protein isolated from the microsomal fraction of a transplantable mouse tumour which stimulates the growth of chick embryonic ganglia in tissue culture. A further recent development in this field is the isolation of an epidermal growth factor (EGF) from NGF (ref. 8). It would be interesting to see the relation, if any, between EGF and the chalones referred to previously.

H. Kirkman discusses the role of sex hormones in hair follicle tumorigenesis (chaetepithelioma induction) with particular reference to the scent gland of the Syrian hamster; and A. W. Horton, P. A. van Dreal and E. L. Bingham put forward the theory that certain long chain hydrocarbons accelerate (promote), and certain polycycloparaffinic compounds delay, carcinogenesis by an effect on the immune response. The evidence for this interesting theory is indirect and corroboration is certainly



necessary because others have shown that gross interference with immune competence, as by neonatal thymectomy, has only a modest enhancing effect on tumour-induction by carcinogenic polycyclic hydrocarbons<sup>9,10</sup>.

It has been known for a long time that exposure to ultra-violet radiation predisposes to skin cancer in man. F. Urbach, R. E. Davies and P. D. Forbes (Philadelphia) review the evidence for the cause and effect relationship. In particular, they distinguish between surfaces which absorb and those which reflect ultra-violet radiation and the significance of reflected radiation in the genesis of cancer. They suggest that whereas ultra-violet radiation may be the sole cause of squamous carcinoma, a second factor is probably involved in the genesis of basal cell carcinoma. J. H. Epstein (San Francisco) describes the induction of skin cancers in hairless mice by irradiation with wavelengths in excess of 3200 Å: rays of these longer wavelengths, which are normally erythrogenic, may induce cancer in the presence of photo-sensitizers with particular action spectra.

Other chapters are concerned with the malignancy of adrenal tumours (H. Pinkus, Detroit, Michigan), the relation between phenotype and susceptibility to particular histological types of skin cancer (G. A. Gellin, A. W. Kopf and L. Garfinkel, New York, N.Y.) and the induction of skin cancer in Simian primates (R. L. Dobson and J. S. Pinto, Portland, Oregon).

As in all books which consist of several invited contributions, the quality and value of chapters vary widely. Apart from this limitation the book is well edited, well illustrated and well produced. F. J. C. ROE

<sup>1</sup> Monod, J., and Jacob, F., *Cold Spring Harbor Symp. Quant. Biol.*, 26, 389 (1961).

<sup>2</sup> In *The Nucleohistones* (edit. by Bonner, J., and Ts'o, P. O. P.) (Holden-Day, San Francisco, 1964).

<sup>3</sup> Bullough, W. S., *Cancer Res.*, 25, 1683 (1965).

<sup>4</sup> Iversen, O. H., Aandahl, E., and Elgo, K., *Acta Pathol. Microbiol. Scand.*, 64, 508 (1965).

<sup>5</sup> Fleming, H. S., *Yale J. Biol. Med.*, 29, 396 (1957).

<sup>6</sup> Levi-Montalcini, in *The Chemical Basis of Development* (edit. by McElroy, W. D., and Glass, B.), 646 (Johns Hopkins University Press, Baltimore, 1958).

<sup>7</sup> Cohen, S., and Levi-Montalcini, R., *Proc. U.S. Nat. Acad. Sci.*, 2, 571 (1957).

<sup>8</sup> Cohen, S., in *Metabolic Control Mechanisms in Animal Cells* (NCI Monograph 13), 13 (U.S. Government Printing Office, Washington, 1964).

<sup>9</sup> Miller, J. F. A. P., Grant, G. A., and Roe, F. J. C., *Nature*, 199, 920 (1963).

<sup>10</sup> Grant, G., Roe, F. J. C., and Pike, M. C., *Nature*, 210, 603 (1966).

respectively, to features with an appropriate extent in the horizontal or vertical direction. He proposes that the subsequent routing of the output of one of these cells is controlled by one or more "memory" cells, each of which has the ability, probably in an all-or-none way, to close one output route. The reader must refer to the book for details. My feeling is that the idea is interesting and not impossible, but that much more discussion could be given of the possible physiological basis and also how the networks given (for example on page 40), which consist of but a few cells, are supposed to be integrated into the overall nervous activity in the brain.

A great deal of use is made in the book of words taken from outside biology, especially from computing. I belong among those who (page 12) "think that it would be wiser to avoid the computer language in neurology". The difficulty, it seems to me, is that if words such as "code", "store" and "address" are precisely defined in terms of nerve cells, their connexions and firing patterns, then one is not merely using a new language but is making a definite hypothesis about brain function. If, as in this book, they are incompletely defined, then it is often difficult to discover the meanings of sentences in which they occur. I am constantly coming up against problems like that presented by page 19 where "representation" is stated to be a much stronger term than "engram", yet they seem to mean the same thing. Then again, I am worried by the remark on the preceding page about biochemists searching for codes and finding them in DNA. It gives a totally false impression of the development of molecular biology. DNA and its hereditary significance were known before there was any "search for codes". The only such search was for the genetic code. It is true that this was often referred to as the "coding problem" but only as a convenient shorthand, and knowledge of communication theory played no part whatsoever in its solution. The problem was to find the rules of translation between two types of linear sequence the components of which were already known. There can be, as yet, no equivalent search relating to memory (see page 18). Similar matters arise in many parts of the book and the result is that I find it unnecessarily obscure. This is a pity because it contains a great many interesting observations and ideas.

J. S. GRIFFITH

## HOW TO REMEMBER

### The Memory System of the Brain

By J. Z. Young. Pp. vi+128. (London: Oxford University Press, 1966.) 28s. net.

THIS book contains a slightly revised version of the Hitchcock lectures delivered by J. Z. Young at the University of California in 1964. It could serve equally as a summary of, or an introduction to, the extensive experiments on the octopus by Young and his colleagues and Young's views on the nature of the changes accompanying learning. As Young has constantly emphasized, it is very desirable for those of us who are primarily interested in mammalian brains to pay close attention to the results obtained from work on one of the most highly intelligent invertebrates. The octopus, having evolved its brain independently of the mammals, gives us a very interesting example with which to compare and contrast the latter. In particular, its memory is physically divided—matters concerned with vision are stored in quite separate places from those concerned with touch. There does not seem to be any parallel to this in the mammalian brain.

Young draws attention to the numerous cells in the optic lobes, which typically have dendritic fields oriented horizontally or vertically and which he believes to respond,

## HOW TO SEE

### The Functional Organization of the Compound Eye

Edited by C. G. Bernhard. (Proceedings of the International Symposium held in Stockholm, October 1965.) (Wenner-Gren Centre International Symposium Series, Vol. 7.) Pp. xiii+591. (Oxford, London and New York: Pergamon Press, Ltd., 1966.) 126s. net.

THE meetings between Jan Swammerdam and Antoni van Leeuwenhoek in 1674, during which it is probable that they discussed the image-forming properties of the faceted eye, prompted Chairman C. G. Bernhard in his opening address to suggest that the meeting that this book reports might aptly be called the Second Symposium on the Compound Eye.

The subject matter of the book is divided into four parts. The first is concerned with optics and the control of light admission; the second with rhabdom structure, photochemistry, colour discrimination, and reaction to polarized light; the third with receptor excitation and quantum sensitivity; and the fourth with integration of the visual output. An author index is provided, but there is no subject index.

According to Müller's "mosaic" theory, which receives most support from behavioural experiments with arthropods, the functional unit of the faceted eye is the om-

matidium. The ommatidium, however, contains a number of long reticular cells (usually seven or eight) which continue through the basilar membrane as primary visual axons. Evidence is presented by a number of contributors that the image formed by the corneal facet and the cone can be analysed by these sense cells. Thus, Burt and Catton consider that the separation of the rhabdomeres (the inner aspects) of the reticular cells is adequate to resolve diffraction patterns which, as they point out, are imaged at three levels. These images offer little basis for form vision, and are considered to mediate sensitivity to movement. This, in their view, leaves the eccentric cell in each ommatidium with the task of assessing the average illumination entering each facet, thus providing a basis for the mosaic theory.

The inner aspects of the retinulae form the photo-sensitive rhabdom. This is generally supposed to be the site of visual pigment and, from its coaxial location in the light path and from its fine structure—an organized lipoprotein membrane system—it is analogous to the outer segment of the vertebrate receptor cell. Eguchi and Waterman in their analysis of the fine structure of the crustacean rhabdom provide anatomical evidence of a two-channel system for the discrimination of linearly polarized light, and Waterman also contributes a study of polarotaxis and photoreception in crustacea.

Physiological and photochemical aspects of visual excitation are considered by Goldsmith and Fernandez in a review that also lists all available information on the properties of crustacean visual pigments. Electrophysiological studies in the *Apis* drone during light- and dark-adaptation by Naka and Kishida suggest that the photochemical reaction in the rhabdomere produces a signal proportional to the logarithm of the light intensity. Reichardt contributes a paper on the detection of quanta by the compound eye of the fly *Musca* and concludes that the receptors behave as one-quantum receptors, that is, spatial and temporal summation do not occur.

Trujillo-Cenóz and Melamed add new details (in dipterans) about the fine structure of the ommatidia and of their projection on the intermediate retina or lamina. Nunnemacher surveys the fine structure of the optic tracts of seven decapod crustacea. De Voe presents a non-linear model of sensory adaptation in the non-compound eye of the Wolf spider while Ratliff, Hartline and Lange provide two very valuable papers on the dynamics of lateral inhibition in *Limulus*. Other aspects of inhibition and excitation in this animal are given in papers by Purple and Dodge and by Adolph. Leutscher-Hazelhoff and Kuiper describe an interesting phenomenon of repetitive firing in the optic lobe of the blowfly and suggest that this could help the fly to estimate distances.

The remaining papers form a supporting background to this report on vision in the compound eye. The advances made in anatomy through the application of the electron microscope, and to the quantitative description of activity in retinal and central neurons by models expressed as computer programmes are, perhaps, the most striking feature of this valuable book. H. J. A. DARTNALL

## OBITUARIES

### Dr. Gordon Malloch

ON November 8, 1966, Dr. John Gordon Malloch died at his home in the South of France, where he had lived since his retirement in 1964.

Malloch was born in Scotland and obtained his early education at Watson's College. On coming to Canada he attended the University of Alberta where he obtained his B.Sc. and M.Sc. degrees in agricultural biochemistry. He

later obtained his Ph.D. at the University of Minnesota for a study of the amylase enzymes of wheat flour. Throughout the period of his graduate work and later he was closely associated with the many problems undertaken by the Grain Research Committee of the National Research Council of Canada, to ensure that new forms of wheat, developed for rust resistance and other features, retained their qualities for such uses as bread baking, so as to maintain the high reputation of Canadian wheat in the international market.

In 1932 Malloch was appointed to the staff of the then Division of Biology and Agriculture of the National Research Council (NRC), where he organized a cereal research laboratory. At the beginning of the Second World War he assumed responsibility for co-ordinating a large section of NRC's work concerned with chemical warfare in addition to supervising a group concerned with protective clothing and related problems. Malloch was appointed as Canadian scientific representative at the British Commonwealth Scientific Office in Washington towards the end of the war and later to the London liaison office. For his work during the war, he was awarded the M.B.E.

From 1945 to 1962 Malloch was in charge of the Canadian Scientific Liaison Office in London, one of the units of the British Commonwealth Scientific Office. In this position it was his responsibility to establish contact not only with the British scientific community, but also with scientific organizations on the continent of Europe. With his broad interests, his congenial disposition and his love of travel he rapidly became a personal friend of scientific leaders in most of the capitals of Europe; he became as much a member of the European scientific community as a representative of Canada.

During his stay in London he was chairman of the working party of the British Commonwealth Scientific Committee and during many years his industry and enthusiasm contributed greatly to the success of that organization. He also represented Canada on the Executive Council of the Commonwealth Agricultural Bureaux.

Malloch always took great interest in the scientific activities of the Organization for European Economic Co-operation (O.E.E.C.) and was, for several years, chairman of its committee for applied research, undertaking pioneer work in establishing a network of co-operative research projects between the member countries. He was also a member of the task force which in 1957 made a report to NATO that resulted in the establishment of the NATO Science Committee. In 1962, when the Organization for Economic Co-operation and Development (O.E.C.D.) was established, Malloch was appointed to Paris where he took on the dual responsibility of scientific counsellor at the Canadian embassy and scientific adviser to the Canadian permanent delegation to the O.E.C.D. From 1962-64 he represented Canada on the NATO Science Committee.

Malloch was one of the first scientists to make a career of scientific liaison, and during his long period in London he did much to establish the position of the scientific attaché. His skill as a negotiator, his talents as a committee member and his understanding of international relations made him an ideal scientist to operate in the grey area where scientific relations impinge on government policy. He had an instinctive feeling for international negotiation and, in his attempts to establish co-operation in specific fields of research, showed real understanding of the different needs of the participating countries and of how research really works. Malloch was an outstanding example of the best type of science administrator whose concepts and initiatives stemmed from an earlier and unwavering love of research.

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W. H. COOK  
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# Implications of the Oblateness of the Sun

by

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Recent measurements of the oblateness of the Sun have been used to determine its quadrupole moment and thus its effect on the perihelion of Mercury. It is shown that these calculations could well be incorrect and that the measured oblateness is that expected if the Sun is slowly rotating throughout, since the surface is not one of constant potential.

DICKE has measured the oblateness of the Sun and found a difference between the equatorial and polar radii of 35 km, which amounts to 5 parts in  $10^5$  of the radius of the Sun<sup>1</sup>. In principle a measurement of the oblateness can reveal the quadrupole moment of the Sun, and because a distorted Sun produces a perihelion advance of the planets<sup>2,3</sup>, the contribution to the perihelion advance of Mercury from an oblate Sun can be determined. From the measured oblateness Dicke deduced a contribution to the perihelion advance of Mercury of 4" arc/century, which implies a mean rotation period for the inside of the Sun of 1.8 days. Because Dicke's results are of great importance both in connexion with the test of the general theory of relativity and with theories of stellar rotation, it is necessary to examine in detail the deductions from a measure of the oblateness to the value of the quadrupole moment and this is done in this article.

Dicke's deductions were made by assuming that the surface of the Sun is one of constant total potential, gravitational plus rotational, that is

$$\Psi = \frac{GM}{r} + \frac{a_2}{r^3} P_2(\cos \theta) + \Omega^2 \frac{r^2 \sin^2 \theta}{2} = \text{constant}$$

Because the surface value of  $M$ ,  $R$  and  $\Omega$  are known, the oblateness then reveals the value of  $a_2$ , the quadrupole moment. Eight kilometres of the measured 35 km are due to the surface value of  $\Omega$  so that 27 km remains to be explained. Any criticism of Dicke's argument must then devolve on the assumption that the surface is one of constant potential and this is the point which I shall examine.

The equation of hydrostatic support for a uniformly rotating star can be expressed as

$$\frac{\nabla P}{\rho} = -\nabla \varphi + \Omega^2 w = -\nabla \Psi$$

from which it follows that  $P$  and  $\rho$ , and thus the temperature  $T$ , are constant on surfaces  $\Psi = \text{constant}$ ; if the surface were then a surface of constant  $T$ , then this would be one of constant  $\Psi$  and Dicke's result could hold. The radiative flux

$$F = -\frac{4ac}{3} \frac{T^3}{\kappa \rho} \nabla T = -\frac{4ac}{3} \frac{T^3}{\kappa \rho} \frac{dT}{d\Psi} \nabla \Psi$$

is not constant, however, on an equipotential surface because  $\nabla \Psi$ , the effective gravity, varies between pole and equator and therefore the flux varies between pole and equator. With the usual definition of the surface as that point where  $T = T_e$ , with  $T_e$ , the effective temperature, given by

$$F = \sigma T_e^4$$

then we readily see that the surface  $T = T_e$  cannot be an equipotential, because  $T$  is constant over such a surface whereas  $T_e$  varies. In fact, because  $T_e$  is lower at the equator than the pole we have to go out further in the equatorial direction than the polar direction to get to the point where  $T = T_e$ . Because in the surface layers of a star we have approximately

$$T^4 = \frac{3}{4} T_e^4 \left( \tau + \frac{2}{3} \right) \tau = \int_{\tau}^{\infty} \kappa \rho dr$$

with  $\tau$ , the optical depth,  $= 2/3$  at the surface, then we have to go an extra distance

$$\Delta r = \frac{\Delta \tau}{\kappa \rho} = \frac{16}{3} \frac{\Delta T}{T} \frac{1}{\kappa \rho}$$

In the surface layers of the Sun  $\kappa \rho \sim 10^{-7}$ , and  $T_e^4$  is proportional to effective gravity, and because the effective

gravity for a uniformly rotating Sun would only vary by  $5 \times 10^{-5}$  of itself over the surface, the temperature difference would only be of the order of  $0.07^\circ \text{K}$ , corresponding to a distance of 600 cm—which is entirely negligible compared with the observed value of 35 km.

If, however, the oblateness were to result from a rapidly rotating interior, as Dicke assumes, the variation of gravity over an equipotential in the rapidly rotating core would be of order  $10^{-2}$  of itself, giving a flux variation of the same value. If flux were conserved along the equator and pole this would give a difference in effective temperature of  $15^\circ$  between pole and equator—Dicke did not observe such a difference.

This argument shows how Dicke's conclusions could be invalidated. If the surface were not one of total potential, then the surfaces of constant temperature and pressure would not coincide, and there could then be a temperature difference along an equipotential surface even though the energy flux is the same at the equator and the pole. If the temperature difference were  $\Delta T$ , then to produce a  $\Delta r$  of 30 km we would need a  $\Delta T \sim 300^\circ \text{K}$ . This would not be observable because the flux and therefore the effective temperature would be the same at equator and pole. The potential surface going through the pole would lie a distance  $\Delta r$  inside the star in the equatorial regions, and going a distance  $\Delta r$  from this potential surface at the equator would take one to the place where  $T$  had dropped to  $T_e$ .

The temperature difference  $\Delta T$  would produce a corresponding pressure difference  $\Delta P$  along a potential surface and thus a pressure force  $\Delta P/R\rho$ . In a direction normal to the equipotential the pressure force  $P/\rho l$ , where  $l$  is the scale height, is largely balanced by gravity  $g$ , and so the force along the surface can be expressed as

$$\frac{\Delta P}{R\rho} = \frac{l}{R} \frac{\Delta P}{P} \frac{P}{\rho l}$$

This pressure force would drive motions until in a steady state it is balanced by the inertial, Coriolis and viscous forces. Because inertial and viscous forces will be negligible, equilibrium is achieved when the Coriolis force balances the driving force

$$2 v_\phi \Omega \sim \frac{\Delta P}{R\rho} \sim \frac{l}{R} \frac{\Delta P}{P} g$$

$v_\phi$  is here the azimuthal component of the velocity field and corresponds to a differential rotation. Replacing  $v_\phi$  by  $\Delta \Omega R$ , where  $\Delta \Omega$  is the difference in angular velocity arising from the differential rotation, we have

$$\frac{d\Omega}{dr} = \frac{\Delta \Omega}{l} = \frac{1}{2} \frac{\Delta P}{P} \frac{g}{\Omega R^2}$$

Now the equation of hydrostatic support in the photosphere is

$$\frac{dP}{d\tau} = \frac{g}{\kappa}, \text{ that is, } P = \frac{g}{\kappa} \tau$$

thus

$$\frac{\Delta P}{P} = \frac{\Delta \tau}{2/3} \sim \frac{1}{2}$$

because  $\Delta \tau \sim 1/3$  to give a distance of 30 km. Therefore

$$\frac{d\Omega}{dr} \sim \frac{1}{2} \times \frac{1}{2} \times 2 \times 10^{-12} = 5 \times 10^{-13} \text{ rad/sec.}$$

Because the equator extends some 30 km farther than the pole, it will have a different angular velocity because there is a greater distance over which  $\Omega$  can vary. The differential rotation will then be such that

$$\frac{\Delta\Omega}{\Omega} = \frac{d\Omega}{dr} \frac{\Delta r}{\Omega} \sim \frac{1}{2}$$

that is, there will be a differential rotation of the order of 50 per cent on the visible surface. The agreement between this value and the observed equatorial acceleration  $\sim 20$  per cent supports the hypothesis that the oblateness is caused by a temperature difference, and resulting pressure difference, over equipotential surfaces.

Moreover, since the pressure gradient along an equipotential surface is positive,  $P$  increases from pole to equator; the Coriolis force must be directed outwards from the rotation axis, hence  $V\phi$  must be positive and so  $\Omega$  increases outwards, giving an equatorial acceleration. Again, more detailed models of the solar photosphere<sup>10</sup> give a change in optical depth of  $3/8$  for a distance of 30 km, and a  $\Delta T \sim 345^\circ \text{K}$  and  $\Delta P/P \sim 0.1$ . The resulting decrease by a factor 5 from our simple estimate decreases  $d\Omega/dr$  by the same factor and consequently gives an equatorial acceleration of 10 per cent, in better agreement with the observed value.

We have still left unanswered the origin of this temperature difference, and the most likely cause is the greater temperature gradient needed to transport the same amount of energy along the pole of the Sun as along the equator, the difference being caused by the stabilizing effect of rotation.

The effect of rotation on the temperature gradient in a turbulent convective medium is very difficult to estimate. Investigations on the onset of convection show that rotation tends to stabilize when the angular velocity  $\Omega$  and gravity  $g$  are in the same direction, but that the rotation perpendicular to  $g$  has no effect<sup>4</sup>. The critical Rayleigh number  $R_c$  at which convection sets in is found to depend on the Taylor number  $\tau$  through a relation of the approximate form

$$R_c = 657 + 8\tau^{2/3}$$

$$R_c = \frac{g}{T} \frac{\beta d^4}{\kappa \nu}, \quad \tau = \frac{4\Omega^2 \cos^2 \theta}{\nu^2} d^4$$

where  $\kappa$  is the thermometric conductivity and  $\nu$  the kinematic viscosity,  $d$  is the depth of the layer,  $\theta$  the angle between  $\Omega$  and  $g$ , and  $\beta$  is the superadiabatic temperature gradient. These results are of no direct use, since in the Sun turbulence is fully developed and  $\beta$  is determined by the condition that the energy flux is carried by the turbulence rather than that convection just sets in.

An estimate of the effect of rotation on turbulent energy transport has been made by Weiss<sup>6</sup> using a cellular model of convection. He found that in order to carry a flux  $F$  in the presence of rotation parallel to gravity, the superadiabatic gradient  $\beta$  had to satisfy the relation

$$\beta = \left[ \frac{8 \Omega F T}{C_p \rho g d^2} \right]^{1/2}$$

whereas when rotation is absent, or we have angular velocity perpendicular to gravity as along the equator, then

$$\beta = \left[ \frac{8 F^2}{C_p^3 \rho^2 d^3} \right]^{1/3}$$

If one inserts typical values for the solar convective zone, one finds that  $\Delta\beta$ , the difference between the superadiabatic gradient along the pole and equator, to be  $4 \times 10^{-8} ^\circ \text{K/cm}$ .

An alternative way of estimating the difference in superadiabatic gradients is to extend an idea suggested by Unno<sup>8</sup> that the largest eddies in the turbulent region are given by the linear theory for the onset of convection, but with the conductivity and viscosity replaced by the eddy conductivity and viscosity of the turbulence, that is, the turbulence so adjusts itself that under its own influence the largest modes are just unstable. This idea, which in the absence of rotation Unno showed to reduce to both the ordinary mixing length theory under certain assumptions, and also to reproduce the analysis of Ledoux,

Schwarzschild and Spiegel<sup>7</sup> under their assumptions, gives an easy way of determining the effect of rotation on the superadiabatic gradient. The results for the onset of instability can be taken over to developed turbulence by just replacing the conductivity and viscosity by their eddy equivalents in both the Rayleigh and Taylor numbers<sup>8</sup>. Since for turbulence the eddy Prandtl number  $P=1$ , and as the turbulence has to carry the flux  $F$ , we have the relation

$$\kappa = \nu = F / (C_p \rho \beta)$$

For typical values for the solar convective zone the eddy viscosity is about  $10^{13}$ , and the corresponding Taylor number about 160. The difference between the superadiabatic gradients along the equator and pole then turns out to be  $3 \cdot 10^{-8} ^\circ \text{K/cm}$ , in satisfactory agreement with the values from Weiss's theory<sup>6</sup>.

Since the solar convective zone has a depth of  $10^{10} \text{ cm}$ , it follows that on travelling this distance up the pole the equator there will be a temperature difference of

$$\Delta T = \Delta\beta \times 10^{10} = 300^\circ \text{K}$$

the value needed to explain the difference of 30 km between the radius of the equator and pole.

An alternative way of expressing these results is to ask what distance one has to travel for the temperature to drop from its value at the base of the convective zone of  $10^6 ^\circ \text{K}$  to its surface value of  $6,000 ^\circ \text{K}$ , when the temperature gradient is steeper up the pole than along the equator by an amount of order  $3 \cdot 10^{-8} ^\circ \text{K}$ , given that the actual temperature gradient is of order  $10^{-4}$  since the temperature drops by  $10^6 ^\circ \text{K}$  in  $10^{10} \text{ cm}$ . This is just 30 km. Thus the greater superadiabatic gradient needed to transport the same flux up the pole as along the equator could well be sufficient to account for the difference in polar and equatorial radii measured by Dicke.

The combination of the arguments given above would seem to support the conclusion that the pressure and temperature are not constant over equipotential surfaces near the surface of the Sun. There is a temperature difference of the order of  $300^\circ \text{K}$  over such a surface and this necessitates a larger equatorial radius than polar radius for the temperature to drop to the same surface value. The resulting pressure difference drives circulation such that in a steady state the Coriolis force on an azimuthal velocity balances the excess pressure force and this produces the equatorial acceleration. The temperature difference is caused by the different superadiabatic temperature gradients required to transport the same flux along the poles and equator of a rotating star. If the above views are substantially correct, then this implies that the surface is not a surface of constant potential so that Dicke's value for the quadrupole moment and resulting effect on the perihelion of Mercury would be incorrect. The Sun would be slowly rotating throughout, producing no detectable effect on the motion of Mercury. On the other hand, it should be admitted that our knowledge of turbulent convection in the presence of rotation is very slight, so the effects discussed here could well be incorrect. However, the fact that the assumption of a temperature difference to explain the shape also gives the equatorial acceleration must lend some support to the views expressed in this article.

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# Random Close Packing and the Heats of Fusion of Simple Liquids

by

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Heats of fusion of inert gases can be calculated using a random close packed model of the liquid and intermolecular potential functions of the Lennard-Jones 6-12 type. Experimental agreement is improved by allowing small decreases in the nearest neighbour distances on melting. This might be expected from the fall in co-ordination, and there is experimental evidence to support it.

THEORIES of the liquid state have usually failed to predict accurately the internal energy at the melting point, and thus also the heat or entropy of fusion. This high density region is the region where simplifying approximations are least valid, although agreement with experiment may be good at low densities. Quasi-crystalline models such as that of Lennard-Jones and Devonshire<sup>1</sup> fit the solid, but fail to reproduce the internal energy in the liquid state, which suggests that such theories are rather descriptions of the solid state. Attempts to construct radial distribution functions mathematically require simplifying assumptions before a solution can be extracted. For example, the Kirkwood superposition approximation<sup>2</sup>, though satisfactory in the low density region, fails seriously for middle and high densities. The Percus-Yevick equation<sup>3</sup> is considerably better throughout, but it also fails to give accurate results at the melting point. Monte Carlo calculations<sup>4</sup> on computers seem to be the most promising approach, giving a better experimental agreement around the melting point and even suggesting the phase transition. There are, however, problems of very slow convergence at high densities, in addition to the reduction in entropy caused by using a periodic repeat cell.

In contrast, a structural approach by way of a random close packed model of the liquid state<sup>5</sup> is easy to handle in the high density region. An attempt has been made to evaluate the heats of fusion of simple liquids from this starting point with interesting and encouraging results.

The heats of fusion of the inert gases at their triple points were calculated assuming a random close packed model of the liquid state. Using a Lennard-Jones (L-J) potential function of the form

$$\phi(r) = 4\epsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6 \right] \quad (1)$$

(Fig. 1) and the random close packing data for 1,005 centres of Scott<sup>6</sup>, the "site-energies" of the twenty-five centres nearest the centre of the mass were calculated. It can be statistically demonstrated that these twenty-five energies form part of the same distribution with a 10 per cent probability. If more centres are chosen, this becomes less plausible; the site energy begins to fall off as the sample boundary approaches the centre. Thus the chosen centres can be taken to be reasonably representative of the whole mass.

A similar calculation of site energy was made for a static face centred cubic lattice.

Because of the truncation of the potential function by the boundary in both the liquid and solid cases (though the latter can be overcome by extending the lattice) a suitable correction should be made. If the density of centres at a distance  $r_0$  and beyond is assumed to be approximately constant, then for a truncation at  $r_0$  from the centre, we apply a correction

$$\Delta E = 4\pi\rho \int_{r_0}^{\infty} r^2 \phi(r) dr$$

where  $\rho$  is the average density of sites. In the liquid  $r_0$  was taken at 4.9 diameters, at which distance the oscillations in the radial distribution function had essentially ceased. For the solid case, a sphere radius  $4.5\sqrt{2}$  diameters was used for the energy calculation;  $r_0$  was also taken at this value.

To facilitate the use of different experimentally determined Lennard-Jones parameters, curves were plotted of  $\frac{\phi(r)}{4\epsilon} : \frac{\sigma}{d}$ , where  $d$  is the distance of closest approach of two atoms in a static model, and  $\frac{\phi(r)}{4\epsilon}$  is the total potential energy of a mole in units of  $4\epsilon$  using a Lennard-Jones potential function. Two curves—one for the liquid and one for the solid—sufficed for all calculations of the heats of fusion of the inert gases considered.

Neglecting any effects resulting from vacancies in either state—these are so small that they can be treated as second order corrections to be applied later—and assuming

$$d_{\text{solid}} = d_{\text{liquid}}$$

we can evaluate the heat of fusion. For

$$\Delta U = -p\Delta V + T\Delta S \quad (2)$$

where  $\Delta U$  is the internal energy change of, say, 1 mole on changing state. Now  $p\Delta V$  can be treated as a further

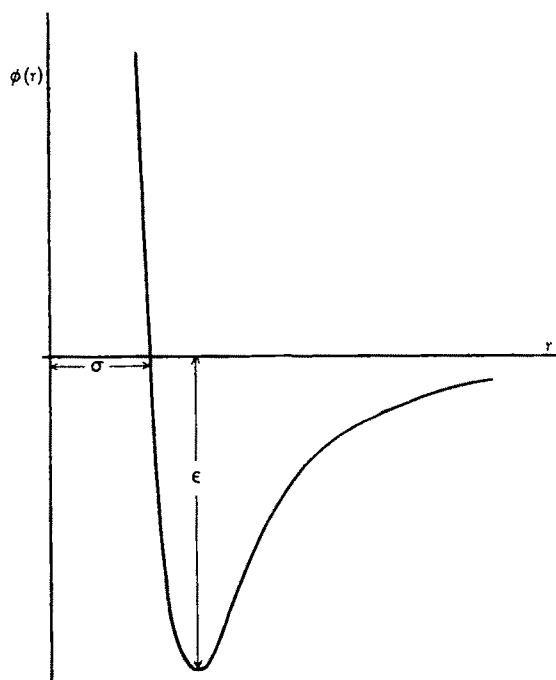


Fig. 1. A Lennard-Jones 6-12 potential function of the form of equation (1), showing the significance of the parameters  $\sigma$  and  $\epsilon$ .



Table 1. INERT GAS LENNARD-JONES PARAMETERS

	$\sigma$ (Å)	$\epsilon/k$ (°K)	Ref. letter in text	Source ref.
Neon	2.749	35.60	A	10
	2.78	34.0	B	11
	2.756	$33.74 \pm 0.18$	C	12
	2.75	$35.64$	D	13
Argon	3.405	119.8	E	14
	3.40	122	F	11
	3.405	125.2	G	15
	$3.409 \pm 0.006$	$119.5 \pm 0.3$	H	16
	3.40	122.4	J	17
Krypton	3.60	171	K	18
	3.597	158	L	19
	3.591	172.7	M	20
	3.591	182.9	N	15
	$3.679 \pm 0.008$	$166.7 \pm 0.5$	P	16
	3.65	166.4	Q	13
Xenon	4.100	221	R	18
	3.963	217	S	19
	4.064	224.5	T	18
	3.92	230	U	13
	$4.070 \pm 0.012$	$225.3 \pm 1.1$	V	16

second order correction at the relevant pressures (all less than 1 atmosphere). Thus the heat of fusion

$$\Delta Q = \Delta U$$

For argon, using the Lennard-Jones parameters (E) (see Table 1), the calculated  $\Delta Q$  value rose from  $(1.097 \pm 0.026)$  kJ mole<sup>-1</sup> for  $d = 3.75$  Å to  $(1.284 \pm 0.030)$  kJ mole<sup>-1</sup> for  $d = 3.94$  Å giving the experimental value of  $1.176$  kJ mole<sup>-1</sup> (ref. 7) at  $d = 3.80$  Å. Now the experimental value of  $d$  is  $3.87$  Å (refs. 8 and 9), at which value  $\Delta Q = (1.251 \pm 0.029)$  kJ mole<sup>-1</sup>. This is 6 per cent too high, a discrepancy not accounted for by the expected error. The implication is a change in the distance of approach of two nearest neighbours, that is, in  $d$ . Such a fall might indeed be expected, as the co-ordination falls on melting.

This postulated change in  $d$  was calculated by taking a value of  $\Delta U$  corresponding to the experimental  $\Delta Q$  value, and reading off from the graph the value of  $d_{\text{liquid}}$  corresponding to a given  $d_{\text{solid}}$ . The error limits on the liquid curve facilitated an estimation of the error in  $d_1$ . The graphical work necessary is shown in Fig. 2.

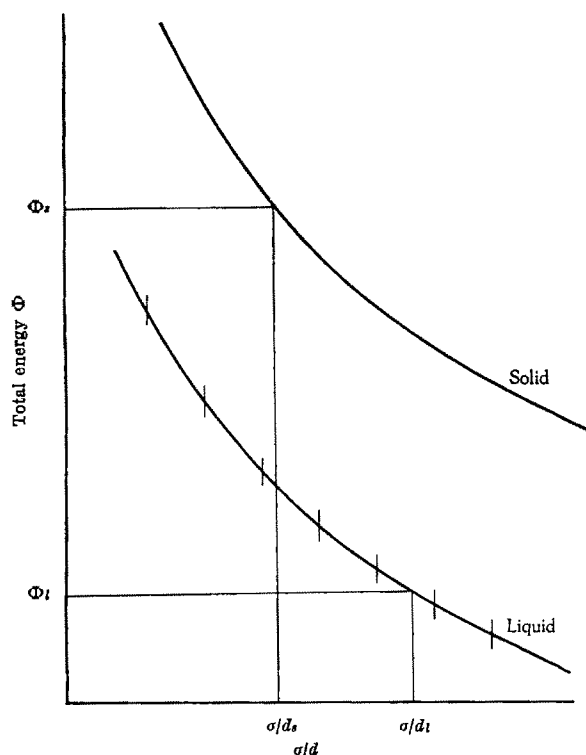


Fig. 2. Schematic representation of the graphical work. Using a chosen L-J parameter  $\sigma$ , and  $d$  value for the solid,  $\Phi_s$ —the energy of a given mass of solid—is read off. Knowing the heat of fusion, we calculate the expected value of  $\Phi_l$  for the same mass of liquid. Hence  $\sigma/d_l$  can be read off from the liquid curve. The error limits on the liquid curve arise from variations in energy of the twenty-five chosen centres and provide an estimate of the possible error in  $d_1$ .

The results of the calculations, using the Lennard-Jones parameters tabulated in Table 1, are set out in Table 2. Postulated  $d_1$  values have been corrected for neglecting the  $p\Delta V$  term in equation (2) and also for the presence of holes in thermodynamic equilibrium in both the liquid and solid states. These corrections together amount to less than  $0.001$  Å, and are really insignificant at this level of accuracy. In some cases, experimental data conflict. Where a value of  $d$  is obtained indirectly, the way in which it is reached is indicated at the foot of the table.

**Neon.** The predicted  $d_1$  value is slightly larger than suggested by experiment, though the order of magnitude is the same. The two extreme sets of Lennard-Jones parameters give a variation in  $d_1$  over  $0.01$  Å. Averaging over the set for  $d_s = 3.20$  Å, we obtain

$$d_1 = (3.18_s \pm 0.00_s) \text{ Å}$$

compared with the experimental  $3.17$  Å.

**Argon.** The second and third sets of data give the best agreement with experiment. The first set of values for  $d_s$  appears, however, to be more reliable, and here the predicted  $d_1$  is slightly lower than found experimentally. Averaging over the five different sets of Lennard-Jones parameters

$$d_1 = (3.84_s \pm 0.01_s) \text{ Å}$$

compared with the experimental  $3.86$  Å. It is worth noting as a general point that parameter sets H and J are given with a quoted error, which inflates the estimated error in  $d_1$ : this would imply all other quoted errors are underestimated.

**Krypton and xenon.** With the heavier noble gases, we are working very close indeed to the bottom of the potential well in  $\phi(r)$  and thus we would expect the calculations to be more sensitive to changes in Lennard-Jones parameters. Moreover, as reference to Table 1 shows, different workers have obtained very different values for these

Table 2 (a). SUMMARY OF NEON DATA

$$\Delta Q = 0.335 \pm 0.002 \text{ kJ mole}^{-1} \text{ (ref. 7)}$$

$$T_f = 24.55^\circ \text{ K (ref. 7)}$$

$$P_f = 325 \text{ mm (ref. 21)}$$

$$\text{Atomic weight} = 20.183$$

$d_s$ (expt.) (Å)	Ref.	L-J ref.	$d_1$ (theory) (Å)	$d_1$ (expt.) (Å)	Ref.	Notes
3.207	9	A	$3.18_s \pm 0.00_s$	3.17	22	(1)
		B	$3.18_s \pm 0.00_s$			
		C	$3.19_s \pm 0.00_s$			
		D	$3.18_s \pm 0.00_s$			
3.202	7	A	$3.17_s \pm 0.00_s$	3.17	22	(2)
		B	$3.18_s \pm 0.00_s$			
		C	$3.18_s \pm 0.00_s$			
		D	$3.17_s \pm 0.00_s$			

Notes: (1)  $d_s$  calculated from density. (2)  $d_s$  calculated from density using the earlier value of volume change on melting.

If we extrapolate the value of  $d_s$  at  $4.2^\circ \text{ K}$  quoted in ref. 22 assuming an expansion similar to solid argon, we find  $d_s \sim 3.21$  Å, which gives support to the values taken.

Table 2 (b). SUMMARY OF ARGON DATA

$$\Delta Q = 1.176 \pm 0.001 \text{ kJ mole}^{-1} \text{ (ref. 7) (see note (1))}$$

$$T_f = 83.78^\circ \text{ K (ref. 9)}$$

$$P_f = 516 \text{ mm (ref. 7)}$$

$$\text{Atomic weight} = 39.944$$

$d_s$ (expt.) (Å)	Ref.	L-J ref.	$d_1$ (theory) (Å)	$d_1$ (expt.) (Å)	Ref.	Notes
3.868	9	E	$3.85_s \pm 0.00_s$	3.86	23	(2-4)
3.868	8	F	$3.84_s \pm 0.00_s$			
3.87	23	G	$3.84_s \pm 0.00_s$			
		H	$3.85_s \pm 0.00_s$			
		J	$3.84_s \pm 0.00_s$			
3.82	24	E	$3.81_s \pm 0.00_s$	3.80	24	(6)
3.82	25	F	$3.80_s \pm 0.00_s$	3.79	25	(6)
		G	$3.80_s \pm 0.00_s$			
		H	$3.81_s \pm 0.00_s$			
3.878	26	J	$3.80_s \pm 0.00_s$	3.86	23	(3)
		E	$3.86_s \pm 0.00_s$			
		F	$3.85_s \pm 0.00_s$			
		G	$3.85_s \pm 0.00_s$			
		H	$3.86_s \pm 0.01_s$			
		J	$3.85_s \pm 0.01_s$			

Notes: (1) This value is taken in preference to that of Eucken<sup>27</sup> ( $1.122$  kJ mole<sup>-1</sup>). It appears to be generally accepted as the most reliable (see ref. 28). (2) From density, which is measured by X-ray and bulk density methods. (3)  $d_s$  calculated from density. (4)  $d_1$  values for this set are calculated taking  $d_s = 3.868$  Å. (5) By neutron diffraction. (6)  $d_1$  is merely quoted.

Table 2 (c). SUMMARY OF KRYPTON DATA

$\Delta Q = 1.636 \pm 0.001$  kJ mole<sup>-1</sup> (ref. 7)  
 $T_l = 115.95^\circ$  K (ref. 9)  
 $P_l = 648$  mm (ref. 7)  
 Atomic weight = 83.80

$d_s$ (expt.) (Å)	Ref.	L-J ref.	$d_l$ (theory) (Å)	$\bar{d}_l$ (expt.) (Å)	Ref.	Note
4.11 <sub>6</sub>	9	K	4.09 <sub>2</sub> ± 0.00 <sub>7</sub>			(1)
		L	4.11 <sub>0</sub> ± 0.00 <sub>7</sub>			
		M	4.08 <sub>9</sub> ± 0.00 <sub>7</sub>			
		N	4.07 <sub>8</sub> ± 0.00 <sub>7</sub>			
		P	4.11 <sub>0</sub> ± 0.00 <sub>8</sub>			
		Q	4.10 <sub>2</sub> ± 0.00 <sub>7</sub>	4.02	29	

Note: (1)  $d_s$  calculated from density.

Table 2 (d). SUMMARY OF XENON DATA

$\Delta Q = 2.295 \pm 0.002$  kJ mole<sup>-1</sup> (ref. 30)  
 $T_l = 161.3^\circ$  K (ref. 30)  
 $P_l = 612$  mm (ref. 30)  
 Atomic weight = 131.3

$d_s$ (expt.) (Å)	Ref.	L-J ref.	$d_l$ (theory) (Å)	$\bar{d}_l$ (expt.) (Å)	Ref.	Notes
4.433	30	R	*			(1)
		S	4.44 <sub>2</sub> ± 0.00 <sub>2</sub>			
		T	4.51 <sub>8</sub> ± 0.00 <sub>2</sub>			
		U	4.42 <sub>2</sub> ± 0.00 <sub>2</sub>			
		V	4.52 <sub>2</sub> ± 0.01 <sub>1</sub>			
4.443	30 (8)	R	*			(2)
		S	4.45 <sub>2</sub> ± 0.00 <sub>2</sub>			
		T	4.51 <sub>8</sub> ± 0.00 <sub>2</sub>			
		U	4.43 <sub>1</sub> ± 0.00 <sub>2</sub>			
		V	4.52 <sub>2</sub> ± 0.01 <sub>2</sub>			
				4.43	31	(3)

Notes: (1)  $d_s$  calculated from density. (2)  $d_s$  calculated from density, using the volume change on melting in ref. 8. (3) X-ray measurement at 163° K (compare  $T_l = 161.3^\circ$  K) implying true  $\bar{d}_l$  slightly lower.  
 \* It is impossible to obtain a fit for this function.

parameters, giving a confused picture. The amount of experimental evidence on neighbour distances is also very sparse, which suggests that caution should be applied in treating the results until more independent measurements are available.

For krypton, the predicted  $d_l$  varies from 4.11<sub>6</sub> to 4.07<sub>8</sub>. This change is in the right direction, but of magnitude insufficient in the light of the experimental 4.02 Å. Error in the parameters is quoted only for set P, implying, as mentioned in the case of argon, that quoted errors in  $d_l$  are underestimated. Averaging over all six sets, for what it is worth,  $\bar{d}_l = (4.09_7 \pm 0.01_6)$  Å.

The situation with xenon is even more uncertain. One set of  $\phi(r)$  parameters makes a fit impossible, while sets S, T and V suggest an increase in  $d$  on melting. Set U predicts a small fall, though this is perhaps one of the less reliable Lennard-Jones functions. The error for set V contains an error quoted for the parameters, again suggesting an underestimate for the others. Experiment suggests a small fall, or little change in  $d$ . The average theoretical  $d_l$  is

$$(4.48_2 \pm 0.04_2) \text{ Å}$$

compared with the experimental value of about 4.43 Å.

In both these cases, the sensitivity to the potential function and the limited amount of experimental data leave an inconclusive picture.

Uncertainty in the density of the random model thwarts any attempt to calculate the volume change on melting, taking the change in  $d$  into account. In order to obtain accurately the average packing density and local density fluctuations, the concept of the Voronoi polyhedron was used<sup>32</sup>. If we draw planes perpendicularly bisecting imaginary straight lines between a centre and its neighbours, and take the smallest closed polyhedron thus obtained (ensuring no part of the polyhedron can be intersected by continuing this process with more distant centres), we will have selected a volume in which all points within the polyhedron are closer to the central point than to any other. This polyhedron is the Voronoi polyhedron associated with the central point: for simplicity, the construction of its two dimensional counterpart is shown in Fig. 3. The average density of the first

four hundred centres thus calculated is 63.48 per cent, while that of the central region—of greater relevance to these "liquid site energy" calculations—is 64.32 per cent. There is still great uncertainty about the maximum density of random packing, a limit which some of our recent work suggests to be statistical rather than absolute; for the densities of the polyhedra are distributed throughout the range from 57 per cent to 70 per cent, with no obvious peak or structural significance attached to the overall average. In such circumstances, a really accurate calculation of  $\frac{\Delta V}{V}$  is not possible. A larger sample may

well improve the situation, when an extension to systems with different density changes on melting—including those which contract—may show interesting results.

The transition from hard spheres to a more realistic potential function attempted here is admittedly rather crude.  $d$  has been taken as the closest approach distance; in fact, this should be modified to take account of thermal fluctuations which would allow molecules to approach closer than this. The kinetic energy leading to such close approaches would, however, be the same in the solid as the liquid, though it might be argued that the generally stronger repulsive fields in the solid resulting from a higher packing density would give a higher proportion of "squashed" molecules in the liquid. This would lead to a slight lowering of the "liquid site energy", giving a greater fall in  $d$  on changing state—required for neon, krypton and xenon, but not argon. In connexion with this, it is worth noting that errors in measuring a hard sphere array do give some centres closer together than  $d$ , thus allowing for a small amount of squashing. Working along these lines it may even be possible to adjust the error function to represent that part of the potential function for which  $r < d$ .

With more data on random packing at different densities, and a simulation of thermal motions in both the liquid and solid states, it may be possible to investigate more fully some of these factors, and to fit accurately the density changes. Without more and accurate data on experimental approach distances and interatomic potential functions, however, this would scarcely at present seem justified.

Probably the most doubtful assumption made here is that of taking the twenty-five chosen liquid centres as a reasonable sampling of the random model. The variation

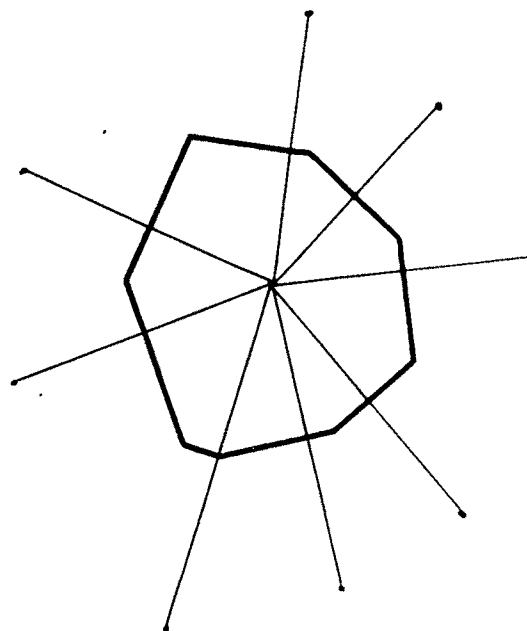


Fig. 3. Construction of a Voronoi polygon.

in "site energy" is perhaps sufficiently large ( $\sim \pm 5$  per cent) to invalidate the concept of an "average" in such a homogeneously irregular array. To allow for this, we could either estimate the reduction in entropy expected from repeating such arrays (which requires assuming no repeating in the real case, which may be a doubtful premise), or use a series of progressively larger samples to see how the entropy of mixing of such samples would change with size. The large variations in the local density of the model suggest the need for a much larger sample to increase the reliability of these calculations: we shall measure such a model ( $\sim 3,000$  centres) in the near future.

In view of the connexion between the change in nearest neighbour distance on fusion and (a) the radial distribution function, and (b) the co-ordination number, this approach by way of the heat of fusion may be a duplicative path. It is, however, more straightforward, less ambiguous and, we suspect, considerably more sensitive to the structural arrangement. We have not attempted to consider the possibility of non-uniqueness of a given "site-energy" with respect to different arrangements: what has been attempted here merely shows how a random model of the liquid state may fit. Moreover, the fact that realistic results can be obtained from an initially apparently unrealistic hard sphere array is very encouraging and worth emphasizing. An extension of the argument to simple metals will provide a more rigorous experimental comparison. In principle we could extend it even further to polyatomic molecules, with directed bonds, though lack of precision in our knowledge of the forces involved would probably make it quantitatively impossible to handle in practice.

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## Aliphatic Hydrocarbons in Pre-Cambrian Rocks

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Analyses of sedimentary rocks from the Pre-Cambrian of South Africa show that they contain a variety of alkanes. Were these hydrocarbons incorporated into the sediments at the time of sedimentary deposition, and are they biogenic in origin?

ALKANES of high molecular weight have been found in shale  $1 \times 10^9$  yr old from the Nonesuch formation<sup>1,2</sup>, chert  $2 \times 10^9$  yr old from the Gunflint iron formation of Ontario, Canada<sup>3</sup>, and rocks (carbonaceous lenses and graphitic silicates)  $2.7 \times 10^9$  yr old from the Soudan iron formation of Minnesota<sup>4-6</sup>. These alkanes include the saturated isoprenoid hydrocarbons, pristane and phytane. The biogenic origin of the organic materials in the Nonesuch shale and the Gunflint chert has been assigned on the basis of fossil micro-organisms contained in them<sup>7</sup> and the hydrocarbons were considered to be remnants of this early life.

We have analysed sedimentary rocks  $3.2 \times 10^9$  yr old from the Fig Tree series of the Swaziland system, eastern Transvaal, South Africa, for aliphatic hydrocarbons, using techniques described in detail elsewhere<sup>8,9</sup>. The carbonaceous silicate rocks from the Fig Tree series were collected by T. C. Hoering in the Sheba gold mine near Barberton, South Africa, and by E. S. Barghoorn in an outcrop near the Daylight mine, 28 km E.N.E. of Barberton. The rocks were black chert (sample No. 1) and a carbonaceous graphitic silicate (sample No. 2) collected by Hoering, and black chert (sample No. 3) collected by

Barghoorn. In addition, an extract obtained at the Geophysical Laboratory of the Carnegie Institute of Washington by Hoering from a core specimen of shale (sample No. 4) was analysed.

The procedure used with samples 1, 2 and 3 was to pulverize the rocks (after surface contaminants had been removed) in a Carver press test cylinder, and extract the organic compounds with benzene-methanol (3:1) in a glass 'Soxhlet'-type extractor. The extract supplied by Hoering was obtained from sample No. 4 by extracting 300-400 g of the crushed shale (core sample) in an ultrasonic generator with *n*-pentane. The solvent extracts were then fractionated on silica gel. The procedure of Meinschein and Kenny<sup>9</sup> was followed except that the aliphatic hydrocarbons were eluted with *n*-pentane in the case of sample No. 4. By carefully blowing the low boiling solvent (*n*-pentane) to a very small volume, many of the relatively low molecular weight hydrocarbons, ordinarily lost by evaporation to dryness when *n*-heptane is used, were recovered.

The eluates were dissolved in benzene and suitable aliquots taken for analysis by gas chromatography and combined gas chromatography-mass spectrometry<sup>10</sup>. For

the gas chromatographic analyses tentative identification of the separated compounds was obtained by (1) coinjection of pristane, phytane, *n*-octadecane, and *n*-octadecene-1 with the samples (samples 1 and 4) on capillaries coated with 'Polysev' (*m*-bis-*m*-(phenoxyphenoxy)-phenoxybenzene); and (2) comparison of the retention times of the compounds in the samples (samples 1-4) with those of appropriate hydrocarbon standards (normal alkanes, pristane, phytane, *n*-hexadecene-1, *n*-octadecene-1, *n*-eicosene-1, and *n*-tricosene-1) chromatographed on the same columns ('Polysev') and under the same conditions. For the graphitic-like silicate (sample No. 2), in addition to the comparison of retention times of eluted components and standards (normal hydrocarbons, pristane and phytane) made with 'Polysev' as the liquid phase, similar comparisons were made using a 15 m packed column with 'SE-30' as the liquid phase.

Mass spectrometric identifications (samples 1 and 4) were made by taking individual mass spectra of the individual components as they were eluted from the 'Polysev' coated capillary<sup>11</sup>. These spectra were compared with spectra of the standards, which were treated in the same manner. Because we did not have a standard, the assignment of norpristane was made by comparing our mass spectrum with available C<sub>18</sub> isoprenoid mass spectra<sup>2</sup>.

Fig. 1 shows the chromatograms obtained using 'Polysev' as the liquid phase in stainless steel capillary columns. The hydrocarbons shown in Fig. 1A were obtained from the internal part of a chert sample (sample No. 1), which was treated with chromic acid cleaning solution before crushing and extracting. Normal alkanes ranging from C<sub>16</sub> through C<sub>26</sub> were found to be present. The peaks designated as *a* and *b* correspond by their chromatographic position to pristane and phytane, respectively. The peak immediately following *n*-C<sub>18</sub> contains Δ-C<sub>18</sub>, but probably also contains norpristane. The shape of the peak clearly shows that it represents at least two compounds. The peaks immediately following *n*-C<sub>18</sub>, *n*-C<sub>20</sub> and *n*-C<sub>22</sub> correspond to the normal C<sub>18</sub>, C<sub>20</sub> and C<sub>22</sub> monoenes, respectively. The retention times do not correspond to the respective *n*-alkylcyclohexanes. The retention times of these cyclic alkanes (on 'Polysev') almost coincide with those of normal alkanes having one more carbon. As indicated, the identification of the olefines was made on the basis of both mass spectrometric analysis and retention times of known hydrocarbons (normal C<sub>16</sub>, C<sub>18</sub>, C<sub>20</sub> and C<sub>22</sub> monoenes). The gas chromatographic analysis of this sample made on the LKB '9000' instrument is shown in Fig. 1E.

A second analytical determination of alkanes was carried out with small pieces (inside and outside) of the same specimen of chert. The decontamination of possible surface contaminants was performed this time not by treating with chromic acid, but by carefully extracting the rock surface with organic solvent (benzene-methanol, 3:1). After extraction the pieces were then dried, crushed, extracted and the extract analysed.

The chromatographic pattern of the paraffinic hydrocarbons recovered from the sample is shown in Fig. 1C. As shown, the chromatographic pattern is the same as that of Fig. 1A. Approximately the same level of hydrocarbons (0.02 p.p.m.) was found in sample No. 1 by the two separate and slightly different analytical determinations. The mass spectrometric results of the second determination were essentially as obtained for the first. Furthermore, in the second analysis, two mass spectra were taken of the peak immediately following *n*-C<sub>16</sub>, one on the ascending side of the peak before inflexion and the other on the descending side. The components of this peak were found, in fact, to be norpristane and C<sub>16</sub> monoene as indicated by the first analysis. The mass spectra of the isoprenoid hydrocarbons of this sample are presented in Fig. 2. They are essentially identical to those given by standards. The controls or procedural

blanks (glass was carried through all analytical steps including pulverization in the Carver press associated with the particular samples) are shown in Fig. 1B and 1D. As seen, the alkane contribution of the blanks amounts to less than 1 per cent and 5 per cent, respectively, of the amounts found in the actual samples.

Sample No. 2 (graphitic-like silicate obtained from Hoering) and sample No. 3 (black chert obtained from Barghoorn) were boiled in benzene-methanol (3:1) to remove any possible surface contamination. The *n*-heptane eluates from these samples were analysed on a 305 m long × 0.76 mm inside diameter stainless steel capillary coated with 'Polysev'. Extremely low levels of hydrocarbons (approximately 0.003 p.p.m.) were obtained. Small peaks representing hydrocarbons in the C<sub>12</sub>-C<sub>18</sub> range were obtained. Peaks having the retention time of pristane and phytane standards were detected in the graphitic-like silicate, but not in the chert. The much lower yields obtained with these two samples show that significant variations in the hydrocarbon content of these rocks exist. In general, variations in hydrocarbon content can be expected from different samples of the same sediment. Significant variations have been observed in our laboratory in rocks from the Gunflint iron formation of southern Ontario. The existence of such rocks deficient in hydrocarbons makes it possible to run ideal analytical controls for samples from the same formation which may be richer in hydrocarbons. The heterogeneity of quanti-

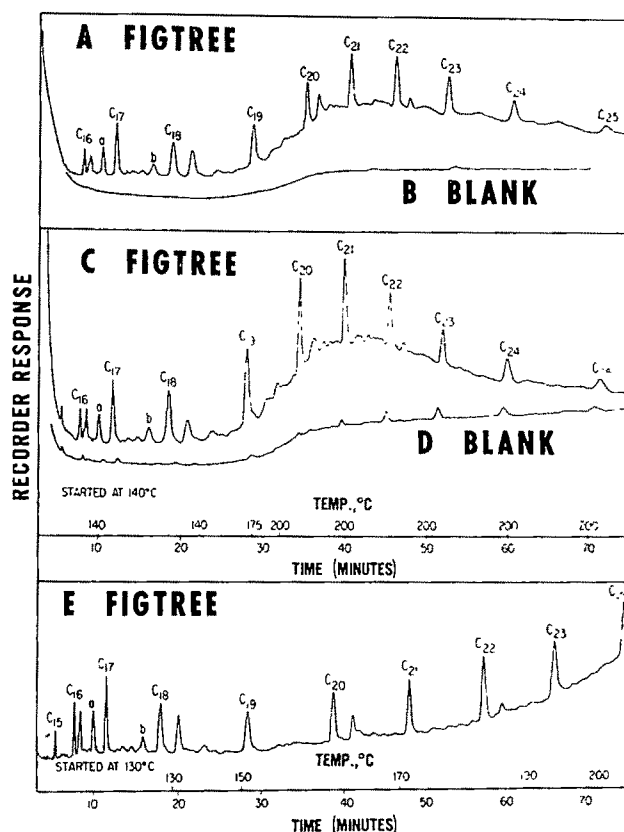


Fig. 1. Gas chromatographic separation of alkanes from the Fig Tree chert with capillary columns ('Polysev'). A, Stainless steel tubing 91.5 m × 0.076 mm, coated with 'Polysev'. F and M model 810 gas chromatograph was equipped with a flame ionization detector. Nitrogen pressure, 1.050 g/cm<sup>2</sup>. No split. Chert extracted (pulverized inside piece), 100.5 g. About half of the *n*-heptane eluate was injected. Range 10; attenuation, 4. Isothermal at 140°C for 22 min, then raised to 200°C at approximately 6°C/min. B, Blank (pulverized glass was substituted for the sample), treated in the same way as the sample. C, Analytical equipment and conditions are the same as for A. Chert extracted (pulverized piece which included some outside material cleaned with solvent), 97.9 g. About half of the *n*-heptane eluate was injected. D, Blank (pulverized glass substituted for sample), treated as sample. E, Stainless steel tubing, 75 m × 0.76 mm, coated with 'Polysev'. LKB '9000' gas chromatograph-mass spectrometer combination. Helium flow, 24 ml./min. No split. Same sample and eluate as in A. About a third of the eluate was injected. Gain, 6. Isothermal at 130°C for 18 min, then raised to 200°C at about 1.5°C/min.

tative distribution of hydrocarbons may be the result of differences in the concentration or composition of organic matter or in the thermal metamorphism of the rocks analysed. This heterogeneity may be taken as an indication that the hydrocarbons were not incorporated into the rocks by a general process of petroleum migration in more recent times.

In order to obtain additional data on Fig Tree Pre-Cambrian sediments, a core of the Fig Tree shale (sample No. 4) was extracted (see previously) more recently at the Geophysical Laboratory of the Carnegie Institute of Washington by Dr. T. C. Hoering, and the resulting hydrocarbons were analysed at Houston. The results obtained were similar to those of Fig. 1 except that more of the low molecular weight alkanes and less of the higher molecular weight paraffins were detected. This is indeed what would be expected by the use of *n*-pentane as a solvent instead of *n*-heptane. The amounts of hydrocarbons were of the order of 0.15 p.p.m.

Aliphatic hydrocarbons are present in the Fig Tree rocks at extremely low levels (0.003–0.15 p.p.m.). Sufficient data are not available to ascertain whether or not the hydrocarbons date from deposition. The treatment of the samples, however, precludes the hydrocarbons being merely surface contaminants. This conclusion is in line with the fact that the  $^{13}\text{C}/^{12}\text{C}$  ratios of the extractable and insoluble organic matter of the Fig Tree rocks are essentially identical<sup>12</sup>. Hoering's comments<sup>12</sup> on the discrepancies for this correlation that occur for several Pre-Cambrian samples, however, indicate that more work on carbon isotope distribution needs to be done before the actual significance of  $^{13}\text{C}/^{12}\text{C}$  ratios can be ascertained. Entities have been found in the Fig Tree shale that have the morphology of fossil bacteria and possibly of algae<sup>13</sup>, and these hydrocarbons may be remains from this early life.

The occurrence of olefines in some samples of Fig Tree chert is somewhat unexpected. Although olefines have been observed in Pennsylvania crude<sup>14</sup>, in general they

are relatively rare in petroleum crudes and sediments. Their presence in the cherty Pre-Cambrian sediment is not easily understood unless one assumes that the olefines can be preserved in the chert matrix for long periods of time or that they may be products of the thermal degradation of biological compounds such as even carbon-numbered alcohols.

The relatively large amounts of pristane found in the sediment may be taken as an indication that the isoprenoid hydrocarbons are probably biogenic. It has been demonstrated, however, that 2-methylbutane (isoprane) and other branched hydrocarbons can be synthesized by Fischer-Tropsch processes<sup>15,16</sup>. Thus it is not impossible that certain isoprenoid hydrocarbons could also be formed abiotically. More work needs to be done on this type of synthesis before advancing any speculations.

To our knowledge no assignment of configuration to the asymmetric centres of fossil pristane and phytane has been made. With the further development of some recent techniques using optically active phases<sup>17</sup> it may eventually be possible to separate the stereoisomers (for example, enantiomers, diastereomers) of pristane and phytane. Then the aforementioned assignments of configuration would be feasible and it might be possible to differentiate between abiologically and biologically derived isoprenoids. Although we do not know the isomeric forms of the isoprenoid structures examined, we do not believe, on the basis of the gas chromatographic and mass spectrometric data, the compounds to be isomers in which methyl substituents are displaced from the positions found in pristane and phytane. Nevertheless, it is possible that our methods might not detect a shift in position of only one methyl group.

After completing this work, we have learned that isoprenoid and paraffinic hydrocarbons have also been detected by other workers in samples of the Fig Tree chert which had apparently undergone a minimum amount of thermal metamorphism (Meinschein, W. G., personal

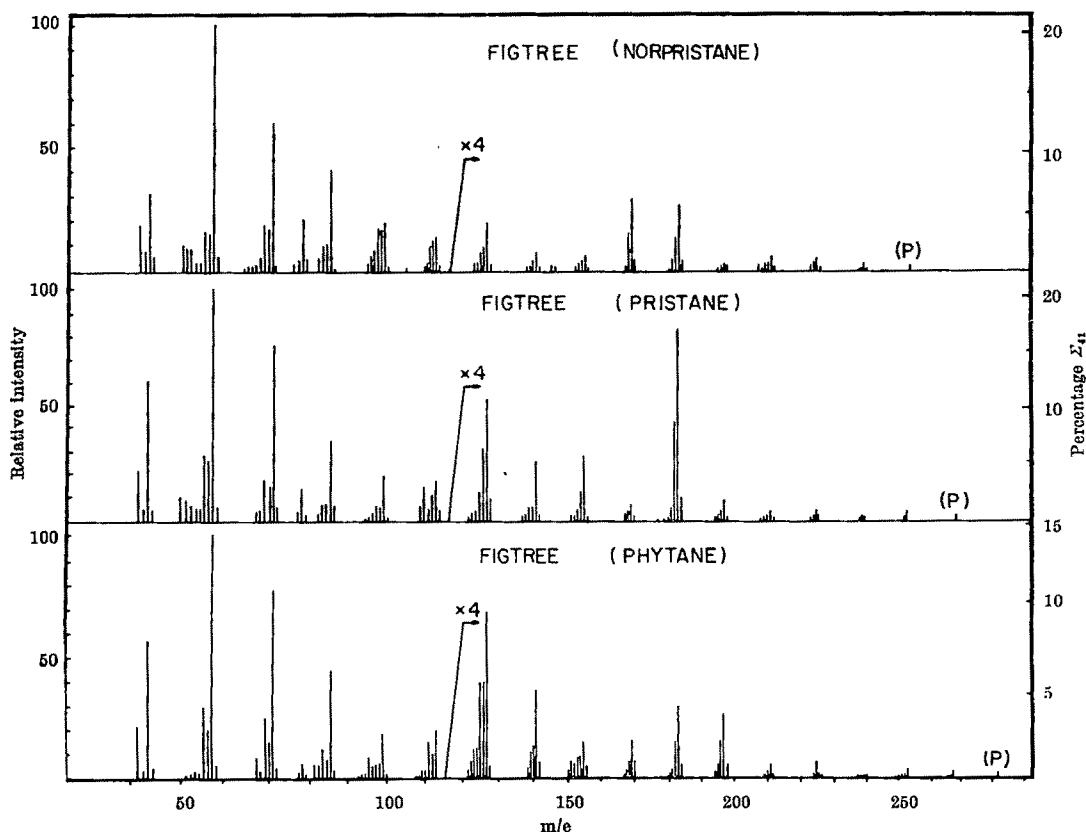


Fig. 2. Mass spectra of saturated isoprenoid hydrocarbons from the Fig Tree chert sample extracted in Houston. Obtained using an LKB '9000' gas chromatograph-mass spectrometer combination.



communication) and in similar rocks which presumably were more metamorphosed<sup>12</sup>. The organic geochemistry of Pre-Cambrian sediments and the problems involved in assessing the role of contamination and metamorphism, and determining whether the hydrocarbons date from sediment deposition or are the result of later infiltrations into the rock, have been recently discussed in some detail by Hoering<sup>13</sup>.

Because of the compact nature of some of these rocks, especially the chert, and the fact that internal samples were analysed in most cases, it is probable that the hydrocarbons are indigenous to the rock. The fact that microfossils have been found in some of these rocks would suggest a biochemical origin for these hydrocarbons which took place about  $3.2 \times 10^9$  yr ago. Additional research will have to be carried out, however, in order to decide in a more definite way whether these hydrocarbons date from sediment deposition and whether they are biogenic or not.

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## Aliphatic Hydrocarbons in Meteorites

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Naturally occurring mixtures of terrestrial isoprenoids and other aliphatic hydrocarbons give the same chromatographic patterns as aliphatic hydrocarbons from carbonaceous chondrites; carbonaceous chondrites Wiik Types II and III show substantially higher contents of aliphatic hydrocarbons than meteoritic graphite nodules and Type I carbonaceous chondrites. Any theory of the origin of hydrocarbons in meteorites must explain these features.

BEFORE the development of suitable analytical techniques for the analysis of trace quantities of organic compounds, a number of investigators of meteorites, including Wöhler, Meunier, Roscoe, Tschermak, Friedheim and Berthelot, reported indication of hydrocarbons in various carbonaceous meteorites<sup>1</sup>. Following Mueller<sup>2</sup>, who in 1953 examined the Cold Bokkeveld meteorite and reported the presence of extractable organic material, several investigators have found traces of organic compounds, particularly hydrocarbons, in carbonaceous and other chondrites<sup>3-9</sup>. These hydrocarbons have been regarded as being remnants of extraterrestrial life<sup>4,5</sup> or products of abiotic synthesis<sup>7,9</sup>. Terrestrial contamination has been considered a possible source for some of the organic compounds<sup>10</sup> but has largely been ruled out for the hydrocarbons<sup>4,7</sup>.

The work reported here was carried out to provide a comparative study on the qualitative and quantitative distribution of isoprenoid and other aliphatic hydrocarbons in carbonaceous chondrites

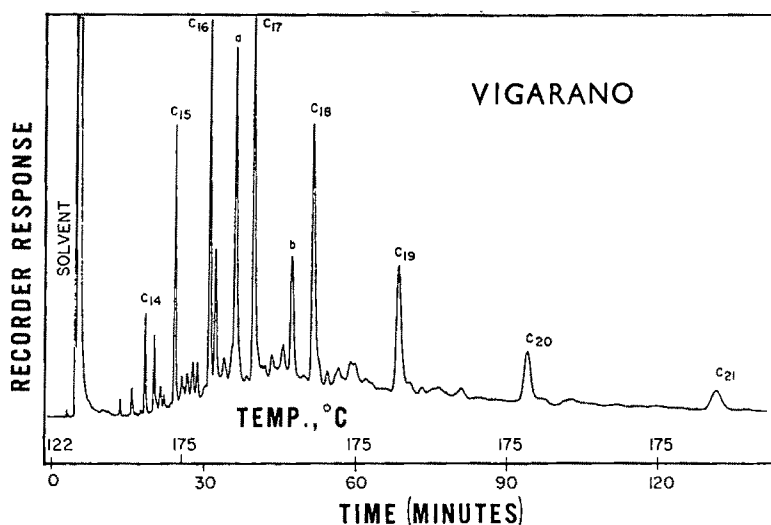


Fig. 1. Example of the gas chromatographic separation of aliphatic hydrocarbons from meteorites. Stainless steel tubing, 152.5 m long  $\times$  0.076 cm inside diameter, coated with 'Polyseal'. 'F' and 'M' Model 810 gas chromatograph equipped with a flame ionization detector. Nitrogen pressure, 1.050 g/cm<sup>2</sup>. No split. Meteorite extracted (Vigarano sample from Nagy), 0.5 g. About 1/5 of the *n*-heptane eluate was injected. Range 10°; attenuation, 2. Programmed from 122° to 175° C at approximately 2.0° C/min. a, Pristane; b, phytane.

and related meteorites and to ascertain, if possible, the origin of these hydrocarbons.

The meteorites (0.2 to 8.6 g) were pulverized to approximately 100 mesh, placed in an all-glass Soxhlet-type extractor and extracted with 50 ml. of a benzene-methanol (3:1) mixture for 8 h. After careful evaporation, the extracts were fractionated by silica gel chromatography, eluting successively with 15 ml. of each of three solvents: *n*-heptane, benzene and methanol. The residues from the *n*-heptane eluates, which usually contained less than 0.1 mg hydrocarbon, were dissolved in a few microlitres of benzene and suitable aliquots (about 1  $\mu$ l.) were taken for analyses.

The gas chromatographic analysis was carried out using long (100 to 1,000 ft.) stainless steel capillary columns coated with different stationary phases, such as 'Apiezon L', 'Polysev', 'Igepal Co-880' and 'SF-96', and other phases specifically suitable for the separation of normal and isomeric aliphatic hydrocarbons. Efficiencies of more than 300,000 theoretical plates were obtained with some of the long columns. 'Barber-Colman Series 5000' and '5320' and 'F and M Model 810' gas chromatographs with flame detectors were used for this purpose. The combined gas chromatographic-mass spectrometric analysis was carried out using 250 ft. long capillary columns, and mainly an LKB '9000' gas chromatograph-mass spectrometer. The mass spectra of the hydrocarbons were obtained at 70 eV as they emerged from the column. Other details of the analytical methods have been described previously<sup>8</sup>.

The meteorites examined belong to the following five groups: non-carbonaceous chondrites; graphite nodules from iron meteorites; and Wiik Types I, II and III carbonaceous chondrites.

The results of this study are recorded in a number of gas chromatograms and mass spectrograms and they are summarized in Table 1. As shown in Table 1, isoprenoid and other aliphatic hydrocarbons were found in varying amounts in practically all the meteorites investigated. The identification of isoprenoid hydrocarbons was carried out by combined gas chromatography-mass spectrometry for the Boriskino, Mighei, Murray, Santa Cruz, Mokoia and Odessa (graphite nodule) meteorites. The identification of these hydrocarbons in the other meteorites was carried out by gas chromatography by comparing their retention times with those of hydrocarbon standards in at least three different columns. See Fig. 1 for an example of a gas chromatographic separation on 'Polysev'.

It will be observed that the concentration of aliphatic hydrocarbons in the graphite nodules is comparable with that of Type I carbonaceous chondrites. Also, the amounts in Type II are comparable with those in Type III carbonaceous chondrites, particularly if the results from the Grosnaja meteorite are excluded. This meteorite contains a large amount of alkanes (415 p.p.m.) resembling industrial paraffin waxes. Thus taking average values, three major concentrations of hydrocarbons can be distinguished which divide meteorites into the following groups: (a) non-carbonaceous chondrites; (b) graphite nodules and Type I carbonaceous chondrites; and (c) Types II and III carbonaceous chondrites. The approximate average values for the isoprenoid and total (isoprenoid + normal) aliphatic hydrocarbons for these three levels are respectively: (a) 0.06 and 1.8 p.p.m.; (b) 0.6 and 8 p.p.m.; and (c) 9 and 60 p.p.m. Thus in going from one of these levels to the next, the isoprenoids increase approximately by a factor of 10-15 and the total (isoprenoid + normal) aliphatic hydrocarbons by a factor of 4-7.

In the case of the graphite nodules the external portions had about five times more hydrocarbons than the internal portions, indicating that the hydrocarbons were acquired from the outside or had been destroyed in the inside. Similar results were obtained with inside and outside samples of the Orgueil meteorite. Significant variations in

the qualitative and quantitative distribution of hydrocarbons for different meteorites and for different samples of the same meteorite were found. Almost all the carbonaceous chondrites which belonged to Wiik Type II, however, gave essentially the same qualitative gas chromatographic pattern although with quantitative variations. Whereas Type I carbonaceous chondrites appeared to contain very small amounts of isoprenoid hydrocarbons, Type II carbonaceous chondrites showed generally high levels. For example, in the Boriskino, Mighei and Santa Cruz meteorites, amounts as high as 20-30 per cent of the total hydrocarbon content were observed. The major isoprenoid compound found in essentially all cases was pristane. No pristane or phytane was found in the Ornans (Type III) and the amounts in the Orgueil (Type I) and in the non-carbonaceous chondrites were almost negligible.

No predominance of hydrocarbons with an odd number of carbon atoms over those with an even number was

Table 1. ISOPRENOIDS AND OTHER ALIPHATIC HYDROCARBONS IN METEORITES

Group	Meteorite	Sample	Isoprenoids (p.p.m.)	Total <sup>a</sup> aliphatic hydrocarbons (p.p.m.)
Non-carbonaceous chondrites	Bruderheim (1)		0.02	0.8
	Chainpur (2)		0.13	2.8
	Indarch (3)		0.09	0.1
	" (3)		0.00	1.1
	Kaňasz (4)		0.12	2.7
	Karoonda (2)		0.11	8.2
Graphitic nodules from iron meteorites	Average		0.06	1.8
	Canyon Diablo (5)	outside	1.33	14.3
	" (5)	inside	0.22	2.8
	Odessa (6)	outside	1.18	15.6
	" (6)	inside	0.44	3.0
	Average		0.79	8.9
Wiik Type I carbonaceous chondrites	Alais (4)		0.33	5.9
	Ivuna (7)		2.42	22.6
	Orgueil (8)		0.72	9.0
	" (8)		0.00	7.8
	" (8)		0.08	4.2
	" (8)		0.04	10.0
	" (9)		0.38	25.0
	" (10)		0.45	4.6
	" (9)	outside	0.00	2.5
	" (9)	inside	0.00	0.1
	" (9)	"	0.02	0.3
	" (9)	"	0.04	2.4
	" (9)	"	0.12	1.0
	Average		0.35	7.3
Wiik Type II carbonaceous chondrites	Al Rais (7)		11.04	49.5
	Bells (7)		2.45	12.2
	Boriskino (7)		18.46	72.8
	" (4)		13.66	38.0
	Cold Bokkeveld (7)		6.79	48.0
	" (3)		3.95	15.5
	Mighei (7)		24.18	127.7
	" (4)		4.97	27.8
	Murray (11)		4.61	35.8
	" (4)		3.67	38.8
	Nogoya (7)		9.78	92.4
	Renazzo (12)		0.15	8.3
	" (13)		0.24	8.9
	Santa Cruz (2)		35.44	150.0
Wiik Type III carbonaceous chondrites	" (2)		4.25	24.7
	Average		9.6	50.0
	Felix (14)		3.15	128.0
	Grosnaja† (4)		3.04	415.0
	Kaba (4)		0.36	11.9
	Lance (13)		14.14	163.0
	Mokoia (2)	replicate	7.75	29.3
	" (2)	replicate	6.69	33.3
	" (4)		3.71	15.8
	" (10)		6.40	82.3
	Ornans (4)		0.00	8.3
	Vigarano (14)		16.63	223.0
	" (10)		30.17	138.5
	Warrenton (4)		0.14	8.2
	Average†		8.1	72.0

<sup>a</sup> Isoprenoid plus normal alkanes.

† The values for Grosnaja have been excluded from the average.

The amounts of hydrocarbons given in this table are those obtained when the procedure described is followed. They do not necessarily represent all of the aliphatic hydrocarbons in the original sample because of some losses occurring during solvent removal after extraction and elution.

Meteorite samples were obtained from the following people.

(1) R. E. Folinsbee, University of Alberta. (2) C. B. Moore, Arizona State University and Ninninger Meteorite Collection. (3) G. W. Hodgson, Research Council of Alberta. (4) G. Mueller, Birkbeck College, University of London. (5) C. F. Lewis, Centre for Meteorite Studies, Arizona State University. (6) G. I. Huss, American Meteorite Laboratory. (7) B. Mason, American Museum of Natural History. (8) E. Anders, Enrico Fermi Institute for Nuclear Studies, University of Chicago. (9) A. Cavaillé, Museum of Natural History, Montauban, France. (10) B. Nagy, University of California (San Diego). (11) E. P. Henderson, Smithsonian Institution, U.S. National Museum. (12) K. Fredriksson, Smithsonian Institution, U.S. National Museum. (13) G. Kurat, Museum of Natural History, Vienna. (14) E. J. Olsen, Chicago Natural History Museum.

observed, with the exception of some samples of the Orgueil meteorite, which showed a slight odd predominance in the  $C_{15}$  and  $C_{22}$ - $C_{27}$  ranges. This may indicate a biological origin in line with optical activity measurements<sup>11</sup>. The distribution of hydrocarbons in the Ivuna, Alais and Warrenton meteorites showed two maxima which indicated two populations of hydrocarbons and implied two different sources or synthetic processes. A number of terrestrial samples including Pre-Cambrian sediments, graphites and crude petroleum were found to contain hydrocarbons giving essentially the same gas chromatographic pattern as those given by the carbonaceous chondrites.

Any theory of process or processes, terrestrial or extra-terrestrial, responsible for the presence of hydrocarbons in meteorites must explain the foregoing qualitative and quantitative variations and similarities. The data on the higher concentration of hydrocarbons in the external part of the graphite nodules and some samples of the Orgueil meteorites are best explained by terrestrial contamination. The data on the predominance of odd carbon number alkanes in some Orgueil samples suggest a biological origin. More research work will be needed, however, before one can state with certainty that the hydrocarbons in meteorites are the result of terrestrial biological contamination. Interesting alternative possibilities are a lunar-terrestrial biological origin postulated by Urey<sup>12</sup> or an abiological extraterrestrial origin obscured or modified by terrestrial (or extraterrestrial) hydrocarbons of biological origin<sup>9,13</sup>.

This work was supported in part by the U.S. National Aeronautics and Space Administration. We thank those who gave meteorite samples (see Table 1). We also thank Professors H. C. Urey, W. F. Libby and other investigators of the group for the analysis of carbon compounds in carbonaceous chondrites, for valuable help or comments.

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## Action of Atomic Hydrogen on Ribonuclease in Aqueous Solution

by

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Atomic hydrogen seems to react with ribonuclease in aqueous solution, not by attacking the molecule at random, but by reacting with specific amino-acids in the enzyme. It seems to have a special affinity for aromatic and sulphur-containing amino-acids, even though these may be some way from the surface of the enzyme.

WE have examined the action of hydrogen atoms on dilute aqueous solutions of ribonuclease (RNase). The results now obtained correlate with those previously obtained for aqueous solutions of simple inorganic and organic compounds<sup>1</sup>, amino-acids<sup>2</sup>, the enzyme proteins chymotrypsin<sup>3</sup> and also cytochrome *c* (ref. 4), which contains a reducible metal ion; and with those recently obtained on solutions of oxidized and reduced glutathione (unpublished work).

For the quantitative study of the reactions of atomic hydrogen with solutes in water, hydrogen ions are produced<sup>1</sup> by a high frequency (30 Mc/s) electrodeless discharge in pure hydrogen at a pressure of 27 mm of mercury and swept through the solution at a pumping velocity of 50 l./min. Oxygen is, of course, excluded from the apparatus. To ensure maximum reproducibility<sup>5</sup> ( $\pm 10$  per cent) the experimental arrangements cause far-reaching recombination before the gas stream enters the solution. Thus a reproducible low concentration of hydrogen atoms, with no other reactive species present, is carried into the solution in a stream of a large excess of inert hydrogen molecules.

The dose rate of hydrogen atoms, in the present series of the order of  $3 \times 10^{-4}$  moles/l. min, was determined exactly before and after each experiment using the ferricyanide dosimeter<sup>6,7</sup>. The solutions were kept at 4° C during experiments to decrease evaporation. The low temperature also helps to decrease reaction by agents other than hydrogen atoms.

The relative rate constants, and from these absolute rate constants, can be calculated from the experimental results according to a procedure described previously<sup>1</sup>. Critical comparison with rate constants of hydrogen atom reactions obtained from radiation chemistry showed that the results agreed within a factor of 2, using a variety of different solutes, over the range  $10^5$ - $10^{10}$  l./mole sec. The present method makes it possible to study the reactions of one of the species active in radiation chemistry and photochemistry separately, in the absence of other reactive species and over a wide range of pH.

Atomic hydrogen is in theory one of the simplest reagents in aqueous solution. Its use allows the study of rates and mechanism with specific substrates without ionic effects and solvent interaction affecting the reagent.

In the present series the substrate was crystalline bovine pancreatic ribonuclease in solutions of 0.5 mg/ml., that is,  $3.7 \times 10^{-5}$  moles/l. Experiments were either at pH 6, with no other solute added, or at pH 1 adjusted by adding hydrochloric acid. The latter does not react chemically with hydrogen atoms (unpublished observations) and influences the results by its pH effect alone. At both pH values control experiments, in which the gas stream was bubbled through the solution but hydrogen atoms were not present (discharge unlit), showed that appreciable enzyme inactivation or product formation was not caused in the absence of hydrogen atoms by bubbling alone.

As in the case of trypsin<sup>8</sup>, we first investigated enzyme inactivation, changes in the absorption and fluorescence spectrum and the formation of sulphhydryl groups and free hydrogen sulphide.

To assay enzyme activity purified RNA was used. We followed the procedure of Anfinsen *et al.*<sup>9</sup>. We used Ellman's unmodified procedure<sup>10</sup>, taking  $\epsilon_{412m\mu} = 1.36 \times 10^4$  for the product with DTNB. Hydrogen sulphide was trapped in the stream of effluent hydrogen in two cooled liquid nitrogen traps arranged in series. More than 90 per cent of the product was found in the first, less than 10 per cent in the second trap. Control experiments with known amounts of hydrogen sulphide evolved in solutions showed that recovery was about 90 per cent, so that our hydrogen sulphide determinations are somewhat low. For the determination of hydrogen sulphide we followed the procedure described previously<sup>2,3,8</sup>, but redetermined  $\epsilon = 1.8 \times 10^4$  for the product with DMPD, using sodium sulphate decahydrate titrated iodometrically as the standard.

Fig. 1 shows the results on enzyme inactivation, sulphhydryl and hydrogen sulphide formation at pH 6. In Table 1 these results are compared with some obtained at pH 1. Brining the enzyme to pH 1 changes its configuration but does not inactivate it irreversibly<sup>11</sup>.

Table 1. COMPARISON OF SOME RESULTS OF TREATMENT WITH HYDROGEN ATOMS AT pH 6 AND pH 1

pH	Dose rate $10^{-5}$ moles/l.min	Total dose $10^{-5}$ moles/l.	Hydrogen sulphide formed, $10^{-5}$ moles/l.	Sulphhydryl groups formed, $10^{-5}$ moles/l.	Inactivation $10^{-3}$ moles/l. (%)
6	28	42	2.7	1.2	0.92 25
1	34	51	10.3	0.3	1.95 58

The observed rates of inactivation at both pH values are similar to but slightly lower than the corresponding values for trypsin<sup>8</sup>. Using the same method of calculation as was used for trypsin<sup>1</sup>, we obtain a value for the rate constant of inactivation by hydrogen atoms at 25° C ( $k_{\text{inact}}^{\text{H} \cdot + \text{RNase}}$ ) of  $3 \times 10^8$  l./mole sec at pH 6, and of  $6 \times 10^8$  l./mole sec at pH 1. By comparison the diffusion controlled limit for the collision between hydrogen atoms and RNase may be estimated (as was done by Braams<sup>12</sup> for the reaction between  $e_{\text{aq}}^-$  and RNase) as approximately  $3 \times 10^{10}$ /mole/sec. This is the theoretical maximum rate at which hydrogen atoms might add to RNase according to equation (1)



to yield a free radical. Not all hydrogen atoms added in this way lead necessarily to inactivation. The rate constant of inactivation now calculated is the overall rate of inactivation and may involve the action of either one or of a number of hydrogen atoms, reacting with various component amino-acids. These reactions of hydrogen atoms with RNase are in competition with the recombination of hydrogen atoms



with  $k$  (recomb.) =  $3 \times 10^{10}$  l./mole sec. Some information on the reactions of hydrogen atoms encountering RNase can be gained from the results on sulphhydryl and hydrogen sulphide formation. As shown in Fig. 1 and Table 1, more than four equivalents of sulphur-containing products ( $-\text{SH} + \text{H}_2\text{S}$ ) are formed for every RNase molecule inactivated both at pH 6 and also at pH 1, where  $k_{\text{inact}}$  increased twofold. While the ratio (inactiv.)/( $-\text{SH} + \text{H}_2\text{S}$ ) changes little, however, the ratio  $\text{H}_2\text{S}/-\text{SH}$  increases from 2.2 at pH 6 to 35 at pH 1.

Comparable results were obtained in the case of trypsin<sup>8</sup>. The ratio between molecules inactivated and sulphur equivalents formed was similar to that obtained by us. This led us<sup>8</sup> to consider the possibility that the correlation is connected with a specific distribution of the damage to the molecules, so that one does not have a population of molecules with random distribution of damage, some fully inactivated, some partly damaged, but the damage is concentrated at certain molecules, which are then completely inactivated, and also involves far-reaching damage to the disulphide linkages (of which there are four in RNase). This possibility was supported by the results on chymotrypsin<sup>3</sup>, where the analysis of the Lineweaver-Burk plots indicated that the action of hydrogen atoms resulted in complete inactivation of some molecules, without the formation (as is the case with ionizing radiations) of partially damaged molecules, with modified Michaelis constants but retaining some enzymatic activity.

Our attempts to obtain reliable Lineweaver-Burk plots in the case of RNase were unfortunately not successful, so that we have no comparable evidence in the present case.

Further comparison of the yields of sulphhydryl and hydrogen sulphide does, however, give some indirect support to these views. For the amino-acid cysteine<sup>2</sup> and the peptide, oxidized glutathione (unpublished observations), the rate constants of hydrogen atoms with  $\text{R}-\text{S}-\text{S}-\text{R}$  for the formation of sulphhydryl and hydrogen sulphide, respectively, at pH 6 were identical for the two

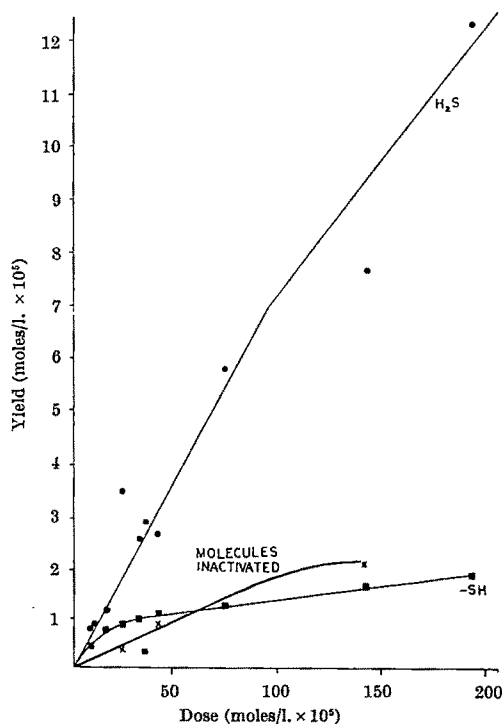


Fig. 1. Formation of sulphhydryl groups, evolution of hydrogen sulphide and inactivation of enzyme in  $3.7 \times 10^{-5}$  molar solutions of RNase at pH 6. Dose rate of hydrogen atoms approx.  $3 \times 10^{-4}$  moles/l. min. min.<sup>-1</sup>.

substrates within experimental error, and gave  $k(-SH) = 1 \times 10^6$  l./mole sec and  $k(H_2S) = 1 \times 10^8$  l./mole sec, so that the yields were in the ratio  $H_2S/-SH$  about 0.1. Investigation of the reaction of hydrogen atoms with the reduced forms of cysteine and glutathione gave for the rates of formation of  $R-S-S-R$  and hydrogen sulphide, respectively, the values  $k(\text{oxidation}) = 6 \times 10^8$  l./mole sec and  $k(H_2S) = 1 \times 10^8$  l./mole sec. For trypsin the ratio  $H_2S/-SH$  rose to unity at pH 6. It was suggested<sup>3,8</sup> that this specific increase in hydrogen sulphide can be correlated with the parallel damage to the aromatic amino-acid components, particularly tryptophan and tyrosine, as indicated by changes in the absorption and fluorescence spectrum.

Tryptophan is absent from RNase. The fluorescence spectrum (Fig. 2) is due to the six tyrosine residues in the molecule. As can be seen from Fig. 2, the percentage decrease in fluorescence, both at pH 6 and at pH 1 (25 per cent and 50 per cent, respectively), happens to be similar to the decrease in enzyme activity. This correlation may again be consistent with specific damage, concentrated on single molecules, in which the fluorescence due to all tyrosines has been eliminated.

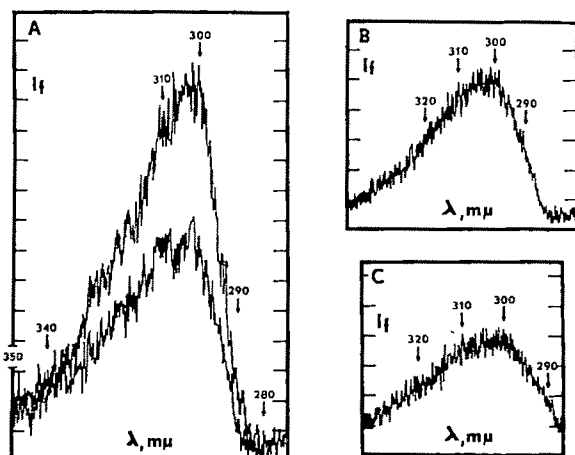


Fig. 2. The effect of hydrogen atom treatment on the fluorescence spectrum of RNase, at pH 1 (A, untreated control and treatment with a total dose of approx.  $50 \times 10^{-5}$  moles/l. of hydrogen atoms; inactivation approximately 58 per cent) and at pH 6 (B, control; C, treated with total dose of approx.  $40 \times 10^{-5}$  moles/l. hydrogen atoms; inactivation approximately 25 per cent).

The difference absorption spectrum after hydrogen atom treatment at pH 6 is shown in Fig. 3. Similar results were obtained at pH 1. The well defined changes in limited regions in the spectrum, and the absence of a generalized background change, should be compared with the results of Smith and Adelstein<sup>13</sup> on the action of ionizing radiations (though at a higher percentage of inactivation). Consistent with the less specific action of ionizing radiations<sup>3,8,14</sup> involving particularly the oxidizing species  $OH\cdot$ , a broad background is seen in the difference spectrum of RNase treated with X-rays<sup>13</sup>. The position of the difference peaks is similar in our case to the X-ray results<sup>13</sup>, and different from the case of trypsin<sup>8</sup>, in which changes in the region due to tryptophan predominated.

Further information on the nature of the process leading to inactivation is provided by the fact that inactivation could not be reversed at all on admission of oxygen. When ribonuclease is reduced by some reagents, such as mercaptoethanol<sup>15</sup>, all four disulphide bonds are reductively opened. This reduced form is almost completely re-oxidized to the enzymatically active form when oxygen is admitted. Using other reducing agents such as borohydride, however, partial reversibility only is obtained. It is possible that the present results are connected with the fact that hydrogen atoms are of the family of one-electron equivalent reagents. Their action on an organic

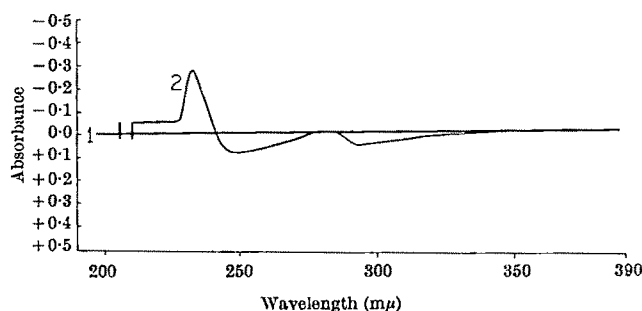


Fig. 3. Difference spectrum after hydrogen atom treatment (total dose of hydrogen atoms approximately  $40 \times 10^{-5}$  moles/l.) at pH 6.

compound, in which stable states are separated by two electron equivalents, must result in the intermediate formation of free radicals<sup>16</sup>. The relevance of this to possible mechanisms of reductive inactivation of biologically interesting substrates, particularly DPN<sup>+</sup> (NAD), has been shown<sup>17</sup>, and borohydride indicated as a reagent operating by a mixed mechanism partly involving one-electron equivalent products. Possibly in the cases where almost full reversibility is obtained after reduction, intermediate free radicals are not produced, and the mechanism is in effect a two-electron equivalent process<sup>18</sup>.

The considerable irreversibility, observable already at low doses and low percentages of inactivation, is illustrated in Fig. 4, where the heating and cooling curves obtained at pH 6 and pH 1 are shown. Native RNase may be heated reversibly within the temperature range used at both pH values<sup>11</sup>, as shown by the control curves. Similar irreversible melting-annealing curves were obtained by Smith and Adelstein after treatment with ionizing radiations<sup>13</sup>. We do not know of a specific interpretation of the correlation between inflexion temperatures, spectroscopic changes and configuration of the macromolecule.

Table 2. SUMMARY OF THE RESULTS OF AMINO-ACID ANALYSIS ON SAMPLES TREATED WITH HYDROGEN ATOMS AT pH 6 AND pH 1

Not decreased at either pH (nor at high dose)

Lysine (10)  
Arginine (4)  
Asparagine + aspartic acid (10 + 5)  
Threonine (10)  
Glutamine + glutamic acid (7 + 5)  
Proline (4)  
Glycine (3)  
Alanine (12)  
Valine (9)  
Isoleucine (3)  
Leucine (2)

Slightly decreased (noticeably at high dose)

Histidine (4)

Decreased	At pH	Approximate percentage of decrease	
		6	1
1/2 Cystine	(8)	25	50
Methionine	(4)	25	60
Tyrosine	(6)	??	50
Phenylalanine		15	45

Dose of hydrogen atoms 40 and  $50 \times 10^{-5}$  molar respectively. Percentage of inactivation, 25 and 55 per cent respectively. Initial concentration of RNase,  $3.7 \times 10^{-5}$  molar. Numbers in brackets are number of component amino-acid in each molecule of RNase (ref. 18).

\* Control and treated samples at pH 6 both much lower than theoretical value.

Decrease relative to control approximately 20 per cent.

To locate the damage in the protein molecule, the effect of the hydrogen atom treatment on the individual amino-acid components was studied by complete amino-acid analysis. The detailed composition and to some extent the configuration of native RNases are known<sup>18</sup>. The amino-acid analyses were kindly carried out by Drs. Lester Smith and John Williams, of the Medical Research Council Laboratory for Molecular Biology, Cambridge. The results are summarized in Table 2. These included amino-acid analyses on untreated controls of pH 6 and pH 1, with



and without performate oxidation, as well as similar determination on samples treated at pH 1 with low ( $40 \times 10^{-5}$  moles/l.) and high ( $200 \times 10^{-5}$  moles/l.) doses of hydrogen atoms, and at pH 1 with a low dose ( $50 \times 10^{-5}$  moles/l.) of hydrogen atoms. The percentages of inactivation at the two low doses were approximately 25 per cent and 55 per cent, respectively. The high dose at which far-reaching inactivation and probably secondary attack on the products resulted was used to indicate the further course of the reaction. The results, given in Table 2, show that of the component amino-acids, only a few, those containing sulphur: half-cystine and methionine, and the aromatic amino-acids, tyrosine and phenylalanine, were considerably decreased, and histidine slightly decreased. The decrease in tyrosine in the amino-acid analysis parallels the decrease in fluorescence.

New product peaks were also observed, as well as apparent increases at the sites of some amino-acids, particularly alanine.

The results of the amino-acid analyses thus also point to the specificity of the damage in the molecule. They are also consistent with the view expressed previously<sup>3,8</sup>, that spatially correlated aromatic and sulphur-containing amino-acids may interact intramolecularly in determining the damage. Because methionine on reacting with hydrogen atoms (ref. 2 and unpublished observations) yields sulphydryl groups and particularly hydrogen sulphide much more slowly than does cystine, it is possible that the methionine affected in RNase is not the major source of the sulphydryl groups and hydrogen sulphide observed, which may originate largely from the half-cystines. The specific involvement of sulphur in the reaction will be discussed separately<sup>16</sup>.

The results thus confirm the correlation between inactivation of enzyme by hydrogen atoms and chemical effect on sulphur-containing and aromatic component amino-acids, as well as histidine, leaving most of the component amino-acids apparently unaffected. It is still not clear whether the damage is more or less randomly distributed among the population, or whether, as is consistent with the results, the population distribution shows a peak at two main points: one of largely unaffected and the other of highly damaged molecules, the latter group showing a correlation between disulphide opening and aromatic amino-acids affected. We hope to continue the investigation of this point.

As to the mechanism by which the encounter between hydrogen atoms and RNase results in such specific damage to components which are not necessarily the ones available on the surface of the protein molecules for primary encounter with hydrogen atoms, we assume<sup>7</sup> that after primary addition of a hydrogen atom to RNase an efficient process of irreversible material transfer, involving a hydrogen atom or probably an electron, will follow within the molecule. This mechanism of the concentration of damage on sensitive sites should be differentiated from mechanisms involving excitation energy transfer. Finally, the radical will be sited on those components, aromatic or containing sulphur<sup>16</sup>, with the greatest affinity. The reactivity of the radical may lead to further intramolecular reactions, as well as intermolecular ones including possibly dimerization of RNase radicals, until electron pairing is complete. When an electron accepting metal ion is present, as in ferricytochrome c (ref. 4), this will become the final site of the one-electron equivalent, so that free radical reactions do not ensue. Otherwise, divalent sulphur<sup>16</sup> and aromatic rings might prove the favoured site for radical formation, through proton equilibria and electron-equivalent transfer.

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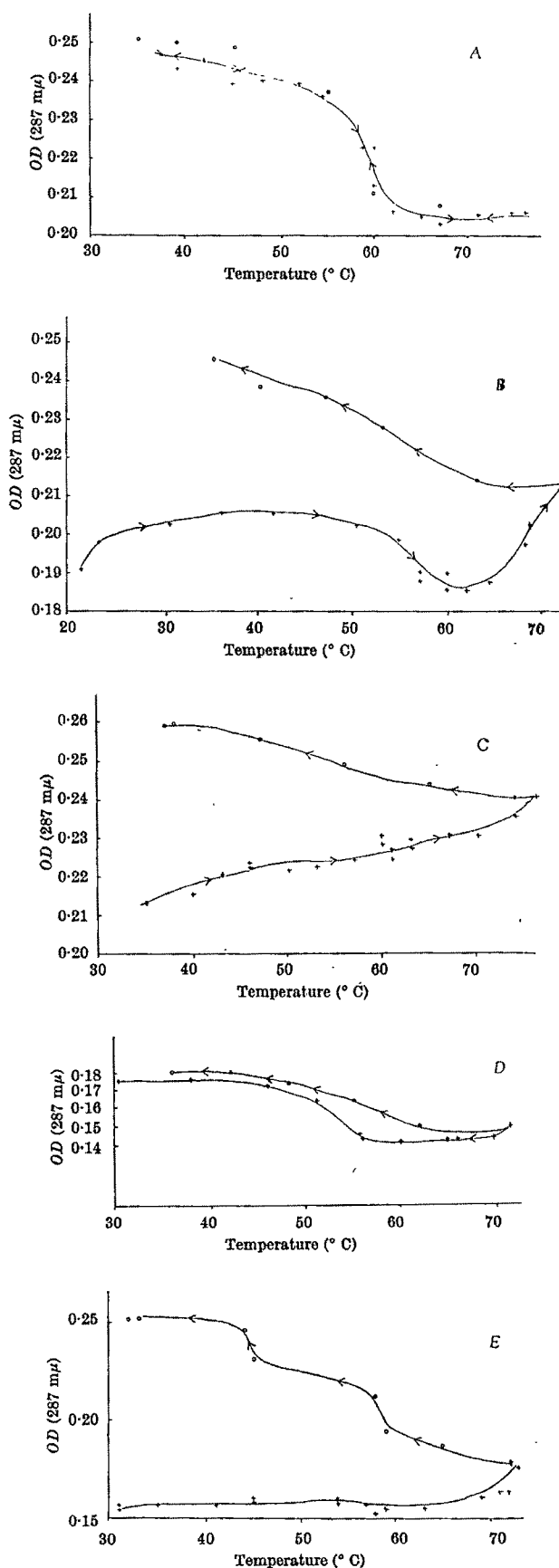


Fig. 4. Changes in the optical density at 287 mμ on heating and re-cooling  $3.7 \times 10^{-5}$  molar solutions of RNase. A, pH 6, untreated; B, pH 6, hydrogen atom dose  $40 \times 10^{-5}$  moles/l.; C, pH 6, hydrogen atom dose  $180 \times 10^{-5}$  moles/l.; D, pH 1 untreated; E, pH 1, hydrogen atom dose  $50 \times 10^{-5}$  moles/l. +, increasing; O, decreasing.

the drafting of this work, and Dr. L. E. Orgel for valuable comments.

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## Chemistry of Crossing-over

by

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The frequency of second division segregation of ascospore colour markers was used to demonstrate the effect of two chemicals (chloramphenicol and 5-fluorodeoxyuridine) on crossing-over in *Neurospora*. The effects of 5-fluorodeoxyuridine show that an impairment of DNA synthesis increases crossing-over.

ONE method by which it should be possible to investigate the chemistry of crossing-over is to use metabolic inhibitors to alter the chemical processes that occur at the time of genetic recombination. In the past, investigations of radiation-induced chromosome aberrations have shown that protein synthesis inhibitors prevent the repair or rejoining of breaks induced by radiation in  $G_1$  cells<sup>1</sup>, whereas the DNA synthesis inhibitor, 5-fluorodeoxyuridine (FUdR), does not<sup>2</sup>. These experiments have been interpreted as indicating that the bonds formed in the repair or rejoining of radiation-induced chromosome aberrations are in protein and not DNA. The mechanism of crossing-over is still unknown, but some models proposed for eukaryotes<sup>3</sup> suggest a mechanism quite similar to the breakage and reunion that occur after X-irradiation. If breakage of chromosomes, followed by rejoining or repair, occurs during crossing-over, then modification of the rejoining or repair processes with various metabolic inhibitors should affect the yield of recombinants. By using this experimental approach during meiosis it should be possible to determine the metabolic processes that are involved in meiotic recombination. In the present experiments we have shown that the inhibitor of DNA synthesis, 5-fluorodeoxyuridine, increases meiotic recombination as reported in brief previously<sup>4</sup>.

Metabolic inhibitors known to affect either DNA synthesis or protein synthesis were tested for an effect on crossing-over with *Neurospora crassa*. Those tested were chloramphenicol, an inhibitor of protein synthesis<sup>5</sup>, and 5-fluorodeoxyuridine, an inhibitor of DNA synthesis<sup>6</sup>. The effect of these compounds on crossing-over was assayed by determining the frequency of second division segregation for an ascospore colour marker (*asco* or *lys*<sup>-5</sup>; FGSC No. 405) in a cross with wild-type (74-OR23-1A; FGSC No. 987). Crosses were made at room temperature (~24° C) in 125 ml. Erlenmeyer flasks containing filter paper cones and 25 ml. of liquid synthetic crossing medium<sup>7</sup> supplemented with DL-lysine (100  $\gamma$ /ml.). The flasks were inoculated with the wild-type strain as the female parent. After the protoperithecia had formed, conidia of the *asco* strain (used as the male parent) and solutions of the chemicals were added simultaneously.

To treat with chloramphenicol a solution of 'Millipore' filter sterilized chloramphenicol was added to the flasks to give a final concentration of 300 or 600  $\gamma$ /ml. in the crossing medium. (Concentrations of 600  $\gamma$ /ml. and greater had been found to give at least a 25 per cent decrease in the linear growth rate of the wild-type strain.) 5-Fluorodeoxyuridine, when used, was added alone or in combination with uridine to give final concentrations of 10<sup>-4</sup>, 5  $\times$  10<sup>-5</sup>, or 10<sup>-5</sup> molar 5-fluorodeoxyuridine and 10<sup>-4</sup> molar uridine. Uridine was added to obviate any possible effects of incorporation of fluorouracil (a breakdown product of 5-fluorodeoxyuridine) into RNA. Treatment with 10<sup>-4</sup> molar uridine was also tested as a control.

Duplicate or triplicate crosses were made for each treatment. Perithecia were dissected in a viscous methyl cellulose solution, and the percentage of second division segregation for *asco* was determined for each of the treatments by analysing 400 asci under a dissecting microscope.

The results in Table 1 show that only 5-fluorodeoxyuridine (either alone or in combination with uridine) gives a significant change in the frequency of second division segregation. No significant effect of the concentration of 5-fluorodeoxyuridine was found over the range of 10<sup>-5</sup>–10<sup>-6</sup> molar either with or without 10<sup>-4</sup> molar uridine. The

Table 1. PERCENTAGE OF SECOND DIVISION SEGREGATION AFTER TREATMENT WITH METABOLIC INHIBITORS

Experiment	Treatment	No. of second division asci	Percentage of second division asci
1	Control	112	28.00
2	Chloramphenicol 600 $\gamma$ /ml.	109	27.25
3	" 800 $\gamma$ /ml.	111	27.75
4	Uridine 10 <sup>-4</sup> molar	93	23.25
5	FUdR 10 <sup>-5</sup> molar + uridine 10 <sup>-4</sup> molar	141	35.25
6	" 5 $\times$ 10 <sup>-5</sup> molar + uridine 10 <sup>-4</sup> molar	128	32.00
7	" 10 <sup>-4</sup> molar + uridine 10 <sup>-4</sup> molar	129	32.25
8	" 10 <sup>-5</sup> molar	136	34.00
9	" 5 $\times$ 10 <sup>-5</sup> molar	126	31.50
10	" 10 <sup>-6</sup> molar	130	32.50

$\chi^2$  for experiment 5 versus 1 = 4.86  $P$  = 0.02–0.03

" " 8 versus 1 = 3.36  $P$  approximately 0.06

" " 5 + 6 + 7 + 8 + 9 + 10 versus 1 + 2 + 3 = 10.3  $P$  approximately 0.001

" " 8 + 9 + 10 versus 1 + 2 + 3 = 7.12  $P$  < 0.01

" " 4 versus 1 = 1.22  $P$  approximately 0.25.

Four hundred asci were used in each case.

results of the present experiments tend to implicate DNA synthesis in meiotic recombination and indicate that an impairment of this process gives an 18 per cent increase in crossing-over. We do not know whether the effect of 5-fluorodeoxyuridine that leads to increased crossing-over occurs at the time of chromosome duplication (the *S* period of the cell cycle), or whether it occurs after duplication and pairing of homologous chromosomes at the time of cytologically observable crossing-over.

No effect was found with chloramphenicol. This does not necessarily imply, however, that inhibition of protein synthesis has no effect on the frequency of crossing-over in *Neurospora*, for we have no direct evidence that the inhibition of linear growth rate obtained with high concentrations of chloramphenicol is caused by a general inhibition of protein synthesis. Furthermore, we have found that puromycin (another protein synthesis inhibitor) did not affect linear growth rate of *Neurospora*.

5-Fluorodeoxyuridine has been found to break chromosomes in higher plants<sup>6</sup>. Such breakage has been postulated to occur either at the end of DNA synthesis (the *S* period)<sup>8</sup> or in *G*<sub>2</sub>, the post-synthetic interphase<sup>2</sup>. The effect of 5-fluorodeoxyuridine, however, is so drastic that many breaks are usually produced in a cell and these do not rejoin. Such an effect, therefore, should not lead to recombination but to genetic loss and death of the affected cells. In *Neurospora* the presence of chromosome aberrations in a cross can be detected by an increase in the percentage of ascospore abortion (light tan or colourless spores). An effect of this type would not be readily detectable in the cross of *asco* and wild-type because all *asco* ascospores are colourless. Evidence for this type of effect in *Neurospora* was sought by analysing a cross of two wild-type strains [74-OR23-1A (FGSC No. 987) and 74-OR8-1a (FGSC

No. 988)] showing a very low percentage of aborted ascospores<sup>9</sup>. None of the chemical treatments increased the percentage of ascospore abortions over the 0.8 per cent found in the control cross. This test suggests that, in *Neurospora*, the concentrations of 5-fluorodeoxyuridine that give an increase in crossing-over do not give any detectable chromosome aberrations.

It is interesting to note that both 5-fluorodeoxyuridine and mitomycin C, which are known to inhibit DNA synthesis in fungi<sup>10,11</sup>, have been found to increase the frequency of somatic recombination in *Ustilago maydis*<sup>11,12</sup>. The present experiments, in which an inhibitor of DNA synthesis increases recombination but a protein-synthesis inhibitor does not, tend to implicate DNA synthesis in genetic recombination during meiosis. They do not necessarily imply, however, that crossing-over in eukaryotes occurs solely in DNA to the exclusion of protein.

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## The Prasinomycins: Antibiotics containing Phosphorus

by

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*Streptomyces prasinus* is found to produce at least five members of a new family of antibiotics, the prasinomycins. These are of high molecular weight and active *in vitro* against Gram-positive bacteria. Single subcutaneous doses provide prolonged protection to mice against *Streptococcus pyogenes*.

A FAMILY of new antibiotics, the prasinomycins, was found to be produced by a streptomycete isolated from a sample of soil collected in Colorado. The organism has been identified as *Streptomyces prasinus*, a member of the green spore colour series of streptomycetes. Contrary to a report of Ettlinger *et al.*<sup>1</sup>, *S. prasinus* does produce an antibiotic, as do other members of this spore colour series, for example *S. hirsutus* and *S. prasinopilosus*. The antibiotic producing activity is, however, specific to the strain because not all cultures examined showed activity.

The cultures were maintained on tomato paste-oatmeal agar slants. When needed, germinator cultures were grown in 15 g soybean meal, 15 g dehydrated mashed potato, 50 g glucose, 0.005 g COCl<sub>2</sub>·2H<sub>2</sub>O, 10 g calcium carbonate and 1 l. of distilled water. After incubation at 25° C for 4 days, 5 ml. was used to inoculate 100 ml. of the fermentation medium in 500 ml. Erlenmeyer flasks.

The fermentation medium consisted of 60 g soybean meal, 50 g glucose, 1 g disodium phosphate, 10 g calcium carbonate and 1 l. of distilled water. The fermentation flasks were incubated at 25° C for 7 days. All incubations were carried out on a rotary shaker, operating at 280 r.p.m. with a 2 in. throw. After production of the antibiotic and its isolation, both agar diffusion and tube dilution bioassays with *Staphylococcus aureus* 209 P were carried out.

Although the activity was present in both mycelium and filtrate at the time of collection, all the activity could be recovered with the mycelial cake by acidification of the culture broth to pH 2 before filtration. The filter cake was extracted with methanol and the extract neutralized to pH 7. The aqueous suspension obtained after evaporation of the methanol was acidified to pH 2 and extracted with chloroform. The chloroform extract, which contained most of the activity, was concentrated

under vacuum to a thick oil and the crude antibiotic precipitated by the addition of ten volumes of acetone. The light tan amorphous powder so obtained showed a minimum inhibitory concentration against *S. aureus* 209 P of 0.15 µg/ml. in a two-fold tube dilution assay. The acetone powder was dissolved in ten times its weight of water, adjusted to pH 9 with sodium hydroxide and washed with *n*-butanol. The aqueous phase was adjusted to pH 5 with hydrochloric acid and washed again with *n*-butanol. The strongly acidic antibiotic was finally liberated by adjustment of the aqueous phase to pH 2, and the resulting free acid extracted into *n*-butanol. The butanolic solution was neutralized with sodium hydroxide and the mixture evaporated to dryness to obtain the crude sodium salt of prasinomycin. At this point it was found that the antibiotic in aqueous solution did not pass through a semi-permeable 'Cellophane' membrane. The crude sodium salt was therefore dialysed to effect further purification. The product obtained had a minimum inhibitory concentration of 0.04 µg/ml. in the *S. aureus* 209 P tube dilution assay. The free acid could be conveniently prepared by removal of the sodium ions by stirring an aqueous solution with 'Dowex 50' (H<sup>+</sup> form).

Considerable difficulty was experienced in finding a suitable partition paper chromatography system which would resolve the antibiotic mixture. It was subsequently found, however, that if doubly acid washed paper (Munktells No. S-302) was used with suitable basic solvent systems resolution of the antibiotic mixture into several bioactive components could be effected. The stationary phase was placed on this paper by dipping it into a mixture of acetone and lower phase of the solvent system (2:1 by volume) and allowing the acetone to air dry at room temperature (2-3 min). The solvent system, *n*-butanol-pyridine-water (4:1:4 by volume), resolved the antibiotic mixture into five bioactive components which could be detected by placing the air-dried chromatogram on the surface of a nutrient agar plate seeded with *Staphylococcus aureus*<sup>2</sup> or by spraying the chromatogram with a 0.001 per cent solution of rhodamine B in 0.25 molar potassium monohydrogen phosphate<sup>3</sup>. The latter technique resulted in white spots on a pink background. The sensitivity of the bioautographic method is about 0.01-0.05 µg. Because of its shorter development time, a *n*-propanol-*n*-butanol-0.2 molar *N*-ethylmorpholine system (2:3:4 by volume) was also found to be useful, although the resolution was not so good (Table 2).

Three of the five components of the antibiotic mixture were isolated by countercurrent distribution in a *n*-propanol-*n*-butanol-0.2 molar *N*-ethylmorpholine system (2:3:6 by volume) under nitrogen. After a thousand transfers, tubes 270-290, which contained a single bioactive component, were removed and combined to give prasinomycin C. After an additional five hundred transfers, tubes 200-240 and tubes 280-320 yielded prasinomycins A and B, respectively. The apparent homogeneity of each component was confirmed by the paper chromatography and the detection techniques already described. To isolate the components as free acids, the solutions were evaporated under vacuum to dryness, taken up in water and treated with 'Dowex 50' ion exchange resin. After filtering off the resin, the solutions were freeze dried and the residue precipitated from methanol by the addition of ethyl acetate to yield approxi-

Table 1. ANALYSES\*

Compound	C	Found (per cent)					Neu- tral equiv.†	Molecular wt.§ (ultracentrifuge)	
		H	N	P	N-OAc			ol	Buffer‡
Prasinomycin A	48.52	6.51	4.42	2.43	6.52	460	1,800	32,000	
Prasinomycin B	50.72	6.70	4.76	2.30	6.33	430	1,650	30,800	
Prasinomycin C	51.36	7.53	4.34	2.19	6.31	646	1,850	31,700	

\* All analyses on anhydrous free acids.

† By titration with sodium hydroxide in water.

‡ Aqueous 1:3 molar sodium chloride-0.1 molar phosphate buffer (pH 6.8).

§ Determined on hydrated forms.

mately equal amounts of prasinomycins A, B and C as colourless, amorphous compounds. Prasinomycins D and E were present in relatively small amounts and have not yet been isolated in pure form.

These antibiotics appeared to have a high molecular weight because they did not pass through a 'Cellophane' membrane on dialysis. In addition, a determination of molecular weight in aqueous buffered solution using an analytical ultracentrifuge<sup>4</sup> gave a value of about 31,000 (Table 1). This value apparently represents an aggregated form, however, because molecular weights in the range of 1,600-1,850 were found for the hydrated forms of the free acids when the determinations were carried out in ethanol solution. The molecular weights determined by the ultracentrifugation method appeared to be highly dependent on the environment of the compounds in solution. For example, after the addition of a small amount of pH 7 phosphate buffer to the ethanol solution, values of 2,100-2,300 were obtained for the molecular weights of the resulting sodium salts. In all the determinations a small amount of a higher molecular weight component (7,200-7,400) could be detected which prevented the calculation of an accurate value for the major component. It is not known whether this minor component represents an impurity or yet another aggregated form. The neutral equivalent of each antibiotic determined by titration with base (Table 1) indicated that several acidic functions were present. These antibiotics are presumably diesters of phosphoric acid which would account for their strongly acidic character. Other acidic functions apparently reside in carboxyl groups because conversion of the acids to the sodium salts results in decreased absorption at 5.80µ (COOH) and increased absorption at 6.10-6.25µ and 7.0µ, characteristic for the transformation of a carboxylic acid to its carboxylate anion. The infra-red spectra of prasinomycins A, B and C are very similar. They all exhibit a strong band centred at 3.0µ (O-H, N-H) as well as absorption at 5.84µ (COOH),

6.0-6.05µ ( $\begin{array}{c} \text{O} \\ \parallel \\ \text{C-NH-} \end{array}$ ), and 6.45µ (N-H). The infra-red spectra indicated the presence of amide groups, and acid hydrolysis liberated acetic acid suggesting the presence of *N*-acetyl functions. All the antibiotics showed absorption in the ultra-violet in the region of 257 mµ (sodium salts), but prasinomycin B was characterized by a much higher extinction coefficient than either A or C (Table 2). The three compounds rapidly formed hydrates on exposure to the atmosphere. The analytical data presented in Table 1 were obtained on the anhydrous forms after drying at 80° C under vacuum. The elementary analyses for the prasinomycins suggest that these antibiotics have empirical formulae in the range (C<sub>62-74</sub>H<sub>98-130</sub>N<sub>5-6</sub>O<sub>32-40</sub>P).

Table 2. PHYSICAL PROPERTIES OF THE PRASINOMYCINS

Compound	[α] <sub>D</sub> <sup>H<sub>2</sub>O</sup>	M.p. (° C)	Ultra-violet, mμ (E <sup>1%</sup> )		I*	R <sub>F</sub>	Distribution coefficient‡
			0.1 N-HCl	0.1 N-KOH			
Prasinomycin A	+0.8°	166-169 d	246 (8.0)	256 (8.2)	0.23	0.25	0.17
Prasinomycin B	+2.8°	167-170 d	246 (42)	257 (90)	0.28	0.25	0.24
Prasinomycin C	+4.4°	178-180 d	244 (7.2)	257 (9.4)	0.36	0.39	0.39
Prasinomycin D					0.38	0.39	0.43
Prasinomycin E					0.45	0.62	0.85

\* *n*-Butanol, pyridine, water (4:1:4 by volume).

† *n*-Propanol, *n*-butanol, 0.2 molar *N*-ethylmorpholine (2:3:4 by volume).

‡ *n*-Propanol, *n*-butanol, 0.1 molar *N*-ethylmorpholine (2:3:6 by volume). Countercurrent distribution run in nitrogen atmosphere.

Acid hydrolysis of prasinomycin *A* gave equivalent amounts of D-glucosamine, 6-deoxy-D-glucosamine and glycine. Hydrolysis of prasinomycins *B* and *C* also gave the same two amino sugars in equivalent amounts but only a trace of glycine. The amino sugars were separated from the hydrolysis products by ion exchange chromatography on 'Dowex 50' (H<sup>+</sup>) eluting with a gradient of 0.2 normal to 2 normal hydrochloric acid. Glucosamine was isolated as the crystalline hydrochloride and identified by comparison with an authentic sample. The 6-deoxy-D-glucosamine was acetylated to give a mixture of  $\alpha$ - and  $\beta$ -tetraacetyl-6-deoxy-D-glucosamine and identified by comparison with an authentic mixture of the  $\alpha$  and  $\beta$  forms. Further efforts to elucidate the structures of these antibiotics are in progress.

The antibiotic mixture, as well as the individual components, are active *in vitro* against Gram-positive bacteria and *Mycobacterium bovis* B.C.G. They have relatively little activity against yeasts, fungi and Gram-negative bacteria. The individual components have the same order and magnitude of activity as the mixture. The prasinomycins are not cross-resistant with penicillin, with any members of the tetracycline family, or with streptomycin, neomycin, chloramphenicol, novobiocin, bacitracin, resistocetin, erythromycin, oleandomycin, carbomycin or methymycin. The *in vivo* effectiveness of the antibiotic mixture when given subcutaneously is

shown by a 50 per cent protective dose of approximately 0.5 mg/kg against *Streptococcus pyogenes* C<sub>203</sub>. No protection is granted to mice when the antibiotic mixture is given *per os*. A remarkable property of the prasinomycin mixture is the prolonged protective effect obtained with a single subcutaneous dose. Protection was still afforded to mice infected with *Streptococcus pyogenes* C<sub>203</sub> 28 days after administration of the antibiotic.

We thank Professor Richard Kuhn for an authentic sample of tetraacetyl-6-deoxy-D-glucosamine. We also thank Dr. J. Kirschbaum for determination of molecular weights, W. Trejo for the identification of the organisms, J. Alicino for elementary analyses, and H. Basch for the biological assays.

*Note added in proof.* Since submission of this manuscript, two other research groups have reported the isolation of antibiotics which appear to be related to prasinomycin: (a) Moenomycin (Wallhauser, K. H., *et al.*, *Antimicrobial Agents and Chemotherapy*, 735-6; 1965); (b) Antibiotic 11837RP (Mancy, D., *et al.*, *Abstr.*, *Ninth Inter. Congr. Microbiol.*, Moscow, July 24-30, 1966, p. 165).

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## Mathematical Interpretation of Heart Kymography

by

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A kymograph—a revolving drum carrying smoked paper—is frequently used to record variations of heart action. Mathematical analysis of the kymograms will provide information about the action on the organ of drugs such as cardiac glycosides.

THE trace of a kymograph can be analysed in a number of ways. The problem is often solved very simply by calculating the percentages, for example, of the increase or decrease in the amplitude of the curve, of the reaction velocity, or of the duration of the reaction. In this article we shall show how a mathematical formulation of the results can be obtained when the influence of cardiac glycosides on the isolated heart of the rat is investigated.

Tests were carried out on thirty male Wistar rats according to the method of Langendorff as modified by Wiegand<sup>1</sup>. After a period of adaptation lasting 2-5 min, the effects on the beat of the isolated heart of a single 10  $\mu$ g dose of strophanthine *g*, digitoxine and convalatoxine were investigated. The dose was administered in a volume of 0.1 ml. of perfusing fluid through a polyethylene cannula inserted into the aortic arch. A further

0.1 ml. of the perfusing solution was used to advance the drug. Coronary outflow was also recorded.

The "envelopes" of the kymograms of the action of the heart after a single dose of a drug, using the Langendorff method, can be approximated by curves which are satisfied by equations of the type (1)-(5)

$$A = [C_1 + C_2(t - t_0)] \exp [-r(t - t_0)] + C_3 \quad (1)$$

where  $A$  is the amplitude of the envelope,  $t$  is the time of the contraction,  $t_0$  is the time at which the drug was administered, and  $C_1$ ,  $C_2$ ,  $C_3$  and  $r$  are constants characterizing the given course.

When  $t = t_0$ , the value of the amplitude when the drug is administered is  $A_0 = C_1 + C_3$ . For  $t \rightarrow \infty$ —that is in a time sufficiently remote from the moment the drug is adminis-

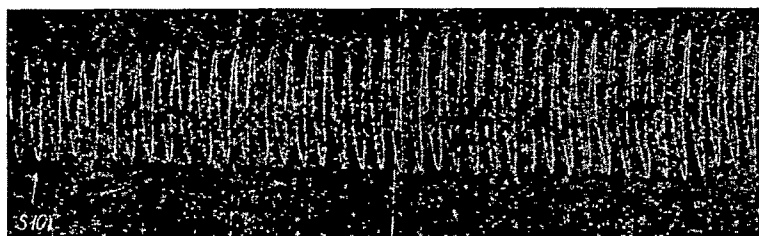


Fig. 1. Recording of the action of the heart after injecting a single 10  $\mu$ g dose of strophanthine.



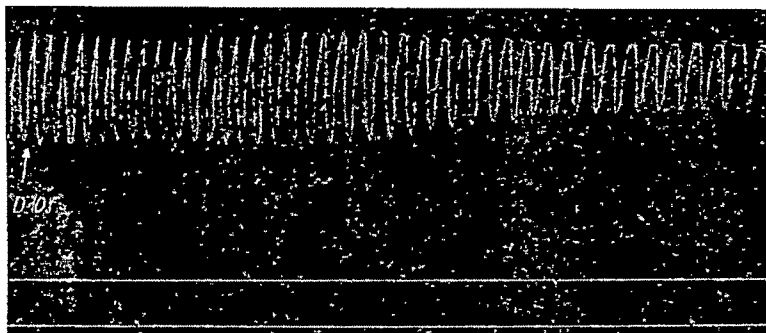


Fig. 2. Recording of the action of the heart after injecting a single 10 µg dose of digitoxine.

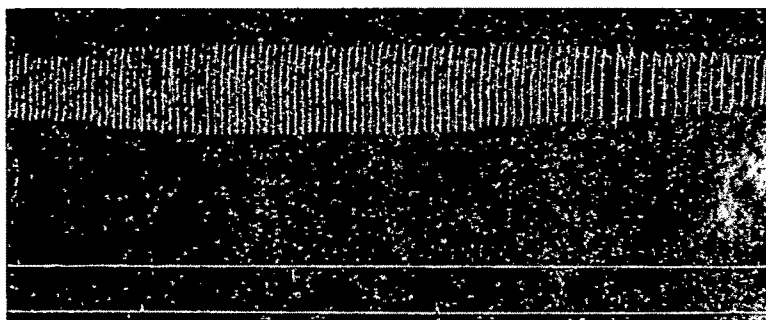


Fig. 3. Recording of the action of the heart after injecting a single 10 µg dose of convallatoxine.

tered—the envelope amplitude returns to normal and is usually established on a different level from that observed before the drug was administered. From equation (1), when  $t \rightarrow \infty$ , the final level  $A_k = C_2$ , and thus  $C_1 = A_0 - A_k$ . Because  $r > 0$ , the curve satisfied by equation (1) has a maximum when  $C_2 > 0$ .

The time derivative of curve (1) for  $t = t_{\max}$ , where  $t_{\max}$ —the moment where the maximum amplitude appears—equals 0, thus

$$\frac{dA}{dt} \Big|_{t=t_{\max}} = \{-C_1 r + C_2[1 - (t_{\max} - t_0)r]\} \exp[-r(t_{\max} - t_0)] = 0$$

$$\text{whence} \quad r = \frac{C_2}{C_1 + C_2(t_{\max} - t_0)} \quad (2)$$

Substituting for  $C_1$ ,  $C_2$  and  $r$  in equation (1) we obtain

$$A = [(A_0 - A_k) + C_2(t - t_0)] \exp \left[ -\frac{C_2(t - t_0)}{(A_0 - A_k) + C_2(t_{\max} - t_0)} \right] + A_k \quad (3)$$

When, after returning to the "norm", the final level of the envelope equals the initial level (normal rate), that is, when  $A_k = A_0$ , we obtain

$$A = C_2(t - t_0) \exp \left[ -\frac{t - t_0}{t_{\max} - t_0} \right] + A_0 \quad (4)$$

Then, for  $t = t_{\max}$ , the following value of the maximum amplitude is obtained  $A = A_{\max \text{ norm}}$ , and from equation (4)

$$\frac{A_{\max \text{ norm}} - A_0}{t_{\max} - t_0} = \frac{C_2}{e} = \alpha \quad (5)$$

where  $\alpha$  is constant for the given normal kymogram course. This constant can be termed a "coefficient of inotropic reactivity", and is dependent on the character, biological activity and dosage of the drug administered, as well as on the properties and "condition" of the heart

examined. Substituting the coefficient  $C_2$  obtained from equation (5) in equation (3)

$$A = [(A_0 - A_k) + \alpha e(t - t_0)] \exp \left[ -\frac{\alpha e(t - t_0)}{(A_0 - A_k) + \alpha e(t_{\max} - t_0)} \right] + A_k \quad (6)$$

If the course proceeds according to equation (6), the reactivity coefficient will be connected with the proportion

$$\frac{A_{\max} - A_0}{t_{\max} - t_0}$$

by a complex equation.

If  $A = A_{\max}$  and  $t = t_{\max}$  are substituted in equation (6) we obtain.

$$\frac{A_0 - A_k}{A_{\max} - A_k} + \alpha e \frac{t_{\max} - t_0}{A_{\max} - A_k} = \exp \left[ \frac{1}{1 + \frac{A_0 - A_k}{\alpha e(t_{\max} - t_0)}} \right] \quad (7)$$

We encountered considerable difficulty in the analytical determination of the coefficient  $\alpha$  from equation (7). Because of this we decided to use a graphical method in order to establish the value of this coefficient, that is, to compare the left and the right hand sides of equation (7). Assuming that  $y$  has the value of the left hand side of equation (7), a straight line is obtained; however, if it has a value equivalent to the right hand side a curve is produced. The point of intersection of the curve and the straight line gives the value of the inotropic reactivity coefficient  $\alpha$  for the course of equation (6).

As has already been established, it is easy to determine all the coefficients of equation (1) or, synonymously, the coefficients of equation (6). Thus the values  $A_0$ ,  $A_k$  and  $t_{\max}$  can be obtained directly from a kymograph as indicated and the coefficient from the intersection of the curve and line using components of equation (7). It must be emphasized, however, that the use of this equation would require that the value of  $A_{\max}$  be determined. By substituting the values  $A_0$ ,  $A_k$  and  $t_{\max}$  in equation (6), we obtain an approximate analytical picture of the

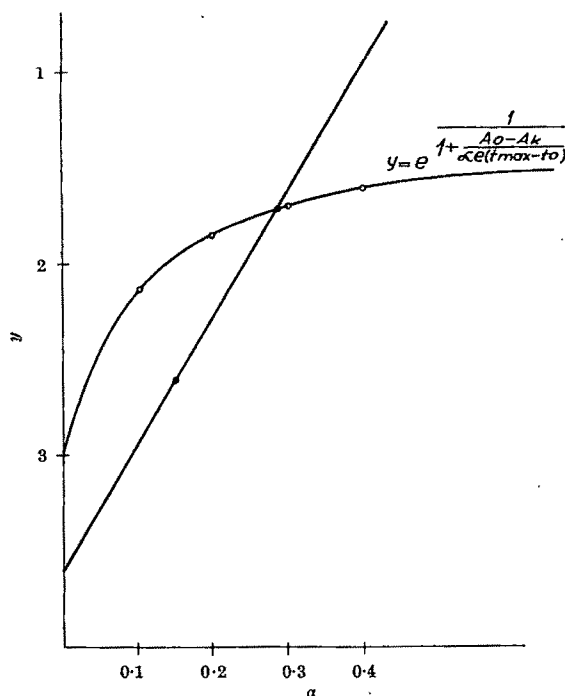


Fig. 4. Calculations for chart on Table 1.

Table 1

$A_0 = 2.4$  cm;  $A_k = 1.45$  cm;  $A_{max} = 3.85$  cm;  $t_{max} = 10$ ;  $t_0 = 4$ .

$$\exp \left[ \frac{1}{1 + \frac{A_0 - A_k}{\alpha e (t_{\max} - t_0)}} \right] = \exp \left[ \frac{1}{1 + \frac{0.95}{\alpha e}} \right]$$

$\alpha$	$\frac{0.95}{\alpha e}$	$1 + \frac{0.95}{\alpha e}$	$\frac{1}{1 + \frac{0.95}{\alpha e}}$	$\lg e$	$\frac{1}{1 + \frac{0.95}{\alpha e}}$	$\exp \left[ \frac{1}{1 + \frac{0.95}{\alpha e}} \right]$
0.1	0.583	1.583	0.633	0.274	1.88	
0.2	0.291	1.291	0.773	0.335	2.16	
0.3	0.194	1.194	0.838	0.363	2.31	
0.4	0.146	1.146	0.872	0.378	2.39	

changed amplitude and the heart beat, which results from the effects of an administered drug.

The frequency of the heart beat after a single dose of glycoside drugs can be analysed in a similar fashion. The changes in frequency over the kymogram course determined from an analytical equation similar to equation (6)

$$f = [(f_0 - f_k) + \alpha_f e (t_{extr} - t_0)] \exp \left[ - \frac{\alpha_f e (t - t_0)}{(f_0 - f_k) + \alpha_f e (t_{extr} - t_0)} \right] + f_k \quad (8)$$

where  $f_0$  is the frequency of contractions at the moment of administering the drug,  $f_k$  is the frequency of contractions after the action of the heart has settled down,  $t_0$  is the moment at which the drug is administered,  $t_{extr}$  is the moment at which the maximum number of contractions occurs and  $\alpha_f$  is the chronotropic reactivity coefficient. This coefficient is similar to the inotropic reactivity coefficient

$$\alpha_f = \frac{f_{extr \text{ norm}} - f_0}{t_{extr} - t_0} \quad (9)$$

where  $f_{extr \text{ norm}}$  is the maximum frequency of vibration of normal heart action, that is one in which the frequency of vibration returns to normal ( $f_k = f_0$ ).

The chronotropic reactivity coefficient can be determined from equation similar to equation (7)

$$\frac{f - f_0}{f_{extr} - f_k} + \alpha_f e \frac{t_{extr} - t_0}{f_{extr} - f_k} = \exp \left[ \frac{1}{1 + \frac{f_0 - f_k}{\alpha_f e (t_{extr} - t_0)}} \right] \quad (10)$$

where  $f_{extr}$  is the maximum number of contractions during the whole kymogram course.

The changes in velocity of the coronary outflow during the action of a heart which had been stimulated by a single dose of the drug were fairly similar for all the drugs which we tested. As shown in Fig. 5, this can also be described by an equation similar to equation (6)

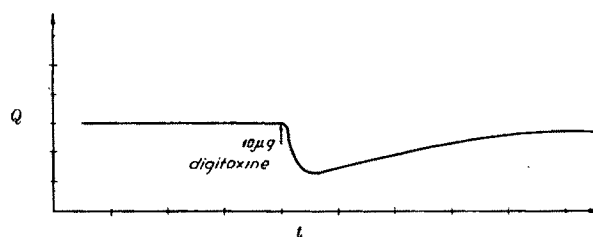
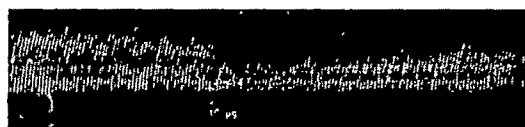
$$Q = [(Q_0 - Q_k) + \alpha_Q e (t_{extr} - t_0)] \exp \left[ - \frac{\alpha_Q e (t - t_0)}{(Q_0 - Q_k) + \alpha_Q e (t_{extr} - t_0)} \right] + Q_k \quad (11)$$

where  $Q_0$  is the velocity of the coronary outflow at the time when the drug was administered,  $Q_k$  is the velocity of the coronary outflow after the action of the heart has settled down,  $t_0$  is the moment at which the drug was administered,  $t_{extr}$  is the time at which maximum velocity of the coronary outflow occurs, and  $\alpha_Q$  is the coefficient of the flow reactivity defined in the same way as the inotropic reactivity coefficients.

Thus

$$\alpha_Q = \frac{Q_{extr \text{ norm}} - Q_0}{t_{extr} - t_0} \quad (12)$$

where  $Q_{extr \text{ norm}}$  is the maximum velocity of the coronary outflow when normal—that is when the velocity of the coronary outflow returns to normal.

Fig. 5. Recording and envelope of a coronary outflow after administering 10  $\mu$ g digitoxine.  $Q$ , Value of the coronary outflow;  $t$ , time.

The reactivity coefficient of the flow can be determined from an equation similar to (7)

$$\frac{Q_0 - Q_k}{Q_{extr} - Q_k} + \alpha_Q e \frac{t_{extr} - t_0}{Q_{extr} - Q_k} = \exp \left[ \frac{1}{1 + \frac{Q_0 - Q_k}{\alpha_Q e (t_{extr} - t_0)}} \right] \quad (13)$$

where  $Q_{extr}$  is the maximum velocity of the coronary outflow for the kymogram course.

If the computed values of the coefficients  $\alpha$ ,  $\alpha_f$  and  $\alpha_Q$  are substituted in equations (8), (9) and (11) from equations (7), (10) and (13) we obtain strict analytical expressions for  $A$ ,  $f$  and  $Q$  as functions of time  $t$ . The remaining coefficients of these equations, for example,  $A_0$ ,  $A_k$ ,  $t_{max}$  and  $t_0$ , can easily be read from the diagrams. By examining several such diagrams for different hearts, various drugs and dosages, we can determine whether a relationship exists between the individual coefficients, and if so to what extent it holds true. It would be invaluable to know, for example, what relationship exists between  $A_0$ ,  $A_k$ ,  $t_{max}$ ,  $t_0$  and  $\alpha$ . It would also be interesting to find out whether, for example, the  $A_k$ ,  $t_{max}$  and  $\alpha$  depend on the primary working conditions of the heart, that is on  $A_0$ , and  $t_0$ , or which of the quantities.  $A_k$ ,  $t_{max}$  and  $\alpha$  depend on the nature and conditions of the heart, and which depend on the dosage of the drug

administered. Furthermore, it would be useful to know the nature and the extent of any such dependencies.

Once this has been done some coefficients can then be eliminated from equations (6), (8) and (11). If these relationships do exist, and they can be defined by analytical formulae, this elimination might be achieved by analytical means. It is possible, however, that these relationships can be defined only by diagrams. In this event equations (6), (8) and (11) could be used in conjunction with the diagrams.

The components discussed here will probably differ according to the drugs used. It should therefore be made clear that the final forms of equations (6), (8) and (11), as well as the diagrams mentioned, will, of course, differ from one drug to another.

Experiments carried out so far show that the coefficients of inotropic reactivity  $\alpha$ , chronotropic reactivity  $\alpha_f$  and flow reactivity  $\alpha_Q$  are constant for hearts of similar properties and condition, treated with equal amounts of the same drug. The value of these coefficients for a similar amount of the same drug can be determined statistically by investigating the effect of the drug on a large number of hearts. Here deviations from the normal values of these coefficients would indicate that the heart is in a pathological condition. This value would be an objective, strictly mathematically determined quantity. For different amounts of the same drug the values of the coefficients  $\alpha$ ,  $\alpha_f$ ,  $\alpha_Q$ , will usually be different. By treating the heart with different doses of drugs and by performing an adequate number of tests we would obtain dependencies

$$\alpha, \alpha_f, \alpha_Q = (\text{dose of drug}) \quad (14)$$

Determination of these dependencies will make it possible to foresee the behaviour of the heart when the amount of drug administered is altered. This will facilitate a strict determination of the amount of drug permissible and most effective for each heart examined. Many dependencies of type (14) will be obtained for different drugs and it should be possible to establish a set of curves determining the effectiveness of various drugs and their eventual quantitative equivalent.

The curve of type (14) will also enable the interrelation between coefficients  $\alpha$ ,  $\alpha_f$ ,  $\alpha_Q$  to be determined, and that between equations (6), (8) and (11)—in other words, between the amplitude  $A$ , of the frequency of contractions  $f$  and the speed of the coronary outflow  $Q$  at every moment of time  $t$ . Determination of this interrelation would be desirable for an interpretation of the phenomena occurring in the heart. It would facilitate the building up of a mathematical model of the heart.

Finally, we emphasize that the equations presented here are, as we mentioned earlier, only approximate and do not pretend to give a full and complete explanation of the phenomena occurring in the heart under the influence of drugs.

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## Fertile Life of Rabbit Spermatozoa in Rat Uterus

by

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Injection of rabbit spermatozoa into the uterus of rat has demonstrated a prolongation of the fertile life of the sperm, sometimes to almost twice that in the rabbit uterus.

THERE is considerable variation between different mammals in so far as the viability of spermatozoa within the female is concerned. Chang<sup>1</sup> has raised the question of the relative importance of the inherent qualities of the spermatozoon, on the one hand, and the characteristics of the female environment, on the other, in the determination of the fertile and motile life of spermatozoa after their entry into the female. Little is yet known, however, of the relationship which exists between spermatozoa and the secretions of the female tract to which they are exposed. Although the integrity of spermatozoa is maintained within the male tract for a period of several weeks<sup>2,3</sup>, spermatozoa remain viable within the female for a much shorter period, which is best measured in hours. In the male and the female, however, the viability of spermatozoa as judged by motility cannot necessarily be taken as an indication of functional competence, because in both of these situations the ability to fertilize is lost well before the capacity for progressive movement. Spermatozoa have been shown to retain their motility for a significantly longer period than their fertilizing ability in the female tract of the mouse<sup>4</sup>, rat<sup>5,6</sup>, guinea-pig<sup>5,7</sup> and sheep<sup>8</sup>. In the rabbit, motile spermatozoa

have been found within the uterus and Fallopian tubes as late as 50 h after insemination<sup>9</sup>. Yet virtually no rabbit spermatozoa remain fertile in the female tract for longer than 30–32 h<sup>2,9,10</sup>, and as judged by litter size, the fertility of many spermatozoa begins to deteriorate some 10 h earlier than this<sup>2</sup>.

In the hope of clarifying further the specificity of the relationship which exists between competent spermatozoa and the environment of the female reproductive tract, rabbit spermatozoa from the cauda epididymidis were incubated in the uterus of the oestrous ferret, cat, guinea-pig and rat. It soon became apparent that the uterine environment in the ferret, cat or guinea-pig is relatively unfavourable to rabbit spermatozoa<sup>14</sup>. The uterine milieu in the oestrous rat, on the other hand, has proved to be a highly suitable climate for the survival of rabbit spermatozoa. It is the purpose of this article to discuss the effect of this favourable heterologous uterine environment on the motile and fertile life of rabbit spermatozoa.

Oestrous and pro-oestrous rats, selected on the basis of their vaginal cytology, were anaesthetized with ether and the uterus was exposed through a ventral mid-line incision. Only those females whose uterine horns were filled with fluid were used as recipients for the rabbit spermatozoa. Spermatozoa taken from the cauda epi-

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didymidis in adult male rabbits were suspended in Hanks solution, and between 4-8 million spermatozoa in 0.03 ml. were injected through the uterine wall immediately above the cervix; the uterus was then ligatured with linen thread to prevent loss of fluid. Usually four to six rats were used in each experiment as recipients of aliquots from a single sperm sample. The spermatozoa were recovered subsequently by withdrawal of the uterine fluids at various times up to 48 h later. The recovered sperm samples were assessed subjectively using a Fuchs-Rosenthal cytometer, and were considered positive if more than 15 per cent of the spermatozoa in the sample showed vigorous progressive motility.

The results of the sperm survival experiments are summarized in Table 1. In general, the survival of rabbit spermatozoa in the rat uterus, as judged by motility, was similar to that in the rabbit uterus. In a few cases, appreciable numbers of rabbit spermatozoa still showed good motility when recovered after 48 h in the rat uterus, but in the majority of instances rabbit spermatozoa survived for a somewhat shorter period in these conditions. Table 1 does not bring out the point that different females whose uteri were filled with fluid showed considerable variation in their respective abilities to support the motility of spermatozoa within the uterus. Thus, at 48 h, only one animal in each group of four to six comparable females possessed highly motile spermatozoa in the uterine flushings. In the others the spermatozoa were either lying free but immotile, or had been ingested by uterine leucocytes. Taken overall, there was a definite difference between apparently similar uterine environments in their ability to support the motility of spermatozoa.

Table 1. SURVIVAL OF RABBIT SPERMATOZOA IN THE FLUID-FILLED UTERI OF OESTROUS RATS

Time in rat uterus (h)	No. of motile spermatozoa samples*
12-18	32/40 (80%)
18-24	22/30 (73%)
24-30	12/18 (66%)
30-36	9/15 (60%)
36-42	7/18 (39%)
42-46	8/26 (30%)
46-48	4/23 (16%)

Numbers in parentheses are percentages of motile spermatozoa samples.

\* Samples containing more than 15 per cent motile spermatozoa.

After examination of their motility, the fertilizing ability of rabbit spermatozoa recovered from the rat uterus was assessed in the following way: 0.03 ml. aliquots of rat uterine fluid containing between 150 and 300  $\times$  10<sup>3</sup> rabbit spermatozoa were inseminated directly into the ampulla of the Fallopian tube in oestrous rabbits<sup>11</sup>. An ovulation injection of human chorionic gonadotrophin ('Pregnyl', Organon) was given to the rabbit either soon before or after tubal insemination. The tubal ova were recovered 1-3 days after ovulation, and were examined for evidence of fertilization. The total fertile life of the spermatozoa in these experiments was estimated to be the length of time for which the spermatozoa were kept in the rat, plus the duration of their residence in the rabbit Fallopian tube before ovulation. The results of the fertility trials are shown in Table 2, the data of which refer only to those experiments carried out after a total of more than 32 h, that is, beyond the normal fertile life of rabbit spermatozoa in the rabbit female tract.

It is evident that after residing previously in the rat uterus, the fertile life of motile rabbit spermatozoa can be extended significantly, in some cases to almost twice the maximum which obtains normally in the rabbit (Fig. 1). Good motile rabbit sperm samples kept in the rat and rabbit for a total of about 45 h always fertilized a majority of ova, and significant numbers of supplementary and supernumerary spermatozoa were found in association with these fertilized ova. After this time, the numbers of spermatozoa about the ova diminished, but the fertilization rate remained high until 50 h, after which

it showed a general decline. Although motile rabbit spermatozoa collected from the rat after 48 h were able to fertilize 80 per cent of ova when placed in the rabbit Fallopian tube only 3 h before ovulation (No. 9), no fertilization was obtained with comparable samples (Nos. 15-17) inseminated similarly 11 h before ovulation.

Table 2. FERTILE LIFE OF MOTILE RABBIT SPERMATOZOA RECOVERED FROM THE RAT UTERUS

Animal No.	Hours of incubation before fertilization In rat uterus	In rabbit oviduct	Total	Percentage of ova fertilized
1	20	12	32	80
2	22	11	33	100
3	24	12	36	80
4	33	11	44	100
5	33	11	44	87.5
6	35	12	47	100
7	39	11	50	70
8	39	11	50	87.5
9	48	3	51	80
10	41	11	52	44
11	42	10	52	10
12	42	11	53	23
13	43	11	54	20
14	44	11	55	87.5
15	48	11	59	0
16	48	11	59	0
17	48	11	59	0

To conclude, in the rabbit and the other mammals investigated so far, the fertilizing ability of spermatozoa in the female declines some time before the capacity for motility. In the foreign environment of the rat uterus, however, rabbit spermatozoa evidently retain the ability to fertilize virtually up to the end of their motile life, which itself is not significantly extended in the rat. It thus becomes apparent that the normal fertile life of rabbit spermatozoa is not a fixed or inherent quality, and that this can be modified within the limits of the motile life, according to the nature of the female environment. The reason for the relative prolongation of the fertilizing ability of rabbit spermatozoa in the rat uterus is not clear. It is possible, however, that the extent of the fertile life of rabbit spermatozoa could be related to the time of capacitation, which must occur before spermatozoa can fertilize an ovum<sup>12,13</sup>. Noyes and Thibault<sup>9</sup> have suggested that some hours after the completion of functional capacitation, a "reversal" (probably better termed "deterioration") of this state may then occur, producing a loss of fertilizing ability in otherwise motile uterine or tubal spermatozoa. It appears from other related investigations, however, that rabbit spermatozoa become only partially capacitated in the uterus of the oestrous rat<sup>14</sup>. Capacitation would not therefore have been completed in the present experiments until 2-4 h after transfer of the spermatozoa from the rat into the rabbit oviduct, and thus only a few hours before the advent of the ova. Thus the prolongation of the fertile life of rabbit spermatozoa residing in the rat uterus might have been a result of the fact that the vulnerable capacitated state was never reached until after the entry of the spermatozoa into the rabbit oviduct and thus only a short while before fertilization.

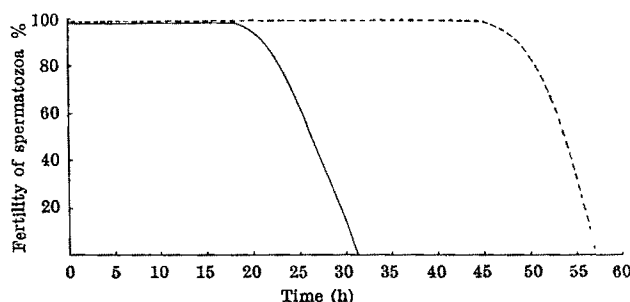


Fig. 1. Graphic representation of the fertile life of rabbit spermatozoa incubated in the uterus of the oestrous rat and rabbit respectively. —, Rabbit sperm incubated in rabbit uterus; ----, rabbit sperm incubated in rat uterus.

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## A Reversible Inhibitor of Nucleic Acid Synthesis

by

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2-Mercapto-1-( $\beta$ -4-pyridethyl) benzimidazole (MPB) inhibits the incorporation of tritiated uridine and thymidine into RNA and DNA in cell cultures. The effect is readily reversed when the compound is removed, and seems to be connected with the reversible fragmentation of the nucleoli seen when MPB is added to cell cultures.

CARTER found that addition of 2-mercapto-1-( $\beta$ -4-pyridethyl) benzimidazole (MPB) (Fig. 1) to the medium of cultured mammalian cells rapidly caused fragmentation and disappearance of nucleoli. This effect was studied in greater detail using Earle's *L* cells cultured in Eagle's medium containing 8 per cent calf serum. Nucleolar disintegration was visible under phase contrast illumination within 15 min of exposure to 40  $\mu$ g/ml. MPB. The effect was equally rapidly reversed by washing in normal medium and individual nucleoli were restored to their original shape and relative positions. When cells were treated for 24 h with 40  $\mu$ g/ml. MPB, and stained with acridine orange, the characteristic yellow nucleolar fluorescence was totally absent and the orange fluorescence in the cytoplasm very much reduced.

These observations suggested that MPB interferes with nucleic acid metabolism, and this was studied using radioactive precursors.

Stationary cultures of Earle's *L* cells were grown in 4 in.  $\times$  0.5 in. stoppered test-tubes on Eagle's growth medium containing 8 per cent inactivated calf

serum. 24 h before use, the cultures were changed to Eagle's medium containing 0.2 per cent bovine plasma albumin, and used 4–6 days after seeding.

Solutions of MPB were prepared by dissolving the crystalline compound in dimethyl sulphoxide at 1,000 times the maximum required concentration, and making serial dilutions from this stock in Eagle's growth medium. These solutions were added to tissue culture tubes, and the synthesis of nucleic acid and proteins measured by the uptake of radioactive precursors. Tritiated uridine, thymidine and lysine were added to tube cultures at concentrations of 0.33, 0.33 and 0.83  $\mu$ c./ml., respectively, at 37° C. Uptake was stopped after 15 min by washing the cells twice with ice-cold saline and once in 10 per cent perchloric acid. The acid was removed by a further wash with saline, and the cells were stripped from the glass by adding 1 ml. of 0.5 per cent trypsin in water and incubating at 37° C for 15 min. 7 ml. of dioxane scintillator containing 10 per cent naphthalene, 1 per cent PPO and 0.25 per cent POPOP were then added, and the radioactivity measured in a liquid scintillation counter. Results

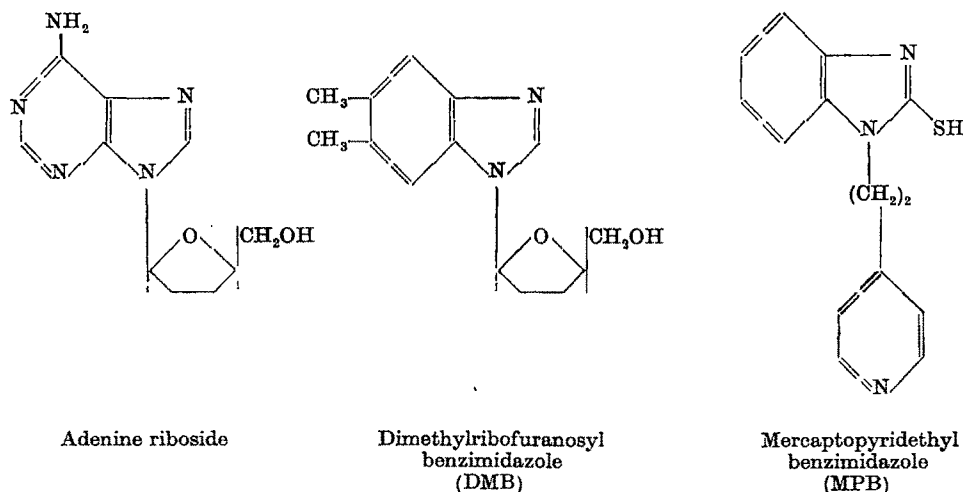


Fig. 1.



were calculated from the mean count on three tubes and the overall coefficient of variation of the method was found to be 7.9 per cent.

RNA synthesis and DNA synthesis are both rapidly suppressed when *L* cells are treated with 25  $\mu\text{g}/\text{ml}$ . MPB. The full inhibition of approximately 85 per cent is reached in less than 10 min, and RNA and DNA synthesis are affected more or less equally (Fig. 1). In comparison, actinomycin acts principally on RNA synthesis and, at 1  $\mu\text{g}/\text{ml}$ , takes more than an hour to produce the maximum inhibition of 85 per cent. After RNA synthesis is suppressed by actinomycin, the dependent protein synthesis of the cell also declines, primarily because messenger RNA is no longer being produced to activate the ribosomes<sup>1</sup>. The reduction in protein synthesis in *L* cells treated with either actinomycin or MPB is very similar (Fig. 2), which suggests that MPB, like actinomycin, acts principally on RNA synthesis and the effects on protein synthesis are secondary. Increasing the concentration of MPB from 25  $\mu\text{g}/\text{ml}$ . to 100  $\mu\text{g}/\text{ml}$ . does not produce any greater inhibition of protein synthesis, again suggesting that the effect is indirect.

The suppression of nucleic acid synthesis by MPB is proportional to the logarithmic compound concentration up to approximately 50  $\mu\text{g}/\text{ml}$ . (Fig. 3). The departure from linearity beyond this point probably occurs because the compound is incompletely dissolved in the tissue culture medium. Table 1 shows the concentration of MPB required to produce 50 per cent inhibition of RNA or DNA synthesis in a range of tissue culture cells. It appears that RNA and DNA synthesis are suppressed approximately equally, although cells such as HeLa and chick embryo fibroblasts may differ by as much as ten times in their sensitivity to the compound.

Table 1. CONCENTRATION OF MPB ( $\mu\text{g}/\text{ml}$ .) REQUIRED TO REDUCE RNA AND DNA SYNTHESIS BY 50 PER CENT IN A RANGE OF TISSUE CULTURE CELLS

	Primary calf kidney cells	<i>L</i> cells	HeLa	BHK 21	Chick embryo fibroblasts
RNA 50 per cent	7.6	6.3	3.1	7.9	50
DNA 50 per cent	9.3	3.5	2.5	4.0	50

It was noticed that preparations of calf kidney cells varied in their sensitivity to MPB and occasionally required 30–40  $\mu\text{g}/\text{ml}$ . MPB to give 50 per cent inhibition of RNA synthesis. The continuous cell lines, however, maintained a uniform sensitivity over a period of several months.

The inhibition of nucleic acid synthesis by MPB, if not continued for more than a few hours, can be reversed completely by a single change of medium. When *L* cells were treated with 25  $\mu\text{g}/\text{ml}$ . MPB, their DNA and RNA synthesis rapidly fell to 16.5 and 21.2 per cent, respectively, of the normal levels. These low rates were maintained in the presence of the compound and after 2 h were 17.8 and 22.2 per cent, respectively. The compound was removed by changing the medium, and nucleic acid synthesis recovered rapidly (Table 2).

After 20 min in fresh medium the rate of RNA synthesis was almost twice the normal value. This rise probably occurs because RNA precursors accumulated within the cells during the period of inhibition are rapidly utilized. The oscillations between 30 min and 120 min in the rates of both RNA and DNA synthesis probably arise during the re-establishment of various feedback mechanisms.

Table 2. RADIOACTIVITY INCORPORATED BY *L* CELLS AFTER TREATMENT WITH 25  $\mu\text{g}/\text{ml}$ . MPB

	10	20	30	60	120
MPB 25 $\mu\text{g}/\text{ml}$ . for 2 h, medium changed	{ DNA 601 (39.4)	986 (64.5)	1,577 (103)	590 (45.4)	847 (55.6)
	{ RNA 2,432 (70.5)	6,435 (188)	6,528 (192)	3,582 (105)	5,186 (152)
MPB 25 $\mu\text{g}/\text{ml}$ ., medium not changed	{ DNA 252 (16.5)	—	—	—	271 (17.8)
	{ RNA 732 (21.2)	—	—	—	771 (22.2)
Controls (no MPB, medium changed)	{ DNA 1,525 (100)	—	—	—	—
	{ RNA 3,457 (100)	—	—	—	—

DNA = tritiated thymidine uptake } 10 min counts corrected for background  
RNA = tritiated uridine uptake } Figures in parentheses are percentage of controls.

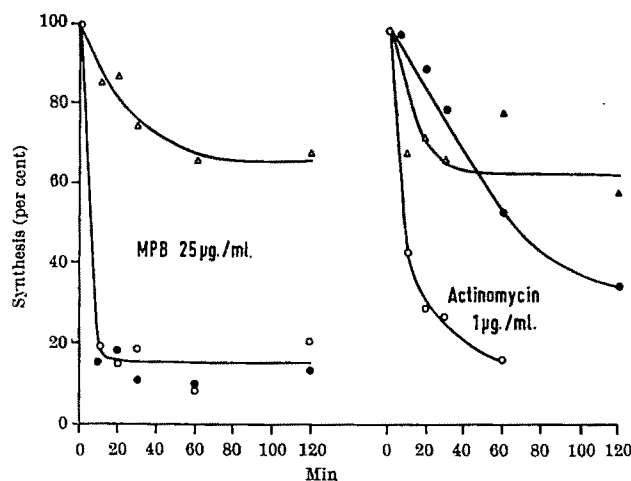


Fig. 2. Inhibition of RNA (●), DNA (○), and protein synthesis (Δ) in *L* cells by MPB (25  $\mu\text{g}/\text{ml}$ .) and actinomycin D (1  $\mu\text{g}/\text{ml}$ .).

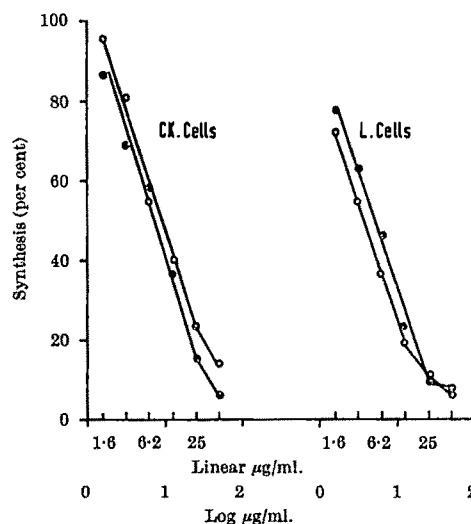


Fig. 3. Inhibition of RNA (●) and DNA (○) synthesis in *L* cells and primary calf kidney cells after 1 h in MPB.

It was interesting to know if MPB inhibited equally the synthesis of high and low molecular weight RNA. *L* cells were grown as monolayers in medical flats and treated with 25  $\mu\text{g}/\text{ml}$ . MPB for 18 h. 1  $\mu\text{C}$ . of tritiated uridine was added to each of fifteen treated flats and also to an equal number of controls. After 4 h at 37° C, all flats were chilled rapidly, drained and the cells scraped from the glass into a *tris* buffer (pH 7.2) containing 0.5 per cent sodium dodecyl sulphate. Impurities were removed by three extractions with phenol at room temperature and RNA precipitated with 2 per cent sodium acetate and 70 per cent ethanol. RNA was reprecipitated three times and finally dissolved in buffer to give approximately 1 mg/ml. A 1 ml. sample was layered on to a 5–20 per cent sucrose density gradient and centrifuged for 18 h at 20,000g. Fractions, each containing ten drops, were collected from the bottom of the tube and their  $OD_{260}$  and

radioactivity were determined. Three molecular species of RNA were recovered with sedimentation coefficients of 27, 18 and 4 S corresponding to high and low molecular weight ribosomal RNA and transfer, or soluble, RNA. The results of one experiment are given in Table 3. The specific activities of the three RNA species from cells treated with MPB expressed as percentages of the corresponding control values are: 27S, 46.7 per cent; 18S, 37.7 per cent; 4S, 54.9 per cent. A second experiment gave similar percentage inhibitions: 27S, 51.2 per cent; 18S, 45.4 per cent; 4S, 38.2 per cent. It seems, therefore, that MPB inhibits the synthesis of ribosomal and transfer RNA equally.

Table 3. SPECIFIC ACTIVITY OF RIBOSOMAL (27S, 18S) AND TRANSFER (4S) RNA ISOLATED FROM *L* CELLS TREATED FOR 18 H WITH 25  $\mu$ g/ml. MPB

	Sedimentation coefficient of RNA	Total weight of RNA ( $\mu$ g)	Tritiated uridine count (c.p.m.)	Specific activity of RNA (c.p.m./ $\mu$ g)	Percentage of controls
Controls	27S	11.2	580	5.19	—
	18S	7.2	290	4.08	—
	4S	5.6	435	7.75	—
25 $\mu$ g/ml. MPB	27S	10.4	250	2.41	46.7
	18S	8.1	125	1.54	37.7
	4S	4.7	200	4.26	54.9

To determine whether or not brief treatment of cells with MPB causes any permanent damage, cells were treated for 4 h with 25  $\mu$ g/ml. of the compound and their growth rate was then compared with untreated cells. As shown in Table 4, *L* cells continue to grow normally following a 4 h exposure to MPB.

Table 4. GROWTH OF *L* CELLS AFTER 4 H TREATMENT WITH 25  $\mu$ g/ml. MPB

	No. of cells per tube	
	Untreated cells	4 h in 25 $\mu$ g/ml. MPB
0 h	$4.35 \times 10^5$	$4.35 \times 10^5$
22 h	$5.15 \times 10^5$	$5.35 \times 10^5$
46 h	$5.81 \times 10^5$	$5.87 \times 10^5$

A consideration of the structure of MPB shows that it bears some resemblance to the nucleic acid ribosides (or deoxyribosides) of adenine and guanine, and also to the dimethylbenzimidazole moiety of vitamin B<sub>12</sub> (Fig. 1). MPB could therefore be acting as an antagonist to the synthesis of nucleic acid purine ribosides or vitamin B<sub>12</sub>. Vitamin B<sub>12</sub> contains adenosyl and dimethylbenzimidazolyl moieties and is involved in a wide range of biochemical pathways, some of which may be important in nucleic acid synthesis. We therefore tried to prevent the inhibition of nucleic acid synthesis in *L* cells by MPB with various mixtures of adenosine, guanosine and dimethylbenzimidazole, but without success (Table 5).

Table 5. SYNTHESIS OF RNA AND DNA BY *L* CELLS TREATED WITH 10  $\mu$ g/ml. MPB

The effects of adding 25  $\mu$ g/ml. adenosine, guanosine, orotic acid and dimethylbenzimidazole (DMB). 10 min counts corrected for background

	RNA		DNA	
	No MPB	MPB 10 $\mu$ g/ml.	No MPB	MPB 10 $\mu$ g/ml.
No additions	24,423	13,270 (54.8)	156,348	58,920 (37.3)
Adenosine	119,868	40,846 (34.2)	142,284	50,728 (35.8)
DMB	19,957	9,915 (50)	124,564	43,876 (35.6)
Adenosine + DMB	100,877	35,292 (35.3)	148,989	45,384 (30.4)
Guanosine	88,711	31,174 (35.2)	121,737	41,862 (34.6)
Guanosine + adenosine	92,620	36,113 (39)	113,982	41,260 (36.4)
Orotic acid	20,724	14,261 (68.2)	149,879	57,712 (37.5)

Figures in parentheses indicate percentage inhibition.

During this work it was noticed that the addition of adenosine, guanosine, or their 5'-phosphates to *L* cell cultures caused a stimulation of RNA synthesis, while the addition of orotic acid or cytidine did not. DNA synthesis was unaffected by the addition of these compounds. Table 5 shows that 25  $\mu$ g/ml. of adenosine caused RNA synthesis to increase five times, and 25  $\mu$ g/ml. of guanosine caused it to increase 2.7 times. In both cases, however, the percentage inhibition produced by 10  $\mu$ g/ml. of MPB is very similar to that found in cells to which no precursors have been added, which suggests that MPB

acts as a non-competitive inhibitor in the overall synthesis of RNA.

The effects of MPB on the phosphorylation of deoxyribosides, and their subsequent incorporation into DNA, were investigated using a kinase and polymerase system isolated from *Escherichia coli*. Tritiated thymidine was converted to thymidine triphosphate with a preparation similar to that of Okazaki and Kornberg<sup>2</sup>. The tritiated thymidine triphosphate was obtained in 90 per cent yield and was used in reaction mixtures containing the remaining three deoxyribonucleoside triphosphates, and a DNA primed DNA polymerase prepared according to Richardson *et al.*<sup>3</sup>. The reaction mixture was incubated at 37° C and after 40 min duplicate 0.1 ml. samples were placed on 1 in.  $\times$  1 in. squares of filter paper which were then washed successively with cold 10 per cent trichloroacetic acid, ethanol and ether. The precipitated material was counted by immersing the papers in a liquid scintillation fluid and counting for 10 min. The radioactive material was identified as DNA by its susceptibility to DNase and resistance to RNase. The results of these experiments are shown in Table 6. It will be seen that 25  $\mu$ g/ml. MPB has no effect on the rate of DNA formation from an equimolar mixture of the four deoxyribonucleoside triphosphates. It was found that DNA synthesis still occurred if individual nucleotides were replaced by the corresponding nucleosides in the polymerase incubation mixture, indicating the presence of nucleoside kinases in the crude polymerase preparation. Thus, with nucleosides in the reaction mixture, the rate of DNA formation is a measure of the nucleoside phosphorylase activity and, using this system, it was shown that MPB had no effect on the conversion of either deoxyadenosine or deoxyguanosine to the corresponding triphosphates. Similarly, MPB was shown to have no effect on the phosphorylation of thymine. Thus, it appears that MPB does not act on the phosphorylation of DNA nucleosides or on the polymerization of nucleotides into DNA. The corresponding RNA systems were not studied. The inhibition of RNA and DNA synthesis by MPB may occur at an earlier stage in purine or pyrimidine metabolism (for example, at the stage of conversion of arylimidazole intermediates to inosine) or MPB may activate an inhibitory feedback mechanism<sup>4,5</sup>.

Table 6. EFFECTS OF MPB ON DNA SYNTHESIS BY AN ISOLATED *E. coli* KINASE/POLYMERASE SYSTEM

Reaction mixture	25 $\mu$ g/ml. MPB	Acid precipitable radioactivity (DNA)
1 dGTP dATP	—	15,699
dCTP dTTP(°H)	+	16,317
2 dG dATP	—	10,375
dCTP dTTP(°H)	+	11,746
3* dGTP dA	—	23,730
dCTP dTTP(°H)	+	27,499

Ten minute counts corrected for background. dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate; dCTP, deoxycytidine triphosphate; dTTP(°H), deoxythymidine triphosphate (tritiated); dA, deoxyadenosine; dG, deoxyguanosine.

\* The reason for the stimulation of DNA synthesis by dA is not known.

Skehel, Hay, Cartwright and Burke have recently suggested (in a personal communication) that MPB acts as a competitive inhibitor in the phosphorylation of uridine to UMP in chick embryo fibroblasts. There may also be an effect on the conversion of UMP to UDP and UTP.

The experimental work for this article was done by Mr. H. Moores. The *E. coli* preparations were kindly supplied by Mr. A. Davies. MPB was first prepared in the laboratories of Midland Tar Distillers, Ltd., and generously supplied for this study.

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<sup>2</sup> Okazaki, R., and Kornberg, A., *J. Biol. Chem.*, 239, 267 (1964).

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<sup>4</sup> Farnham, A. E., *Virology*, 27, 73 (1965).

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## Growth Regulatory Effects of Cellular Interaction

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Euploid human fibroblasts and amnion cells showed contact inhibition of growth in monolayers. In mixed cultures the two cell types did not inhibit each other. Neither inhibited the growth of aneuploid epithelial cells. What is the significance of these phenomena with regard to the growth of malignant tumours?

THE marked diminution in the growth rate of human diploid fibroblasts soon after they achieve complete confluency on a glass surface could be related to the contact inhibition of motion as described by Abercrombie *et al.*<sup>1-3</sup>. Some of the molecular events associated with this decreasing growth rate have been described previously<sup>4</sup>. In contrast, most heteroploid human cells achieve much higher populations and mass/unit surface area, continuing to grow at a logarithmic rate well beyond the point of confluency, with the formation of compacted and multilayered sheets. The present communication describes the mutual effects of human diploid and heteroploid cells in mixed culture, measured in terms of cellular proliferation and protein synthesis.

The cell cultures used in the present experiments are listed in Table 1. They include ten strains of euploid fibroblasts from both foetal and postnatal tissues; euploid epithelial amnion cells in primary or secondary passage; three lines of heteroploid human cancer cells (HeLa, *KB*, *HEp2*), and four "spontaneous" transformants deriving from normal tissues (*FL* and *WISH* amnion, liver and conjunctiva), all with highly abnormal karyotypes, including wide differences in chromosome numbers and morphology within the same culture. There were also six cultures of cells with chromosomal aberrations involving one chromosome, three strains of trisomic and hypodiploid skin fibroblasts, and three epithelial cancer cells, one quadriploid (*RPMI* 2650) and two hypodiploid (*C4* and *C33*). All cultures were divided by trypsinization with 0.25 per cent–0.5 per cent 'Difco' 1–250 trypsin in a "minimal" medium free of calcium and magnesium<sup>6</sup>. Trypsinized

cells were shaken off into growth medium supplemented with 10 per cent serum (5 per cent calf and 5 per cent foetal calf) and with the six nutritionally non-essential amino-acids, each at 0.2 mmolar. Cultures of diploid cells were fed with fresh medium two to three times weekly, and divided approximately two- to eight-fold weekly; while heteroploid cells were fed every 1 to 2 days (depending on the amount of cell growth) and divided ten- to eighty-fold weekly. Proteins were determined by a modified Lowry procedure<sup>7</sup>.

*Interaction of euploid human cells in culture.* As illustrated in Table 2 and Fig. 1A, when several different strains of diploid fibroblastic human cells were simultaneously inoculated, the total cell protein and cell number after 5-8 days were usually the same as when a single cell type was planted. Similar results were obtained when a formed monolayer was superinoculated with cells of other strains (Fig. 1B). As shown by over-laying formed monolayers with other cells pre-labelled with  $^{14}\text{C}$ -thymidine, the latter not only adhered to the underlying cellular substrate, but remained adherent during the following 5-8 days of incubation, during which they did not significantly multiply. Ten different diploid fibroblast strains in thirty-three different combinations gave qualitatively similar results (Table 3). One strain (E-196) was, however, significantly less effective than the others in inhibiting the growth of superimposed fibroblasts.

Like the serially propagated diploid fibroblasts, amnion cells in primary culture formed a well defined monolayer of epithelioid cells, after which there was a marked decrease in growth rate. Nevertheless, such monolayers

Table 1. HUMAN CELL CULTURES USED IN PRESENT EXPERIMENTS

Euploid		Minor chromosomal aberrations		Gross chromosomal aberrations	
Fibroblasts	Epithelioid	Fibroblasts	Epithelioid	All epithelioid	Cancer
		Skin	Cancer	Spontaneous transformants from normal tissues	
Amnion	Amnion				
Foetal adrenal		<i>H46</i> (trisomic)*	<i>RPM1</i> 2050 (quasi-diploid)	Amnion ( <i>FL</i> )	<i>HEp2</i>
Foetal conjunct.		<i>H53</i> (hypodiploid)*		Amnion ( <i>WISH</i> )	HeLa
Foetal bone		<i>H170</i> (trisomic)*	<i>C4</i> (hypodiploid)	Conjunctiva	<i>KB</i>
Foetal lung					
Foetal skin					
Foetal thymus			<i>C33</i> (hypodiploid)		
<i>BAL</i> (skin)				Liver	
Detroit 510					
<i>F1</i>					

The sources of most of the cultures have been listed in a previous communication<sup>6</sup>.

\* Skin culture provided by Dr. Harold P. Klinger.

Table 2. MUTUAL GROWTH INHIBITION OF DIPLOID HUMAN CELLS IN CULTURE WHEN SIMULTANEOUSLY INOCULATED AS DISPERSED CELLS

Cell strain	Cell protein ( $\mu\text{g}$ ) on day						Cell count ( $\times 10^4$ ) on day					
	0*	2	4	6	8	12	0	2	4	6	8	12
(1) Detroit 510	112	205	285	365	437	592	7-4	20	28-5	36	42	54
(2) Embryonic bone	100	107	177	192	225	342	7-6	18	21-6	27	30	39
(3) BAL 3 (skin)	97	200	342	387	510	572	9	22	33	36	40	63
(4) Embryonic conjunctiva	107	155	262	447	552	912	10-2	22	39	42	72	75
1 + 2	275 (212)	252 (312)	365 (462)	442 (557)	472 (662)	612 (934)	22 (15)	30 (38)	39 (50)	45 (63)	51 (72)	66 (93)
3 + 4	257 (204)	312 (355)	357 (604)	432 (834)	462 (1,062)	742 (1,484)	16 (19)	32 (44)	42 (72)	48 (78)	66 (112)	72 (138)
1 + 2 + 3 + 4	420 (416)	372 (667)	494 (1,066)	342 (1,391)	582 (1,724)	792 (2,418)	45 (34)	52 (82)	84 (122)	84 (141)	87 (184)	75 (231)

Numbers in parentheses represent theoretical values if different cell lines grew independently, uninhibited by presence of other cells.

\* Twenty-four hours after planting.

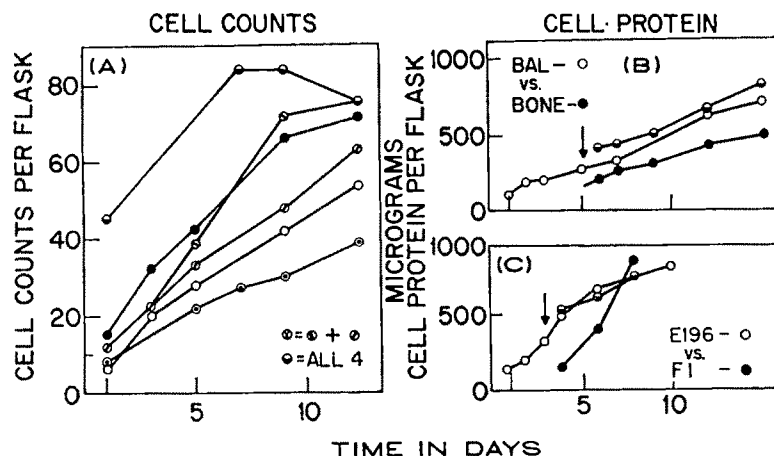


Fig. 1. Mutual growth inhibition of different strains of diploid human fibroblasts in mixed culture. (A) ○, Detroit 510; ◻, BAL; ◻, foetal conjunctiva; ◊, foetal bone; ●, BAL+foetal conjunctiva inoculated simultaneously; ◼, all four strains inoculated simultaneously. In (B) and (C) arrow indicates time of super-inoculation. (◼, Mixed cultures.)

usually failed to inhibit the growth of superimposed diploid fibroblasts, including the amnion-derived fibroblasts which regularly appear in the course of serial passage and eventually overgrow the epithelioid cells (Table 4 and Fig 2A).

**Interaction of euploid and aneuploid human cells in culture.** Fibroblastic skin cultures of trisomic and hypodiploid cells showed contact inhibition of growth, and gave mutual inhibition with euploid fibroblasts (see Table 4). In contrast, a quasi-diploid cancer cell (*RPMI* 2650) and two hypodiploid cancer cells, all epithelial, behaved like those with grossly abnormal karyotypes as described below: they attained much higher population densities than euploid cells in culture, and their growth was either not demonstrably inhibited by contact with the latter, or was inhibited to only a minor degree (see Table 6).

In general, the growth rate of most human cells with marked chromosomal aberrations does not decrease sharply when they form a complete monolayer. Instead,

they continue to grow and form a highly compacted layer in which the number of cells/cm<sup>2</sup> greatly exceeds that attained by euploid cells; and many such cultures form multilayered cell sheets. (With these heteroploid cells, the maximum cell population is determined by unknown factors presumably not directly related to intercellular contact. In some cultures the entire cellular sheet sloughs off the glass; sometimes large areas in the culture become necrotic; and individual cells may come off the multilayered sheet in large numbers.)

The growth of such heteroploid cells was usually not greatly inhibited by contact with diploid cells, whether the latter were epithelial amnion cells (Table 4) or fibroblastic (Tables 5 and 6), and whether the heteroploid cells were inoculated simultaneously with much larger numbers of diploid cells in mixed culture, or superimposed as a few discrete cells on to a completely formed and essentially non-growing layer of diploid cells. Although there was sometimes partial inhibition of the heteroploid cells in

Table 3. MUTUAL INHIBITION OF GROWTH BY DIPLOID HUMAN CELLS IN CULTURE

Cell strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) Foetal adrenal	+						+							
(2) Foetal bone		+					+							
(3) Foetal conj.			+				+			±	+	+	+	0, 0
(4) Foetal lung ( <i>WI</i> 38)				+	+			+						
(5) Foetal skin ( <i>E</i> -196)				0, ±	+		±		+			±, +		
(6) Foetal thymus					+	+								
(7) Post-natal skin ( <i>BAL</i> )			+				+	+	+	±				0, 0
(8) Post-natal skin (Detroit 510)		+	+				+	+	+	±				
(9) Post-natal skin ( <i>F1</i> )			+	±			+		+				+	
(10) Amnion fibroblasts		+						+		+				
(11) <i>H</i> 46 (trisomic)											+			
(12) <i>H</i> 53 (hypodiploid)					±							+		
(13) <i>H</i> 170 (trisomic)						0	0	0	0	0	+	+	+	+
(14) Amnion epithelial†	0	±	0	0	0	0	0	0	0	0	+	+	+	+, +

Cells inoculated either simultaneously (see Table 2 and Fig. 1) or sequentially (one strain overlaid on to established monolayer of another). +, Definite inhibition of growth after 6 days (< 25 per cent of control value, and usually < 10 per cent). 0, No significant inhibition of growth after 6 days (> 75 per cent of control values: see text page 1102). ±, 25 per cent to 75 per cent inhibition.

All cells used were fibroblastic except (14).

\* Self-inhibition on completion of monolayer.

† In primary or secondary passage.

Table 4. FAILURE OF EPITHELIOID AMNION CELLS IN PRIMARY CULTURE TO INHIBIT THE GROWTH OF SUPERIMPOSED CELLS, WHETHER DIPLOID OR HETEROPOID

Cell line	1*	4	6	8	11	Cell counts on day
(1) Amnion cells (primary epithelial)	1,075	975	935	835		68
(2) <i>BAL</i>	290	495	865	885		80
(3) Embryonic bone	93	415	710	1,185	1,005	124
(4) Amnion (fibroblastic)	93	405	1,025			57
(5) Cancer <i>RPMI</i> 2650 (quasi-diploid)	93	285	650			243
(6) Amnion- <i>FL</i> ("transformed", heteroploid)	117	650	1,455			276
1+2	925 (1,365)	1,225 (1,470)	1,655 (1,800)	1,605 (1,720)		158
1+3	1,095 (1,168)	1,215 (1,390)	1,275 (1,645)	1,385 (2,020)	1,405 (1,900)	173 (148)
1+4	1,375 (1,168)	1,275 (1,380)	1,765 (1,960)			175 (192)
1+5	1,075 (1,168)	1,095 (1,210)	1,325 (1,585)			117 (125+)
1+6	995 (1,192)	1,685 (1,725)	2,225 (2,390)			304 (311)
						334 (344)

Numbers in parentheses indicate theoretical value if there were no mutual inhibition; contrast Table 2.

\* Twenty-four hours after superinoculation of the primary amnion culture. The secondary inocula were all at  $10 \times 10^4$ /ml. ( $2 \times 10^4$ /cm<sup>2</sup>), except for the *FL* cell, which was inoculated at  $6 \times 10^4$ /ml. because of its more rapid growth. The freshly trypsinized primary amnion cells had been planted 8 days previously at  $50 \times 10^4$  cells/ml., only a fraction of which, however, survived.

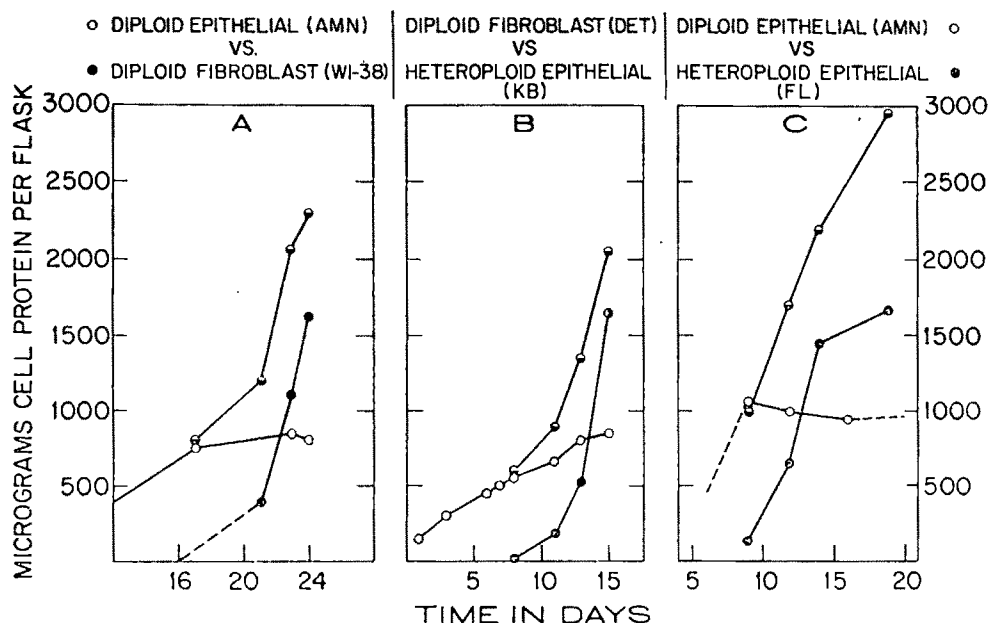


Fig. 2. Lack of mutual growth inhibition between human diploid fibroblasts, diploid epithelial (amnion) cells, and heteroploid cells deriving from normal (*FL*) and cancer (*KB*) tissues. ●—○, Mixed culture.

mixed culture (Table 6), that inhibition requires qualification. Even when the 6 day cumulative growth of heteroploid cells in mixed culture was as little as, for example, 25 per cent of that in the control, the average generation time had been prolonged only from, for example, 24 h to 36 h. It is nevertheless clear from the data of Table 6 that the escape of heteroploid human cells from growth inhibition by euploid cells is not an all or none phenomenon, and that the growth of some heteroploid cells was significantly inhibited by intimate association with some diploid cells (for example, *WI 38* versus *FL*, and *E-196* versus *FL* in Table 6).

In these mixed cultures, the heteroploid cells sometimes grew in compacted and discrete masses instead of the diffusely spread and confluent sheet of the pure culture, indicative of a limitation of their lateral movement, rather than on their growth and division (Fig. 3). Most of these heteroploid cells spread on a glass surface in pure culture and so it seems that their binding affinity to each other is less than that to glass, but greater than their affinity to a diploid monolayer. Published data about the relative mutual adhesiveness of malignant and of non-malignant cells are confusing. Although Coman<sup>17</sup> found cancer cells to have a decreased adhesiveness to each other, malignant mouse and rat cells have recently been reported to form larger aggregates in suspension than their non-malignant

counterparts<sup>18,20</sup>. Not all the heteroploid cells grew in this manner in mixed culture; some separated as they divided to form an "invasive" growth of discrete heteroploid cells which followed the ordered stratification of the diploid fibroblastic monolayer (Fig. 4). This difference in pattern of movement of the heteroploid cells was not, however, associated with differences in the rate of growth. Whether growing as compacted islands or as "invading" cells, many of the heteroploid cell lines grew essentially as rapidly in mixed culture as when they were inoculated alone (see Fig. 2B and Table 6).

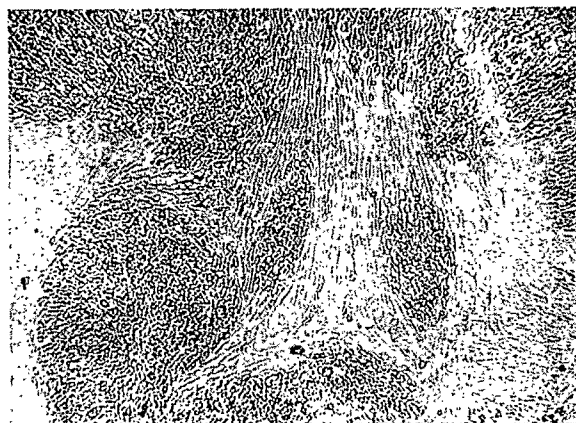


Fig. 3. Failure of contact-inhibited diploid human cells to inhibit the growth of heteroploid cells simultaneously inoculated. The fibroblasts were planted at  $40 \times 10^4/\text{ml}$  and  $8 \times 10^4/\text{cm}^2$ , sufficient to form a complete monolayer within a few hours. The heteroploid cells were planted as discrete cells at  $1/40$  of that level. The progressive growth of the heteroploid cells is shown below; the photograph was taken on day 5.

Strain	No. of cells/cm <sup>2</sup> × 10 <sup>4</sup>	μg of cell protein* on day				Heteroploid cells × 10 <sup>4</sup> on day 6
		1	3	5	6	
Diploid alone (Detroit 510)	8	390	458	537	569	—
Heteroploid alone (HeLa)	0.2	—	69	223	459	170
Diploid + heteroploid			513 (527)†	676 (676)	1,030 (1,028)	192

\* In  $15 \text{ cm}^2$  culture flask.

† Numbers in parenthesis indicate expected total cell protein if diploid cells did not inhibit growth of heteroploid cells. The measure of agreement between the calculated and experimental values is fortuitous.

Table 5. FAILURE OF AN ESTABLISHED MONOLAYER OF DIPLOID HUMAN FIBROBLASTS TO INHIBIT THE GROWTH OF SUPERIMPOSED HETEROPOID HUMAN CELLS

	Cell protein (μg on day)			No. of heteroploid cells on day 5 ‡
	0†	3	5	
(1) Diploid alone*	546	731	841	—
Heteroploid alone	—	224	981	$168 \times 10^4$
(2) Conjunctiva	—	88	374	—
(3) HeLa	—	141	531	—
(4) KB	—	124	851	$150 \times 10^4$
(5) Liver	—	—	—	—
Diploid and heteroploid				
1+2	609	941	1,511	$117 \times 10^4$
1+3	554	801	1,141	—
1+4	613	951	1,301	—
1+5	578	871	1,551	$200 \times 10^4$

Detroit 510 cells were planted at  $10 \times 10^4/\text{ml}$  and  $2 \times 10^4/\text{cm}^2$ , in a  $15 \text{ cm}^2$  flask. Four days later, the formed monolayers were superinoculated with small numbers of heteroploid cells ( $0.5 \times 10^4/\text{ml}$ , and  $0.1 \times 10^4/\text{cm}^2$ ).

\* Daily proteins in the diploid cultures before superinoculation measured 173, 348, 474 and 546 μg.

† On day of superinoculation.

‡ Determined by cloning the trypsinized cells: the heteroploid cells grew out to form characteristic compact clones readily distinguished from the occasional diploid cell which survived in the small inoculum used for cloning.



Table 6. EFFECT OF DIPLOID HUMAN CELLS ON THE GROWTH OF SUPERINOCULATED ANEUPLOID CELLS

Type of heteroploid cell superinoculated →	Conjunctiva	Deriving from normal tissue Liver	FL	WISH	HeLa	KB	Deriving from cancer tissues 2650	C33	C4
Underlying monolayer			Heteroploid cell growth expressed as percentage of that in control culture (based on cell proteins* unless otherwise indicated † ‡)						
Foetal adrenal			13, 10†	24, 46†	128	23			
Foetal conjunctiva			42, 50†	59, 78†		12			78
Foetal lung (WI 38)			0, 6†		17†, 16‡			68	48
			9†, 8‡						
Post-natal skin (BAL)		60		75, 70‡		127	20, 90†		
		80		82†		80, 100†			
Post-natal skin (Detroit 510)	73	116			39	50			
	73	83			32	59			
						72			
Foetal skin (E-196)			0, 6†	75	75	80	59	58	
			37, 14†	34, 40†	18, 3†	47			
				67, 104†	67, 25†, 72‡	100			
Primary amnion (epithelial)			88			100	65		

All the heteroploid cells here used were epithelioid (see Table 1). Small numbers of the heteroploid cells were superinoculated on to a formed monolayer of diploid cells.

\* For example, proteins in diploid monolayer, 800 mg; proteins of heteroploid, 1,600 mg; proteins of combined culture, 2,200 mg: growth of heteroploid in mixed culture,  $\frac{1,400}{1,600}$  88 per cent of that in control. This calculation assumes that the cells in the diploid monolayer were unaffected by the superinoculated heteroploid cells. If appreciable numbers were killed, then the amount of heteroploid growth would be correspondingly greater than the values indicated here. The occasional disparities between the values for heteroploid cells based on cloning and on proteins suggest that this may in some experiments indeed have been the case.

† Based on clonable heteroploid cells.

‡ Based on cell counts.

The mutual effects of heteroploid and diploid cells in culture have been investigated a number of times, with somewhat discrepant results. Most work involved species other than man, and many related to cellular movement rather than growth. Abercrombie *et al.*<sup>1-3</sup> observed that the outward movement of cells from mouse sarcoma 37 and Crocker sarcoma 180 explants was not inhibited by contact with cells similarly growing out of normal mouse or chick tissue explants, and suggested that this lack of inhibition could explain the tumorigenicity of these sarcomas *in vivo*. Although Barski and Béléhradek<sup>4</sup> found that the progressive movement of sheets of malignant mouse cells was inhibited when they came into contact with sheets of normal cells, a few "sentinel" cells penetrated into gaps among the normal cells to establish islands of invasive growth. Stoker<sup>5,6a</sup> found that the growth of hamster cells transformed by polyoma virus was inhibited when they were superimposed on a monolayer of normal hamster or mouse cells, and suggested that the discrepancy with Abercrombie's results could be related to the non-metastatic nature of the tumours formed by the polyoma-transformed cells.

In our experiments, human fibroblasts, whether diploid or with minor chromosomal aberrations, and epithelial amnion cells in primary or secondary culture, all showed a marked diminution in growth rate on the formation of a confluent monolayer on a glass surface. In mixed culture, most fibroblast strains similarly and indiscriminately inhibited each other's growth. In contrast, despite the inhibitory self-recognition of amnion cells and fibroblasts, they did not inhibit each other: whatever the "trigger" mechanism which reduces the rate of cellular growth in these confluent fibroblast and epithelial cell cultures, it is apparently initiated only by contact with cells of the same general type. This type-specific "recognition" is reminiscent of the sorting out of mixed cells in suspension to form homotypic clusters<sup>10-15</sup>. Such cohesive association may be an essential preliminary to the inhibition of macromolecular synthesis and growth in confluent surface cultures of euploid human cells, whether fibroblastic or epithelial. Whereas fibroblasts of different tissue origin would presumably adhere to initiate the growth-inhibitory process, there would be no such intimate association of fibroblasts and amnion cells.

The heteroploid epithelial human cells investigated here, whether originally derived from normal or cancer tissues, continued to grow rapidly even after they had formed a confluent monolayer. The juxtaposition of these cells did not initiate growth inhibition, and the limits on growth *in vitro* were set only later, by factors not yet defined. The growth rate of these heteroploid cells was usually either unaffected, or only slightly reduced, by

intimate contact with fully grown cultures of fibroblastic or epithelial diploid cells. Cancer cells with relatively minor chromosomal aberrations behaved like those with a grossly abnormal karyotype in their continuing growth even when surrounded by, and in intimate contact with, other human cells of either the same or unrelated types.

The dense clumps of growth sometimes formed by heteroploid human cells in the midst of a diploid monolayer (Fig. 3) contrast sharply with the limited growth of the surrounding normal cells, and resemble the similar piled-up cell plaques formed in fibroblastic cultures by some virus-transformed cells<sup>16</sup>. The pattern of growth appears analogous to a clump of tumour cells growing in an organ, surrounded by the normal cells from which they arose. Furthermore, there is a similarity between the invasive growth of cancer cells *in vivo*, and the occasional "invasion" of diploid fibroblasts by the uninhibited spreading growth of superinoculated heteroploid cells. Most cultured human cancer cells are heteroploid, and have escaped from contact inhibition of growth; and it is tempting to relate their partial escape from growth controls *in vivo* to that escape from contact inhibition. On this basis, the proximal cause of the malignant transformation would be an alteration in the cell surface in consequence of which it escapes from contact inhibition of growth; and its invasive properties would presumably reflect a similar escape from contact inhibition of move-

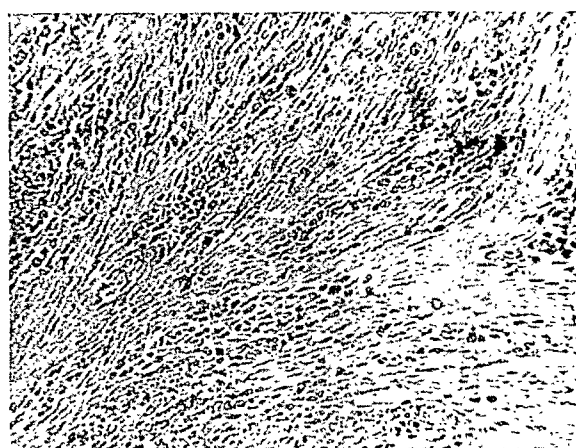


Fig. 4. Failure of a diploid fibroblast monolayer (foetal adrenal) to inhibit the growth of superinoculated heteroploid cells (amnion WISH). Human adrenal cells planted were at  $8 \times 10^4/\text{cm}^2$ . After 6 days, when the cells had achieved a confluent monolayer, they were overlaid with WISH cells at  $4 \times 10^4/\text{cm}^2$ . The photograph was taken 6 days later.

ment. The possible growth-regulatory significance of contact inhibition *in vivo* is not excluded by the finding that high concentrations of serum relieved contact inhibition in a spontaneously transformed mouse culture<sup>26</sup>, or that a perfused culture of human embryonic lung cells grew beyond the limits ordinarily posed in cultures fed intermittently<sup>27</sup>. If contact inhibition of growth *in vitro* can sometimes be reversed by methods less drastic than culture subdivision, this lends plausibility to the thesis that it may be a flexible growth-regulatory mechanism *in vivo* as well.

This perhaps simplistic interpretation poses a number of problems. (a) It is by no means certain that intercellular contact is the important growth-regulatory mechanism for normal cells *in vivo*; and if it is not, it would appear unlikely that escape from contact inhibition is the new element which permits cancer cells to grow in the whole animal. (One could make the *ad hoc* assumption of an associative rather than a causal relationship: that the surface change evidenced by escape from contact inhibition of growth *in vitro* is responsible also for the escape from unspecified growth-regulatory mechanisms *in vivo*.) (b) Many heteroploid human cells, as we have shown, originally derived from normal tissue and which arise in culture by "spontaneous" transformation, have also escaped from contact inhibition of growth, yet, unlike cells cultured from human cancers, most of these do not produce tumours on inoculation into, for example, the cheek pouch of the normal hamster<sup>21-23</sup>. (The validity of the latter negative result is, however, debatable in view of the complications introduced by species differences and rejection phenomena. With these human cells there may be no valid test of malignancy short of reinjection into the original donor; the validity even of this could be questioned.) (c) Finally, Stoker<sup>9</sup> has shown that although polyoma-transformed and tumorigenic hamster cells have escaped from contact inhibition of growth, in the sense that they pile up in culture to achieve abnormally high population densities, their growth is inhibited by contact with a fully compacted layer of the parent cell. Qualitatively similar results have been obtained in some (but not all) strains of virus-transformed human cells. There is thus reason to believe that escape from contact inhibition of growth may not be the sole or perhaps even the usual immediate determinant of malignant behaviour *in vivo*.

Escape from contact inhibition of growth *in vitro* is, however, only one of many parameters which distinguish cultured heteroploid cells from their diploid counterparts (Table 7), whether these heteroploid cells derive from cancers, are viral transformants, or have arisen spontaneously. It is true that these changes are not invariable. Thus (a) not all heteroploid cells can be grown in suspension. (b) Similarly, although enhanced cystathionase activity, endowing the cells with the capacity to grow in a cystine-free medium, is a frequent concomitant of chromosomal aberrations<sup>5</sup>, a quasi-diploid (RPMI 2650)

and a hypodiploid (C4) human cancer line which had escaped from contact inhibition of growth, and which had many of the other properties of grossly heteroploid cells, did not show that enhanced capacity to synthesize cystine. (c) Several non-human cells (a mouse fibroblast<sup>24</sup>, a rabbit lens cell (personal communication from A. Shapiro), and several sub-lines of hamster kidney cells<sup>25</sup>) which had transformed spontaneously in culture, with karyotypic and many associated phenotypic changes, continued to show marked contact inhibition of growth, with little or no cell proliferation after the formation of a complete monolayer, and correspondingly low maximum population densities. (d) The appearance of new antigens, a frequent consequence of viral transformation, has not yet been shown to be a regular occurrence in either cancer cells or "spontaneous" transformants, although some examples have been reported. Despite these and other exceptions, the phenotypic changes listed in Table 7 show a high degree of correlation, and some may well be causally related. An altered surface composition or structure in particular may underlie several of the differences regularly observed between human diploid cells and those with an abnormal karyotype.

The working hypothesis remains tenable (see Boveri<sup>28</sup>) that genomic changes of widely varying causation and extent, and frequently evidenced in visible chromosomal aberrations, can sometimes produce phenotypic changes which permit the altered cell to escape from normal control mechanisms *in vivo*, and thus determine the character of malignancy. Whether the specific change is primarily metabolic (analogous to the enhanced cystathionase activity of heteroploid cells), or whether, for example, a surface change leading to escape from contact inhibition of growth is the immediate determinant of the malignant transformation, should perhaps remain an open question.

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Table 7. COMMON\* PHENOTYPIC DIFFERENCES BETWEEN PARENT AND "TRANSFORMED" CELLS IN CULTURE

	Parent euploid cells	Transformants "spontaneous" virus-induced carcinogen-induced cancer
Karyotype	Euploid	Aneuploid
Life expectancy	Finite	Immortal
Malignancy	0	+
Plating efficiency	<1%	50%-100%
Surface properties		
Contact inhibition of movement and growth	+	0
Maximum population density/cm <sup>2</sup>		Increased
Growth in suspension	0	+
Arrangement of fibroblasts in packed culture	Ordered	Random
Morphological changes		+
New antigens		+
Biochemical activities		
Alkaline phosphatase		Increased
Cystathionase		Increased

\* Common, but not invariable, in that not all transformants show all the phenotypic changes listed.

## LETTERS TO THE EDITOR

## ASTRONOMY

## An Experimental Test of the Mechanism of Radio Emission from Cosmic Ray Showers

THE suggestion by Askaryan<sup>1</sup> that a detectable radio pulse should be emitted by an extensive air shower was based on the coherent emission from a negative charge excess in the shower front. A more detailed analysis by Kahn and Lerche<sup>2</sup> showed that a radio pulse may also be emitted by the transverse current in the shower front which results from separation of charges in the Earth's magnetic field. Using a restricted model of the shower, consisting of a ring of charges, they showed that the radiation resulting from separation of charges would be expected to dominate over that from an excess of charges.

Radiation from separation of charges would be polarized perpendicular to the Earth's magnetic field, while that from an excess of charges would be polarized radially from the shower centre. Measurements of radio polarization have been obtained by Jelley *et al.*<sup>3</sup>, and by Allan and Jones<sup>4</sup>, who found no clear evidence for an east-west polarization, and by Borzhovski *et al.*<sup>5</sup>, who claimed to have found statistical evidence in favour of it.

A further test of the relative importance of the effects of the geomagnetic field and the separation of charges is to compare the magnitudes of pulses radiated by showers travelling along and perpendicular to the Earth's magnetic field. We have therefore compared the radio pulses received in identical aerial systems directed to the north and south at Jodrell Bank, where the dip angle is 68°. The aerials were corner reflectors two wavelengths long, with a beam-width of 45° directed at 45° to the zenith. The receiving equipment and the Geiger-Müller counters used to detect the shower front were similar to those used in the original detection of the radio pulses, except that further counters were added to form seven equilateral triangles extended along an east-west line 175 metres long. This line also contained the aerials, mounted back-to-back 29 m from the centre. Coincidences were registered whenever the three counters of any one triangle were simultaneously struck, and the two receiver outputs were then photographed. About eight coincidences per hour were obtained with this system, which corresponds to a threshold energy of about  $5 \cdot 10^{16}$  eV.

The analysis consisted of recording the pulse heights in a channel centred on the expected position on the oscilloscope time base, with a width corresponding to the measured time jitter of  $\pm 0.5$   $\mu$ sec which resulted from the variable delay in the Geiger-Müller counters. A reference channel 10  $\mu$ sec later was also analysed in the same way. The results of this analysis are presented in Table 1 as the number of pulses in each channel the amplitude of which exceeds one of three specified levels, increasing by 6 decibels in peak power.

We note that there is a consistent excess of large pulses in the two signal channels but that there is no evidence of any difference between the north and the south channels.

Table 1

Level	1	2	3
South signal channel	210	38	2
North signal channel	247	36	1
South signal—south reference	41	17	2
North signal—north reference	43	14	1

Although the statistics are poor, it is already clear that radio pulses arriving across the lines of the geomagnetic field are not usually stronger than those which arrive along them. The expected ratio of the numbers in the lower two rows of Table 1 is about 7:1 if the shower numbers vary as  $\cos^7$  (zenith angle) as recorded by Macleod<sup>6</sup> and the radio pulses wholly arise from the effects of the geomagnetic field. We must therefore conclude that the effects of the separation of charges do not dominate as Kahn and Lerche suggest they do. One possible explanation of this discrepancy between theory and observation is that the showers contain a higher excess of charges than was suggested by Askaryan, consisting, perhaps, of electrons of lower energy than those normally recorded experimentally.

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A Galactic Discontinuity at  $l^{\text{II}} = 140^\circ$ 

THERE is substantial evidence of a major discontinuity in the structure of the Galaxy at  $l^{\text{II}} = 140^\circ$ . This evidence is provided by large changes in the numbers of Wolf-Rayet stars, dust-embedded stars and interstellar globules, in the wavelength dependence of the position angles of interstellar polarization of starlight, and in the orientation of the polarization vectors of galactic radio waves on each side of that longitude.

The Wolf-Rayet stars provided the first evidence of the discontinuity. It was pointed out by Vorontsov-Velyaminov<sup>1</sup>, and confirmed by Roberts<sup>2</sup> and by Stephenson<sup>3</sup>, that the anticentre region from  $l^{\text{II}} = 140^\circ$  to  $l^{\text{II}} = 225^\circ$  is completely devoid of Wolf-Rayet stars (Fig. 1a).

Hoffleit<sup>4</sup> and Blanco and Williams<sup>5</sup> discovered groups of stars in which the brightest are the most heavily reddened, apparently by circumstellar obscuring clouds. A survey by Reddish<sup>6</sup> has shown that such dust-embedded stars occur in a considerable number of associations and very young clusters, but that none of these are in the range of galactic longitudes from  $l^{\text{II}} = 136^\circ$  to  $l^{\text{II}} = 253^\circ$  (Fig. 1b). In addition to this general relationship with the distribution of Wolf-Rayet stars there is a more detailed relationship in that Wolf-Rayet stars are most often found in systems containing dust-embedded stars.

Estimates of the masses of interstellar globules, of the circumstellar clouds around dust-embedded stars, and of the shells around Wolf-Rayet stars, show that the masses are similar and of the same order as those of early type stars<sup>6</sup>.

These observations support the views that massive stars form from globules and that Wolf-Rayet stars are very young objects in the last stages of contraction to the main sequence. They indicate that the region from  $l^{\text{II}} = 140^\circ$  to  $l^{\text{II}} = 250^\circ$  is not at present active in the formation of massive new stars.

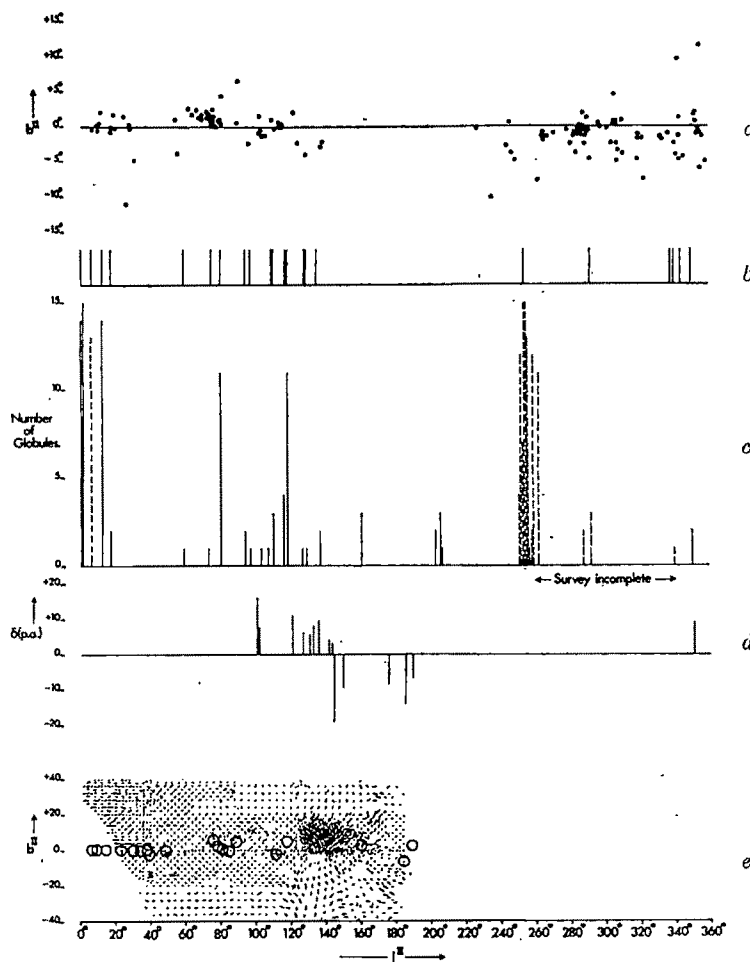


Fig. 1. The distributions with galactic longitude of: *a*, Wolf-Rayet stars; *b*, associations and young clusters containing dust-embedded stars; *c*, globules (broken lines indicate provisional values); *d*, the rotation with wavelength of the p.a. of the polarization vector of starlight, from  $1/\lambda = 3.04\mu^{-1}$  to  $1/\lambda = 1.05\mu^{-1}$ ; *e*, polarization vectors of galactic radio noise at 408  $\mu$ c/s.

The discovery of these relationships indicated that a survey of the distribution of globules in young clusters and associations would be useful. The survey is being carried out by M. E. Sim at the Royal Observatory, Edinburgh, for the sixty-six clusters and associations investigated for dust-embedded stars, and is three-quarters complete. The results show a substantial deficiency in the numbers of globules found in associations and young clusters in the longitude range  $l = 120^\circ$  to  $l = 250^\circ$  (Fig. 1c).

Measurements of the wavelength dependence of the position angles of the interstellar polarization vectors of starlight, reported by Coyne and Gehrels<sup>7</sup>, show a systematic dependence on galactic longitude for about half the stars. With increasing wavelength, the vectors rotate clockwise when  $l < 144^\circ$ , anticlockwise when  $l > 144^\circ$  (Fig. 1d). It has been suggested that the effect may be the result of a longitudinal compression of the spiral arms at the smaller longitudes, and a rarefaction at the greater ones<sup>8</sup>. The possibility that the effect is due to the angle of viewing the spiral arms would seem to be discounted by the coincidence of the longitude of null rotation with the boundary of the zones of deficiency of Wolf-Rayet stars, dust-embedded stars, and globules, which indicate a change in the structure of the spiral arm.

The polarization of the galactic radio noise<sup>9,10</sup> at frequencies of 408 Mc/s and 610 Mc/s is very strong at  $l = 141^\circ$ ,  $b = +6^\circ$ , a region which appears to be the

focal point of a pattern of symmetry in the polarization (Fig. 1e).

A large change in the apparent space densities of Wolf-Rayet stars, dust-embedded stars, and globules, and in the structure of the interstellar medium as indicated by the polarizations of starlight and radio noise, occurs close to  $l = 140^\circ$ . These changes indicate a major discontinuity in the structure of the Galaxy at that longitude. It is possible that a similar discontinuity in the reverse sense occurs at  $l = 250^\circ$ .

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## Measurements of OH Emission Sources with an Interferometer of High Resolution

PREVIOUS experiments at Lincoln Laboratories, M.I.T.<sup>1</sup>, California Institute of Technology<sup>2</sup> and Jodrell Bank (to be published) to determine the angular size of the OH emission regions have used interferometers with baselines of about 0.5 km ( $\sim 3,000$  wavelengths). None of the emission sources was resolved which showed that their angular sizes were less than 20 arc secs. Between January 9 and January 27, 1967, measurements were made on a baseline of 127 km ( $7.0 \times 10^5$  wavelengths at 18 cm) in a direction approximately north-south (hour angle,  $353.8^\circ$ ;  $\delta$ ,  $-37.2^\circ$ ). This represents an increase of 200 in resolving power compared with the previous measurements.

The telescopes used were the Mark II at Jodrell Bank and one of the 25 m aerials of the Royal Radar Establishment. Parametric amplifiers gave an overall system noise of about 160° K on each telescope. Both feed systems could be remotely switched to receive left-hand or right-hand circularly polarized radiation. Linear polarization was also measured using oppositely polarized feeds at the two ends of the baseline. The phase stability of the system, although not capable of giving absolute phase measurements, was adequate to give a steady fringe rate over periods of several minutes and consequently the fringe rate was automatically slowed from a natural fringe frequency of about 40/sec to 1/min. Six adjacent 3 kc/s bandwidth filters and a 200 kc/s bandwidth filter centred on the narrow band frequencies were used. The output of the various channels was fed directly into an *Argus* 100 computer which integrated the output over intervals of 4 min.

Observations were made of the features in the 1,665 Mc/s spectra of the OH emission sources near W3, W24-B2, W49 and W75, and in the 1,667 Mc/s spectrum of W49. The positions used for this investigation were those given by Rogers *et al.*<sup>1</sup> and others obtained at Jodrell Bank (to be published). Fringes were observed over a wide range of hour angles for the strong features in all the spectra investigated.

Fringe amplitudes were converted into flux densities by comparison with the fringes obtained from two calibrating sources, 3C 273 B and 3C 454.3. Both these sources are known to be unresolved<sup>3</sup>, and were assumed to have flux densities of  $26.9$  and  $11.5 \times 10^{-26}$  Wm<sup>-2</sup> (c/s)<sup>-1</sup>, respectively. Zero baseline fluxes of the OH spectral features were measured with the Mark II telescope alone and were calibrated by observations of Cassiopeia A ( $2.02 \times 10^{-23}$  Wm<sup>-2</sup> (c/s)<sup>-1</sup>). The fringe visibilities near maximum resolution of the stronger components of the OH sources were found on initial reduction to be greater than 0.6. On

the simplifying assumption that the emission regions have a gaussian brightness distribution the half-power widths of W3, W49 and W75 are less than 0.1 arc seconds. In the case of W24-B2 the maximum projected baseline is smaller and the width is less than 0.2 arc seconds. Final reduction of the data is expected to give more accurate fringe visibilities.

Information on the angular size of the various components can also be derived from observations of fringe amplitude as a function of hour angle. Data for the strong component in the spectrum of W3 at +249 kc/s in right-hand polarization (Institute of Radio Engineers convention) have been reduced in some detail and show a fringe amplitude which does not change by more than 15 per cent between projected baselines of 0.4–1.0 times the maximum. In addition the maximum baseline flux is within 15 per cent of the zero baseline flux. It is thus highly probable that the component is unresolved in all position angles, and has a fringe visibility greater than 0.85. The corresponding diameter of the equivalent gaussian source is less than 0.05 arc seconds.

The linear sizes corresponding to these derived limits of the angular sizes can be calculated from the distances of the HII regions which have been identified with the OH emission sources. Values are given in Table 1. In the case of the nearer OH sources (W3 and W75) the corresponding linear diameters are less than about  $6 \times 10^{-4}$  parsec (pc) and for the more distant OH sources (W24-B2 and W49) the linear diameters are less than about  $10^{-3}$  pc. The upper limit to the diameter of the apparent emitting area of the +249 kc/s right-hand component of W3 is  $5 \times 10^{-4}$  pc which is only about the diameter of the orbit of Pluto.

Lower limits to the equivalent brightness temperatures  $T_B$  of spectral components in the various OH sources can be derived from the measured angular diameters and flux densities. Values are given in Table 1 for the strongest component of each source.  $T_B$  is typically  $> 3 \times 10^9$  °K and for the +249 kc/s right-hand feature of W3 it is  $> 10^{11}$  °K which is a factor of  $5 \times 10^4$  greater than the limits set for this feature in previous experiments.

Further information about the structure of each OH emission source can be deduced from measurements of the relative phases of the spectral components as a function of hour angle. The sources W3, W49 and W75 show significant changes of relative phase between components.

Table 1. DIAMETERS OF COMPONENTS OF OH EMISSION SOURCES

OH source near	Assumed distance (kpc)	Angular diameter ("arc)	Linear diameter (pc)	$T_B$ of strongest component (°K)
W75	1.5	$< 0.1$	$< 7 \times 10^{-4}$	$> 6 \times 10^9$
W3	2	$< 0.05$	$< 5 \times 10^{-4}$	$> 1.0 \times 10^{11}$
W24-B2	10	$< 0.2$	$< 1.0 \times 10^{-3}$	$> 3.5 \times 10^9$
W49	15	$< 0.1$	$< 7 \times 10^{-3}$	$> 1.6 \times 10^{10}$

### W3 RH polarization

Jan. 24, 1967

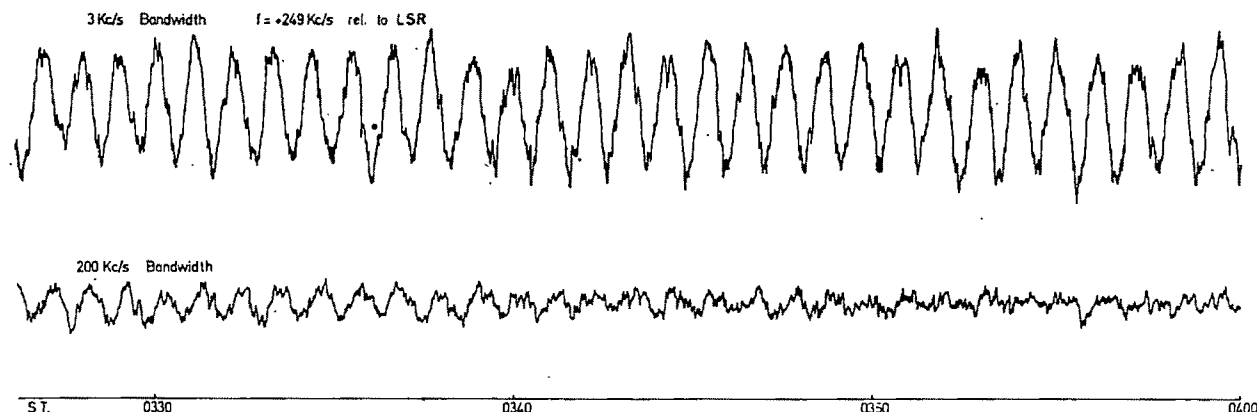


Fig. 1. 200 kc/s and 3 kc/s bandwidth fringes obtained with right-hand polarization on W3 on January 24, 1967. The 3 kc/s channel is centred on the +249 kc/s right-hand component. The variation in amplitude on the 200 kc/s channel results from the change of relative phase of the various spectral components of W3 as a function of time.



(So far no analysis has been made of W24-B2.) This was also evident on the 200 kc/s bandwidth output which showed changes of amplitude over periods of an hour. The effect is illustrated in Fig. 1, which is a facsimile of a section of the 200 kc/s and 3 kc/s bandwidth analogue records obtained for W3. In this source, for example, it is found that the +255 kc/s left-hand component is displaced 1.4" relative to the +250 kc/s left-hand component. This displacement is thirty times greater than the upper limit of the apparent size of the components. In the case of W49 the two major complexes found at shorter baselines to be separated by 100 arc seconds were clearly discernible by their different fringe rates. Within complex A (R.A. = 19h 07m 50.0s,  $\delta = 09^\circ 01' 12''$ , 1950.0 co-ordinates) the angular separation of the strongest spectral features was found to be about 0.1 arc seconds. The linear separation of the components in W3 and W49A is about 0.01 pc.

From these observations a picture emerges of compact OH emission sources near HII regions with overall dimensions of about  $10^{-2}$  pc. Within these sources narrow spectral components of characteristically high left-hand or right-hand circular polarization are emitted from smaller regions with apparent diameters of less than  $10^{-3}$  pc. Such a source configuration would be consistent with a maser amplification process for the OH emission (see, for example, ref. 4). On the basis of a maser process the observed brightness temperature limits are equivalent to an amplification factor ( $\ln A$ ) greater than twenty which would imply that the emission of a particular frequency component would come from a very small fraction of the volume of the OH region. A narrowing of the emission lines by a factor of more than 5.5 would result from this amplification. If the overall spread of velocity in the spectra of the sources is representative of the width of the unamplified emission, then the ratio of this spread to the width of individual lines (typically 5–40) would be the true narrowing factor. The present data are consistent with these values.

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## PLANETARY SCIENCE

### Recent Changes in the Magnetic Dipole Moment of the Earth

IN the course of an investigation into magnetic secular variation, a number of spherical harmonic analyses were performed on successive 5 year means of results from eighty magnetic observatories. From the first three spherical harmonic coefficients the moment of the equivalent dipole of the geomagnetic field ( $M$ ) can be deduced in terms of the radius of the Earth ( $a$ ) from the relation  $Ma^{-3} = \{(g_0^0)^2 + (g_1^1)^2 + (h_1^1)^2\}^{\frac{1}{2}}$ .

The same set of observatories was used at each epoch in order to avoid spurious effects due to different conditioning of the equations. Great care was also taken to remove discontinuities at an observatory due to changes of site or standard. In view of these precautions it is considered that the change of moment between epochs is well determined, although no special claim is made for the accuracy of the absolute value.

The results are given in Table 1.

Table 1

Epoch	$Ma^{-3}$ $\gamma$	$\Delta Ma^{-3}$ $\gamma/\text{yr}$
1942-5	31,324	$-7 \pm 1$
1947-5	31,291	$-7 \pm 1$
1952-5	31,257	$-18 \pm 1$
1957-5	31,194	$-17 \pm 1$
1962-5	31,111	

Table 2

Epoch	$Ma^{-3}$ $\gamma$	$\Delta Ma^{-3}$ $\gamma/\text{yr}$
Nagata <sup>1</sup> 1922-1955	—	-12
Nagata <sup>1</sup> 1945-1960	—	-15
Finch and Leaton <sup>2</sup> 1955	31,200	—
Leaton <sup>3</sup> 1955	—	$-11 \pm 1$
Nagata <sup>1</sup> 1955-1960	—	-17
Leaton <sup>4</sup> 1960	—	$-12 \pm 1$
Nagata <sup>1</sup> 1958-1962	—	-16
Hurwitz <i>et al.</i> <sup>5</sup> 1965	30,981	—
Leaton <i>et al.</i> <sup>6</sup> 1965	30,987	$-16 \pm 2$

It is of interest to compare these results with those from other sources (Table 2).

The suggestion by Procopiu<sup>7</sup> and others<sup>8-10</sup> that the dipole moment passed through a minimum some time between 1930 and 1952 has been questioned by Nagata and Rikitake<sup>11</sup> and by Leaton<sup>3</sup>. The figures in Table 1 clearly indicate that the dipole moment continues to decrease, and suggest an increasing rate of decrease. It is tempting to suggest an eventual field reversal within the next few thousand years.

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### Rotational Speed of the Upper Atmosphere, from the Orbits of Satellites 1966-51A, B and C

THE rotational speed of the upper atmosphere at heights near that of the perigee of a satellite can in principle<sup>1</sup> be evaluated from the change  $\Delta i$  in the orbital inclination  $i$  of the satellite. If  $\Lambda$  denotes the ratio of the atmospheric angular velocity to the Earth's angular velocity,  $\Delta i$  is given<sup>2</sup>, as a first approximation, by

$$\Delta i \approx 0.007 \Lambda \sin i \Delta T \text{ degrees}$$

where  $\Delta T$  is the change in orbital period, in minutes. In practice the change in inclination,  $\Delta i$ , is often too small to be accurately measurable, and the most accurate values of  $\Lambda$  are likely to be obtained from the orbits for which  $\Delta i$  is largest. We therefore seek orbits which (i) are near-polar and (ii) show a large change in orbital period.

This method of determining atmospheric rotational speed was applied<sup>2,3</sup> to twenty satellites in the years 1958-65, and the results showed that the atmosphere at heights of 200-300 km was on average rotating faster than the Earth: the mean value of  $\Lambda$  was 1.29, with a root mean square scatter of 0.17. These previous values of  $\Lambda$  were averages over the whole lifetime of the satellites, often 2 or 3 years, because the orbital data were not accurate enough to reveal the change in  $i$  over shorter time intervals. Since  $\Lambda$  may vary with time, there is a clear need for more accurate values over shorter time intervals.

Of all the satellites so far launched, those sent into orbit by an *Atlas-Agena* vehicle on June 9, 1966, are potentially the best for determining  $\Lambda$ . In this launch three objects were placed in orbit: 1966-51A, the *Agenda* rocket, with additional payload; and two small satellites, *Secor* 6 (1966-51B) and *ORS* 2 (1966-51C), which both transmitted radio signals for about 3 months. Initially, all three objects were in polar orbits with periods of about 125 min and perigee heights near 190 km. Because the perigee heights were so low, the decrease in period has been rapid: 1966-51A came down on December 3, 1966, and the other two are expected to decay before July 1967.

The orbital data at present available consist of orbital elements determined by the National Aeronautics and Space Administration for 1966-51B and C during the first 3 months of their lives, and U.S. Air Force "five-card elements" for all three satellites. The best values of inclination are the NASA elements for 1966-51C, shown in Fig. 1. The theoretical variation of  $i$  has been calculated by the methods described in ref. 3, and the value of  $\Lambda$  which best fits the observed values has been chosen. The resulting theoretical curve, with  $\Lambda = 1.5$ , is shown in Fig. 1. The decrease in orbital inclination is usually most rapid when perigee is near the equator, and Fig. 1 clearly shows this effect for 1966-51C. Most of the decrease in inclination occurred in August 1966 when perigee was near the sunset line and at latitudes less than 40°. So 1966-51C suggests that  $\Lambda = 1.5 \pm 0.1$  at sunset at heights near 200 km at latitudes less than 40° in August 1966.

The data for the other two satellites, though not in conflict with 1966-51C, are less accurate. Preliminary results are  $\Lambda = 1.1 \pm 0.2$  from 1966-51A, averaged between June and December 1966, and  $\Lambda = 1.2 \pm 0.2$  from 1966-51B,

averaged between June 1966 and February 1967. We hope to derive a better orbit for 1966-51A from the large number of visual observations available; a better value of  $\Lambda$  from 1966-51B should be obtainable after its decay.

The value obtained for 1966-51C is interesting because it refers to a much shorter time interval than any previous determination by this method. Also the high value of  $\Lambda$ , 1.5, which corresponds to a west-to-east wind of over 200 m/sec at the equator, suggests that there is a particularly strong west-to-east wind at about sunset, a conclusion which is consistent with the small number of results from vapour trails at heights near 200 km.

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### Stony Meteorites bearing Maskelynite

Its discoverer, Gustav Tschermak, assigned the name "Maskelynite" to an isotropic substance with the chemical composition of plagioclase, which occurred in the Shergotty achondritic stony meteorite<sup>1</sup>. He first regarded this as a new cubic mineral, but later, finding it also in several chondrites and realizing that it formed pseudomorphs after plagioclase, he reinterpreted maskelynite as remelted or otherwise transformed plagioclase glass<sup>2</sup>. Because its amorphous character was not confirmed and no chemical analyses were made of the chondritic variety, however, Tschermak's reassessment of the nature of maskelynite was not universally accepted by meteorite petrographers.

During a reinvestigation of maskelynite-bearing meteorites undertaken at the British Museum (Natural History), London, concentrates of maskelynite were prepared from two achondrites, Shergotty and Zagami, and from the chondrite Château-Renard. Chemical analyses of the two former confirmed their labradoritic composition (both recalculating in terms of feldspar end members to  $Or_2Ab_{48}An_{51}$  molecular per cent), while the latter has the composition of a plagioclase close to the albite-oligoclase boundary ( $Or_5Ab_{88}An_9$ ). Long exposure X-ray powder photographs of all three concentrates possessed no diffraction lines, indicating that maskelynite lacks a crystalline structure. Taken with the textural evidence of pseudomorphism, these new data substantiate Tschermak's final conclusion.

Maskelynite is known in only three achondrites, Shergotty, Zagami and Padvarninkai. The first two stones are closely similar in texture and mineralogy. Each contains two monoclinic pyroxenes, augite and pigeonite, both showing extensive compositional zoning (Fig. 1); and also two opaque oxide minerals, ilmenite and titanite.

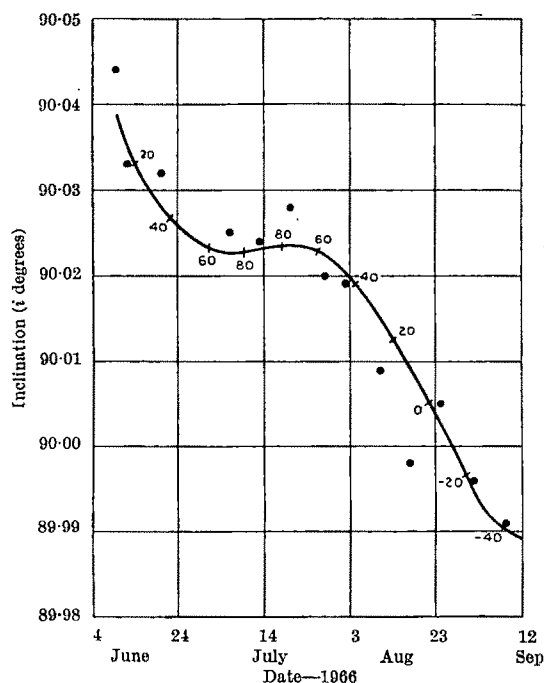


Fig. 1. Values of orbital inclination for 1966-51C, with theoretical curve ( $\Lambda = 1.5$ ). ●, Values from NASA bulletins; —, theoretical curve. Numbers on the curve indicate perigee latitude in degrees.

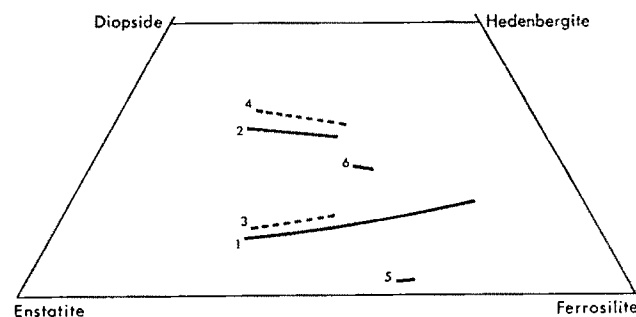


Fig. 1. Pyroxene quadrilateral (portion of a molecular per cent triangular plot of calcium, magnesium and iron) showing compositional trends of (1) pigeonites in Shergotty, (2) augites in Shergotty, (3) pigeonites in Zagami, (4) augites in Zagami, (5) ferrohypersthene in Padvarninkai, (6) subcalcic ferroaugite in Padvarninkai. Based on electron probe microanalyses.

magnetite (averaging 65 molecular per cent ulvöspinel content). A second isotropic substance with refractive index 1.465–1.475, probably silica glass, occurs as a rare constituent in Shergotty but is apparently absent from Zagami. By comparison Padvarninkai differs significantly; it is a brecciated stone<sup>3</sup>, and although texturally similar to Shergotty and Zagami, it lacks titanomagnetite and contains comparatively unzoned ferrohypersthene and subcalcic ferroaugite as its two pyroxenes (see Fig. 1). Furthermore, it retains some untransformed bytownite plagioclase ( $An_{85}$ ) and the refractive index of its maskelynite ( $n=1.571$ ) suggests a bytownitic composition quite different from that of the Shergotty and Zagami maskelynites. Besides extending the known chemical variation in maskelynites to cover the greater part of plagioclase compositional range, these observations reveal that maskelynite occurs in two distinct kinds of achondrite. Padvarninkai is probably best regarded as a brecciated howardite, but Shergotty and Zagami together constitute a unique class.

Plagioclase of albite-oligoclase composition occurs as a product of recrystallization in numerous chondrites<sup>4–6</sup>. In several cases this plagioclase is replaced by isotropic maskelynite, Château-Renard being a well known example. Of thirty-three chondrites bearing maskelynite recognized in the British Museum collection, no fewer than twenty-eight display black veins or brecciation structures, and of the remaining five, four are such small stones that they could be derived from veined or brecciated bodies. In seven chondrites maskelynite is restricted to the vicinity of veins or brecciation zones while plagioclase remains elsewhere. These features suggest a close genetic relationship between vein-brecciation structures and the plagioclase-maskelynite transformation, and since ultra high pressure shock provides the most acceptable explanation for the geometrical and microscopic characteristics of the former<sup>7</sup>, a shock origin for chondritic maskelynite appears highly likely.

In achondritic meteorites there are fundamental difficulties in understanding a purely thermal transformation of plagioclase to maskelynite without eutectic or eutectic-like melting between feldspar and coexisting mafic minerals<sup>8</sup>. The synthesis by shock of maskelynite-like glasses from albite<sup>9</sup> and bytownite<sup>10</sup>, and the reproduction during explosive shock experiments on a gabbro of textural relationships similar to those in Shergotty<sup>10</sup>, strongly support the alternative—a shock origin for maskelynite—in both chondritic and achondritic meteorites. In this respect it is interesting to note that the average refractive indices of the three analysed maskelynites ( $n=1.544$ , 1.540, 1.502 in Shergotty, Zagami and Château-Renard respectively) are significantly higher than those of glasses of the same composition synthesized by fusion at atmospheric pressure, a feature also recorded in the synthetic shock-produced bytownitic maskelynite.

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## THE SOLID STATE

### Ring Configurations in a Random Network Model of Vitreous Silica

The properties of liquids and glasses may, for many purposes, be classed together. Both lack the extensive regularly repeating groups of atoms that characterize crystalline arrays. X-ray diffraction patterns of both, instead of showing the sharp rings or spots associated with crystal structures, show diffuse bands indicating that the type of order existing in them is limited to a short range.

When considering the structures of such irregular aggregates of atoms, they can be very roughly divided into two types, associated and non-associated. The non-associated types comprise atoms the interactions of which are non-directional: the simplest examples of these are the inert gas liquids, the spherical non-polar atoms of which interact with non-saturating and non-directive forces. The structures of these liquids can be derived to a good approximation simply from packing considerations: this has been done very successfully in the work of Bernal<sup>1–3</sup> and Scott<sup>4,5</sup>. A characteristic of this type of liquid, in contrast to the unique co-ordination in the crystal, is the existence of a distribution of co-ordination numbers, to which the thermodynamic properties are related.

In associated liquids, on the other hand, the interactions between the atoms are directional: they form bonds which occur in definite vectorial positions in space, and there is a limit to the number of bonds which can be formed to a molecule. In this type of liquid the directed forces lead to the formation of an extensive set of rings linking up the molecules. Examples of this type of liquid are water which forms hydrogen bonds, and vitreous silica, both of which consist of tetrahedral arrangements of atoms. It has been suggested by Bernal<sup>6</sup> that in the associated liquids and vitreous silica the statistical variation is not in the co-ordination number but in the number of molecules forming rings.

Using a random network model of vitreous silica, the data concerning which have been published previously<sup>7</sup>, an investigation into the distribution of types of rings which occur in the model has been carried out.

In crystals the rings are regularly repetitive and the types of rings in the immediate neighbourhood of each atom are limited. In tridymite and cristobalite, for example, only hexagonal rings of silicon atoms exist, chair-shaped in the case of cristobalite and chair- and boat-shaped in the case of tridymite. In vitreous silica, on the other hand, rings of four, five, six, seven and more silicon atoms may exist.

When looking at a crystal model such as cristobalite or tridymite it is visually obvious which rings form the neighbourhood of a particular atom. In a random network model, however, which consists of complex and highly interconnected sets of rings, the choice of rings is not always obvious. For this reason it was decided initially to look at the most generalized set of rings so that these could be regrouped in various ways later.

One hundred silicon atoms were selected as centres and the number of rings of each type around each silicon centre were computed using the London University Institute of Computer Science Atlas computer. Rings with up to nine silicon members were selected. For the purposes of the count a ring was defined as any closed path of atoms within the model, each atom of which is connected by an oxygen to two and only two other silicons in the path, and in traversing the path each atom is crossed only once. This meant that an arrangement such as that in Fig. 1 would be counted as one six-fold and two five-fold rings.

In a general count of rings, when the acceptable rings are not limited to those containing only closest neighbours the number of  $n$ -fold rings to a centre increases

with the number of members,  $n$ , in the ring: this is shown in Fig. 2.

A more significant way of looking at the rings is to consider the rings which are formed of the close neighbours of each centre. There are a variety of ways in which this could be effected, for example, the ring count could be restricted to those atoms which were within, say, three Si-Si neighbour distances from a particular centre, or one could limit the number of rings to one, two or more rings to a vertex pair. One way of doing this is to consider each of the pairs of vertices of the  $\text{SiO}_4$  tetrahedra in turn and limit the counts of rings to the shortest path connecting the atoms in each of the pairs. This limits the number of rings to a centre to six. The distribution of rings found for a count of this sort is shown in Figs. 3 and 4.

Fig. 3 shows that five-folds predominate, because the average number of rings to a centre is 2.77 for five-folds, 2.27 for six-folds, 0.94 for seven-folds and 0.02 for eight-folds. Fig. 4 shows the way in which the five-, six- and seven-fold rings are distributed among the centres. There

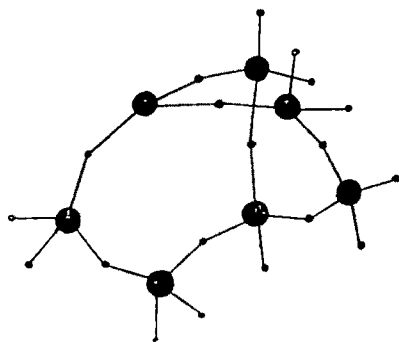


Fig. 1. Portion of vitreous silica model showing one six-fold and two five-fold rings of silicon atoms.

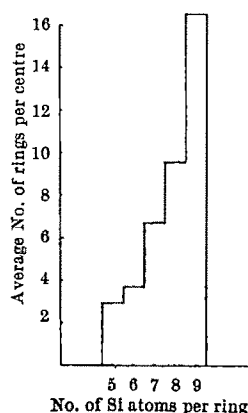


Fig. 2. Histogram showing average number of rings to a centre for different ring types. (Unlimited path analysis was used.)

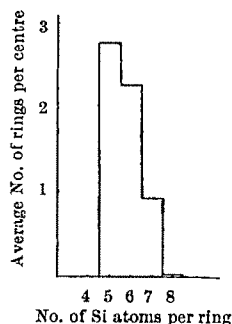


Fig. 3. Histogram showing average number of rings to a centre for different ring types. (The shortest path analysis was used.)

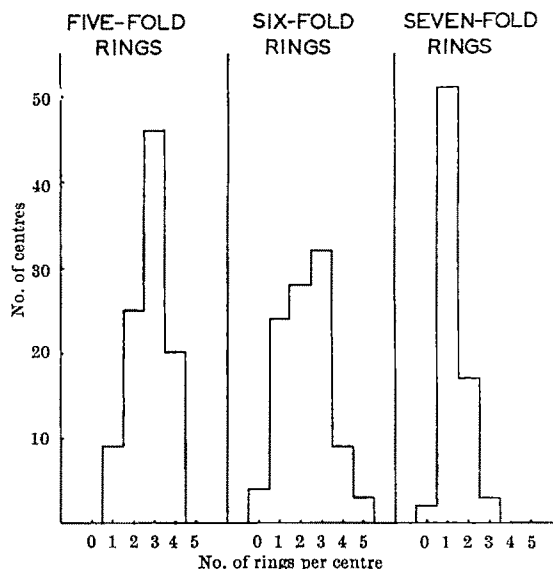


Fig. 4. Histograms showing the way in which five-, six- and seven-fold rings are distributed among the centres (in shortest path analysis)

were only two eight-folds and so they were not included in Fig. 4.

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### Carbon Fibre Composites

EFFECTIVE use of any fibres requires that they be incorporated into a matrix that develops the full strength of the fibres. Much attention, rightly enough, has been given to carbon fibres because of their low density and high strengths and elastic moduli. Rolls-Royce, Ltd., have announced a fibre with tensile strength of 250,000 lb./in.<sup>2</sup> and modulus of 60 million<sup>1</sup>. Several U.S. suppliers have fibres commercially available with strengths to 180,000 lb./in.<sup>2</sup> and moduli to 25 million. In time, fibre properties may approach 500,000 lb./in.<sup>2</sup> strength and 90 million modulus. These properties of fibres are very attractive, but it is the composite properties that are of ultimate importance in the use of the fibre.

We have been working with carbon composites for more than a year, and the results show both deficiencies and strong points for these composites. Our matrix has been exclusively epoxy resin with an amine curing agent, and with a vacuum-temperature precleaning of the fibre to remove volatiles and occluded gases before impregnation with resin. An important relationship found so far is the inverse correlation of composite shear strength with fibre modulus; that is, the greater the fibre modulus, the smaller the shear strength. Fig. 1 graphically illustrates these results. We do not yet know why this inverse relationship holds true. We found that the critical surface tension of 68 million modulus fibres was between 44 and 48 dynes/cm. This value, because it is greater than the surface tension of most epoxy resins, should result in

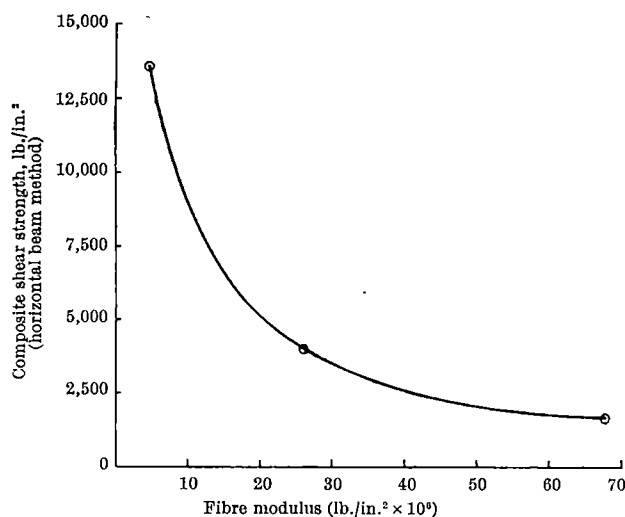


Fig. 1. Carbon fibres composite shear strength versus fibre modulus. Epoxy resin, amine cure.

spontaneous wetting. Observation of the epoxy meniscus with graphite fibres and the spreading of epoxy on bulk graphite confirms this wetting. Other researchers<sup>2</sup> have reported that surface treatments of graphite fibres with nitric acid resulted in a 50 per cent increase in composite shear strength. There is evidence to indicate that this improvement was brought about by improved bonding to acidic sites created by the oxidation. This increased value, to about 6,700 lb./in.<sup>2</sup>, is still far too small to develop the full strength of the fibres. Shear strength is important in a composite because in compressive applications a mode of failure involves shearing the fibre-matrix bond, allowing the then unsupported fibres to buckle. An engineering rule of thumb is that the shear strength should be 75 per cent the compressive yield strength of the matrix to avoid this type of failure. This indicates 15,000 lb./in.<sup>2</sup> shear strength and greater, far more than the values now being attained.

The fatigue resistance of high modulus (low shear strength) composites is relatively untested, but we expect it to be a direct function of the type of test. If the test applies a high shear load, we would expect the composite to fail quickly. We have shown this in one test which involved the twisting of a half ring, imposing high shear and low bending stresses. Samples fail in shear in the first cycle of this test. The same types of samples, however, when exposed to a fatigue test imposing low shear and high tensile or compressive stresses may last much longer. The low modulus fibres in composites give good fatigue life in the half ring twist test—as good as or better than glass. This is further evidence of the good shear bond.

An additional point is that carbonaceous composites seem reasonably resistant to degradation by water. This is in contrast to the first results obtained with glass-epoxy composites, which in the early days of development could be severely degraded by boiling in water. This prompted the whole research effort on glass finishes, which eventually resulted in silanes and others which made the glass-epoxy composites relatively impervious to water. It appears that no such road need be followed with the carbon fibres, at least for water resistance purposes.

Now, as for shear strength of the high modulus graphite composites, that may be another story.

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## PHYSICS

### X-ray Diffraction Experiments in Nano-second Time Intervals

DEBYE-SCHERRER X-ray powder patterns are conventionally obtained on photographic emulsions in times of the order of hours. When special techniques are used, the time required can be reduced to minutes or perhaps seconds. In this communication, we report the recording of X-ray powder patterns on photographic emulsions in nanosecond time intervals.

The pulsed X-ray machine used in these experiments was constructed following the principles described by Blumlein<sup>1</sup> and Fitch and Howell<sup>2</sup>. The machine generated a 50 kV, 50 kAmp electron current pulse with a pulse duration of about 20 nsec. The bremsstrahlung obtained with a tungsten anode was 30 mr. per pulse at a distance of 1 m from the anode. The anodes used in the diffraction experiments were made of titanium and iron. Additional details of the construction of this X-ray machine will be published elsewhere.

The feasibility of pulse X-ray diffraction was shown in a Bragg reflexion experiment with a single crystal of lithium fluoride. The 200 reflexion was recorded on 'Eastman KK' emulsion. The titanium  $K\alpha$  radiation from a single pulse of the X-ray source was used. The experimental arrangement and results are shown in Fig. 1. Because the expected decrease in diffracted X-ray intensity in going from the single crystal experiment to a Debye-Scherrer experiment was about three orders of magnitude, it was not clear that a photographic record of the lithium fluoride powder pattern could be obtained. Using a pellet of lithium fluoride powder in the experimental arrangement shown in Fig. 2a, the 220  $K\alpha$  reflexion was, however, clearly evident on the emulsion, as shown in Fig. 2b. The 220  $K\beta$  and 200  $K\alpha$  lines were also recorded, but the 220  $K\beta$  was too weak to show clearly on the film. Additional confirmation of these results was provided by observing the 400 and 222 reflexions from a pellet of sodium chloride with the experimental arrangement already described. The reflexions from larger

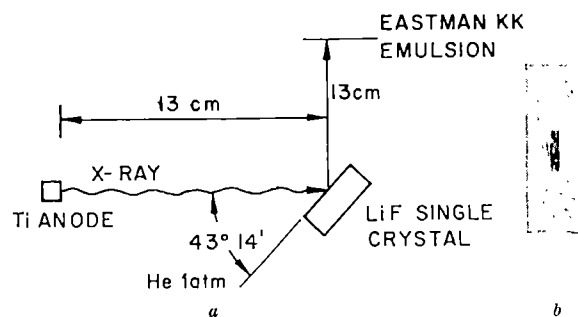


Fig. 1. a, Experimental arrangement. b, Recorded Bragg reflexion.

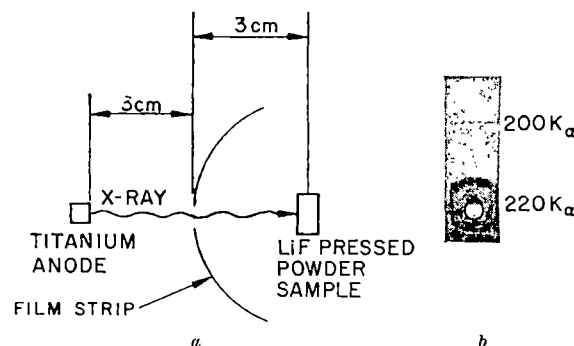


Fig. 2. a, Experimental arrangement. b, Recorded powder pattern.



*d*-spacings were not observed because of absorption by the sample. By using an iron anode and tipping the pellet of lithium fluoride 45° from the vertical, the 220, 311, 222 and 400 reflexions were observed. All these records were made with a single pulse of X-rays lasting about 20 nsec.

Further work is under way to extend the technique to dynamic experiments in which X-ray diffraction information is obtained while the sample is undergoing structural change. The technique could, for example, be used to investigate phase changes in materials during the passage of shock waves.

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### Stimulated Emission with a Non-dissociated Europium $\beta$ -Dicetonate

EARLIER it was reported that laser action was obtained at 6111 Å with the sodium salt of europium-tetakis benzoyl-acetonate ( $B_4EuNa$ ) (ref. 1). Spectroscopically, this is the best chelate now known for obtaining stimulated emission: its luminescence spectrum presents only one strong very sharp line, namely 6 Å, in solution at 77° K (ref. 2).

Some spectroscopic characteristics of this chelate contrast with those of its organic cation homologues. The part played by the cation is important and raises the problem of the exact origin of the luminescence spectrum observed.

We have investigated the luminescence at 77° K and the conductance at 295° K of fresh\*  $B_4EuNa$  in pure dimethyl formamide (DMF) and in a mixture of DMF and ethanol (1:3) which makes laser action possible. It is assumed that during the fast cooling of the solutions, the equilibrium reached at 295° K does not noticeably change.

In these two solvents, the  $B_4EuNa$  is partially decomposed and this leads to the formation by the *tris*-ligand of an adduct with the solvent:  $B_3Eu$ , DMF. The 5798 Å line of the *tris*-ligand luminescence spectrum enables its concentration to be determined.

The method of calculation has been described by Brecher, Samelson and Lempicki<sup>3</sup> and consists of comparing heights of the  $^5D_0 \rightarrow ^7F_0$  luminescence lines corresponding to the *tris* and *tetakis*-ligand forms. This method was established for the case when only one form of *tetakis* chelate was present. The case of  $B_4EuNa$  is more complex because two *tetakis* forms have to be considered (see later). Close examination of the spectra reveals that besides the 5798 Å line only one other line is observed with  $B_4EuNa$  at 5801 Å (Fig. 1). We conclude that the 5801 Å line is common to both *tetakis* forms. The 5798 Å line obtained with a  $10^{-2}$  molar solution in DMF is nineteen times less intense than that of a solution of  $B_3Eu$  at the same concentration in the same solvent. In the mixture of DMF and ethanol, this line is only 4.4 times less intense. From these values it can be deduced that there are 7 per cent of  $B_3Eu$  in DMF and 30 per cent in the mixture of DMF and ethanol.

The results of conductance measurements given in Table 1 show that the dissociation of  $B_4EuNa$  is much less than for the other chelates. The considerable decomposi-

Table 1. MOLAR CONDUCTANCE AT 295° K

	$C = 10^{-2}$ moles/l. DMF	$C = 10^{-2}$ moles/l. DMF + EtOH	$C = 2 \times 10^{-3}$ moles/l. Acetonitrile
$BNa$	10	19	20
$B_3Eu$	1.8		
$B_4EuNa$	15.8	10.2	24
$(BTF)_3EuNa$	29.3		103
$B_3EuP$	8.5		47.5
$(BTF)_3EuP$	29.3		119

tion into the *tris*-ligand form<sup>3</sup> explains the low conductance of  $B_4EuP$ . It should be noted that the compound  $BNa$  is clearly not totally dissociated in these solvents.

Considering the size of the  $B_4Eu^-$  ion and the high dielectric coefficient of the solvents, the existence of ion pairs is very improbable. Thus the presence of a molecular form in the solution is demonstrated.

By observing modifications in the relative heights of the  $^5D_0 \rightarrow ^7F_2$  luminescence lines as one passes from one solvent to the other, it is possible to classify the chief lines into several groups within each of which there is a constant intensity ratio (Table 2). The 6111 Å line can be attributed to the molecular form because: (1) it also exists in crystallized  $B_4EuNa$ ; (2) it does not appear in benzoyl-trifluoroacetate which is known to be largely dissociated<sup>5</sup>; and (3) it is greatly diminished in the solution of  $B_4EuNa$  in N-ethylacetamide which has a very high dielectric constant ( $\epsilon = 135$  at 20° C). In the last case, two strong lines similar to those appearing with the dissociated fluorinated chelates are observed at 6122 Å and 6132 Å.

Table 2. CHARACTERISTIC MAXIMA AND SHOULDERS (IN BRACKETS) OF THE LUMINESCENCE OF  $B_4EuNa$  SOLUTIONS AT 77° K (IN Å)

Line group	$^5D_0 \rightarrow ^7F_2$	$^5D_0 \rightarrow ^7F_0$	Attribution
1	6111-6142-6160	5801	$B_4EuNa$ (molecular)
2	(6115)-6135	5801	$B_3Eu^-$
3	(6122)-6177	5798	$B_3Eu$ , DMF

By combining results of conductance and luminescence it is possible to calculate approximately the proportion of each of the three luminescent species  $B_4EuNa$ ,  $B_3Eu^-$  and  $B_3Eu$ , DMF which are in equilibrium.

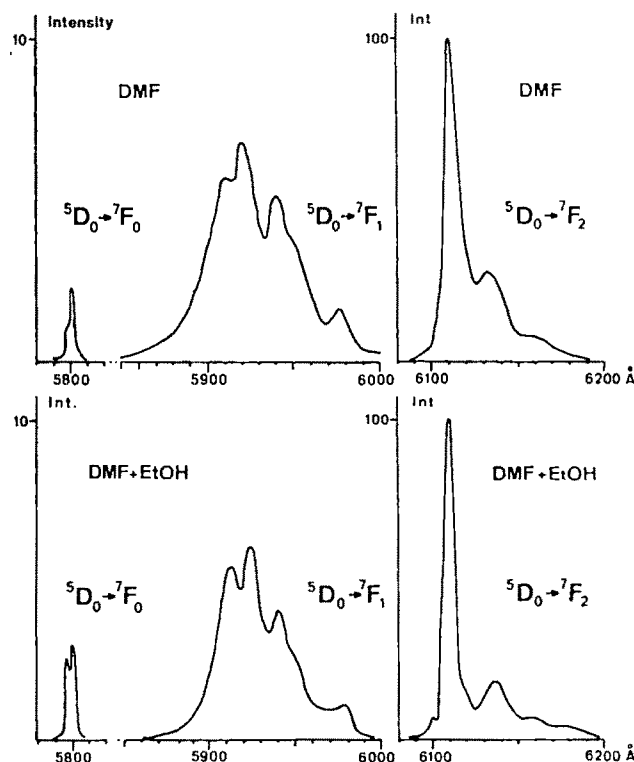
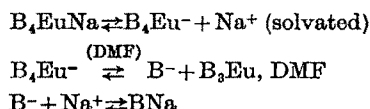


Fig. 1. Fluorescence spectra of 0.01 molar solutions of  $B_4EuNa$  at 77° K (uncorrected for the photomultiplier spectral sensitivity).

\* The solutions are unstable and vary at room temperature. Within a few weeks, the ligands break: B → acetophenone + ethyl acetate (ref. 4).



**DMF solvent.** The limiting ionic conductance of  $\text{Na}^+$  and of a few anions in DMF is known<sup>7</sup>. For anions (which are not solvated in DMF) it is approximately proportional to the reciprocal cube root of the ion mass (if anions have similar densities). This calculation gives, for the limiting molar conductance  $\Lambda_0 = 55 \Omega^{-1} \text{ mole}^{-1} \text{ cm}^2$  for  $\text{B}_4\text{EuNa}$  and 73 for  $\text{BNa}$ . The Onsager equation<sup>8</sup> for the monovalent weak electrolyte is

$$\Lambda = \left[ \Lambda_0 - \left( \frac{82}{(\epsilon T)^{1/2} \eta} + \frac{8.28 \times 10^5}{(\epsilon T)^{3/2}} \Lambda_0 \right) (\alpha C)^{1/2} \right] \alpha$$

where  $\Lambda$  is the molar conductance,  $\epsilon$  is the dielectric constant,  $T$  is the absolute temperature,  $\eta$  is the viscosity,  $\alpha$  is the degree of dissociation and  $C$  is the total concentration. This equation makes it possible to calculate the concentration of the  $\text{B}_4\text{Eu}^-$  ion. The conductance due to  $\text{B}^-$  can be neglected.

**DMF + ethanol solvent.** No data have been published concerning the conductances in the mixture of DMF and ethanol, but it is likely that the ion solvation is identical and that the product  $\Lambda_0 \eta$  (limiting molar conductance and viscosity) is the same in both solvents. The measurement with sodium perchlorate gives  $\Lambda_0 = 54$ . We then deduce that  $\Lambda_0 = 48$  for  $\text{BNa}$  and  $\Lambda_0 = 36$  for  $\text{B}_4\text{EuNa}$ . Here the conductance of  $\text{B}^-$  can no longer be neglected. From a plot of the molar conductance against concentration and from the Onsager equation, it is possible to calculate the ionic dissociation constant of  $\text{BNa}$ .

Moreover, to the first approximation, the conductance  $\chi$  of a  $\text{B}_4\text{EuNa}$  solution is the sum of the conductances due to the two species which can be dissociated

$$\chi = \chi_{\text{BNa}} + \chi_{\text{B}_4\text{EuNa}}$$

$$\text{With } \chi = \frac{C\Lambda}{1000}, \text{ we have:}$$

$$C\Lambda = \Lambda_{\text{BNa}} \cdot C_{\text{B}^-} + \Lambda_{\text{B}_4\text{EuNa}} \cdot C_{\text{B}_4\text{Eu}^-}$$

where  $C_{\text{B}^-}$  and  $C_{\text{B}_4\text{Eu}^-}$  are the concentrations of dissociated fractions and  $C$  the total concentration of  $\text{B}_4\text{EuNa}$ .

$\Lambda_{\text{BNa}}$  and  $\Lambda_{\text{B}_4\text{EuNa}}$  are expressed in terms of the corresponding  $\Lambda_0$  by means of the Onsager equation. It is possible to calculate approximately the concentration of the different species by means of the constant  $K = \frac{C_{\text{B}^-} \cdot C_{\text{Na}^+}}{C_{\text{BNa}}}$  and the equation  $C_{\text{B}_4\text{Eu}} = C_{\text{BNa}} + C_{\text{B}^-}$ , where

$C_{\text{B}_4\text{Eu}}$  and  $C_{\text{BNa}}$  are the respective concentrations (Table 3).

The results are in agreement with the increase of the intensity ratio of the 6111 Å and 6135 Å fluorescent lines going from DMF to the mixture of DMF and ethanol. From Table 3, it can be seen that the concentration ratio  $\frac{C_{\text{B}_4\text{EuNa}}}{C_{\text{B}_4\text{Eu}^-}}$  passes from  $\frac{60}{33}$  to  $\frac{53}{17}$ , that is, a factor of 1.7. On the spectra, direct reading of the intensity ratio of the 6111 Å and 6135 Å lines gives 1.5. Considering the approximations used, the agreement is satisfactory.

With benzoyltrifluoro-acetonates in acetonitrile, however, only the ionic form  $(\text{BTF})_4\text{Eu}^-$  is obtained<sup>5</sup>. Recently, T. M. Shepherd<sup>6</sup> has shown that, in a low  $\epsilon$  solvent like benzene, a different spectrum is obtained, dominated by an intense 6111 Å line. This he attributes to an ion pair. We have verified that the same results are obtained with the  $(\text{BTF})_4\text{EuNa}$  in acetonitrile and in benzene. Thus the  $\text{B}_4\text{EuNa}$  appears to be a particular case in which the sodium is much more strongly bonded.

Table 3. APPROXIMATE NUMBER OF MOLES OF EACH SPECIES WHEN 100  $\text{B}_4\text{EuNa}$  MOLES ARE DISSOLVED.

	$\text{B}_4\text{Eu}$ , DMF	$\text{B}_4\text{Eu}^-$	$\text{B}_4\text{EuNa}$	$\text{BNa}$	$\text{Na}^+$	$\text{B}^-$
DMF	7	33	60	6.5	33.5	0.5
DMF + EtOH	30	17	53	19	28	11

Total concentration of europium was  $10^{-2}$  moles/l.

With  $\text{B}_4\text{EuNa}$ , laser thresholds of only 160 J were obtained at  $-150^\circ \text{C}$ , despite the presence in the solution of a considerable quantity of inactive species absorbing a large part of the excitation which is thus lost for stimulated emission, unless transfer, not yet demonstrated, occurs. We consider that this molecular form is of particular interest for obtaining laser action.

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### Comparison of Flow Birefringence Data of Polymers by means of Reduced Variables

Flow birefringence is usually characterized by the variation of the extinction angle  $\chi$  and the birefringence  $\Delta n$  as a function of the velocity gradient  $q$ . This holds in particular for flowing polymer systems, including the limiting cases of very dilute solutions and of bulk polymers in the molten state. In principle,  $q$  can always be eliminated from the two relationships. Although information is lost in this way, the over-riding advantage is that, for small  $q$ , the flow birefringence of polymer systems behaves very much like stress birefringence of rubber-like materials. Such a consideration eliminates the picture of flow, because birefringence is considered as a function only of the stresses.

Let a Cartesian co-ordinate system be chosen with one axis parallel to the direction of laminar shear flow, and a second along the direction of the (constant) velocity gradient and the third axis completes the set. In this co-ordinate system the stress-optical relations read

$$\Delta n \cos 2\chi = C(p_{11} - p_{22}) \quad (1)$$

$$\Delta n \sin 2\chi = C 2p_{12} \quad (2)$$

where  $p_{12}$  denotes the shear stress measured at a plane parallel with the flow direction,  $p_{11}$  and  $p_{22}$  denote normal stresses and  $C$  is the stress-optical coefficient.

It is important to realize that, according to the theory of rubber elasticity, any stress component can be made dimensionless by dividing it by the number of chain molecules per unit of volume times  $kT$ . Applying this principle to equations (1) and (2), the following dimensionless quantities are obtained:

$$E = \frac{\Delta n \cos 2\chi}{3C} \frac{M}{cRT} \quad (3)$$

$$\beta = \frac{\Delta n \sin 2\chi}{2C} \frac{M}{cRT} \quad (4)$$

where  $M$  is the molecular weight,  $c$  is the concentration (that is, the density for the bulk polymer),  $R$  is the gas constant and  $T$  is the absolute temperature. In equation (3) the factor 3 is incorporated. This has been done to give quantity  $E$  the approximate meaning of chain expansion by the following definition<sup>1</sup>:

$$E = \bar{h}^2 / \bar{h}_0^2 - 1 \quad (5)$$

where  $\bar{h}^2$  is the mean square end-to-end distance of the polymer chain during flow and  $\bar{h}_0^2$  is the same quantity at rest.  $\beta$  is very much the same as the well known Bueche-Harding parameter<sup>2</sup>, because  $C$  must be determined by comparison of viscosity and flow birefringence measurements using equation (2)<sup>3,4</sup>.

Dilute solution theory is in accordance with the given quasi-static treatment<sup>5,6</sup>. The same holds for the theory of networks containing cross-links of finite lifetimes<sup>7</sup>. The given reduction (equations (3) and (4)), however, is only meaningful so long as one can speak of separate macromolecules. The same holds for any relationship between  $E$  and  $\beta$ . From Zimm's theory for dilute solution, perfectly flexible macromolecules at low values of  $q$ , Peterlin has shown that

$$E = \epsilon \beta^2 \quad (6)$$

with  $\epsilon$  between 0.136 for impermeable coils and 0.267 for freely draining coils<sup>8</sup>. In actual fact,  $\epsilon$  can take these values only in the case of linear polymers of very narrow molecular weight distribution. For other linear materials  $\epsilon$  should, according to dilute solution theory, rapidly increase with the broadness of the distribution<sup>1,8</sup>.

In Fig. 1 a plot according to equation (6) is presented on a double logarithmic scale. The data were obtained from a series of anionic polystyrenes of different molecular weights. The high molecular weight samples were investigated in monobromo-benzene as a solvent and at a temperature of 25° C (ref. 9). The sample of the lowest molecular weights was investigated in bulk at a temperature of 196° C (ref. 10). The purpose of this graph is to show that the proposed reduction covers pronounced differences in molecular weight, concentration and temperature. Apparently it is valid even for the melt. For  $\epsilon$ , a value of 0.27 is obtained. This is practically within the limits given previously.

It can be concluded from this rather surprising result that even in the melt the polymer molecules behave like separate molecules. The interaction with the neighbour molecules seems to be satisfactorily described by an effective friction factor. The value of this factor is irrelevant when the normal stresses are related to the shear stress.

The coincidence of reduced results shown here is, however, not always found. Concentrated solutions apparently show a more complicated behaviour than dilute solutions or melts, and their  $E$ -values are comparatively higher at corresponding  $\beta$ -values. The results shown in Fig. 1 were obtained for polymer samples which possess a very narrow distribution of molecular weights. With broader distributions coincidence is sometimes obtained for results on dilute solutions and on the melt.

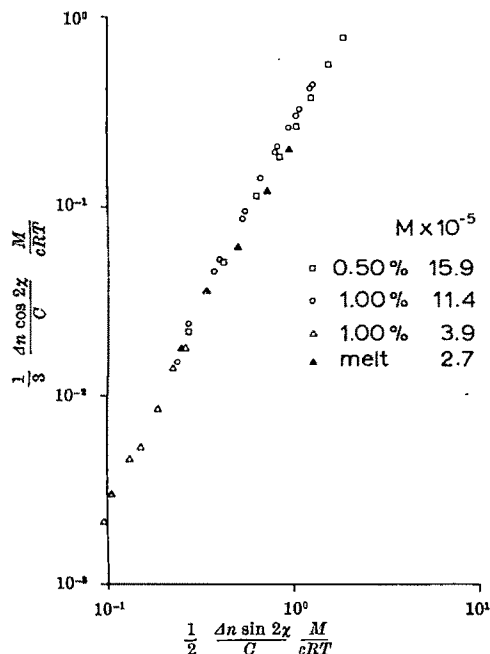


Fig. 1. Double logarithmic plot of the reduced flow birefringence data, obtained on various anionic polystyrenes. The molecular weights are indicated in the graph. Solutions in monobromo-benzene were measured at 25° C, melt measurements were carried out at 196° C.

This matter will be an important point for future investigations. In fact, some theories<sup>11</sup> for the determination of molecular weight distributions are based on the concept of the effective friction factor and on the simple additivity of the contributions of each molecular species to the properties of the bulk.

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### A Moving Body must "appear" Cool

LANDSBERG has recently suggested<sup>1</sup> replacing Einstein's law  $T_{lab} = T_{cm} (1 - w^2/c^2)^{1/2}$  with the law  $T_{lab} = T_{cm}$ , where  $T_{lab}$  is the temperature of a body as measured in laboratory co-ordinates,  $T_{cm}$  is the temperature of the body measured in its rest frame, and  $w$  is the velocity of the body relative to the laboratory. The word "appear" was placed in quotation marks in the title because  $T_{lab}$  may not be the temperature as observed by the casual laboratory observer at all, just as the Lorentz contraction is not seen by a laboratory observer under ordinary visual inspection, no matter how large it is, but is replaced by a rotation<sup>2</sup>. A black body at some temperature  $T_{cm}$  will emit a black body spectrum appropriate to  $T_{cm}$  in its rest frame, which will be seen Doppler-shifted to another temperature by other observers. An observer in front will see a blue-shift and one behind will see a red-shift, in neither case by a factor  $(1 - w^2/c^2)^{1/2}$ .

To see what is the proper definition of  $T_{lab}$  one may proceed either via general principles as Einstein did, or via simple hypothetical experiments. The elegance and generality of the former technique evidently are compensated by some vagueness or appearance of arbitrariness, as evidenced by the fact of Landsberg's objection. Thus a simple experiment is desired. It is impossible, however, to carry on the sort of quasi-static variations and transfers of energy demanded by "thermodynamics" (sometimes called "thermostatistics") between two bodies that are moving rapidly together or apart. The only relevant experiment is one where the relative velocity is transverse to the line of centres of the two objects; then the distance remains constant. Consideration of such a hypothetical experiment leads at once to the conclusion that Einstein's definition is the only one consistent with the zero'th and second laws of thermodynamics. [By the zero'th law is meant that there exists a unique function, called temperature (or a one-to-one function thereof), such that two bodies in thermal contact remain in their initial states if and only if they have the same temperature.]

Consider, then, the following experiment: place a black body  $B_1$  at one temperature  $T_0$  at the centre of a revolving turntable, and place another  $B_2$  at laboratory temperature  $T_{lab}$  on the rim, so as to move with constant velocity  $w$ . Allow radiative transfer between the two through a narrow radial tube. Unless the spectrum radiated by  $B_2$  as seen at  $B_1$  is a black body spectrum of temperature  $T_0$ , and that of  $B_1$  as seen at  $B_2$  is a similar spectrum of temperature  $T_{cm}$ , net radiative transfer will occur, causing one to get hotter and the other cooler. Thus one must define  $T_{lab} = T_0$  when the above spectral conditions hold, and only then. Any other definition leads to a viola-

tion of the second law of thermodynamics. But photons from  $B_2$  suffer a transverse Doppler shift toward the red by a factor  $(1 - w^2/c^2)^{1/2}$  as seen at  $B_1$ . Thus  $T_{\text{cm}}(1 - w^2/c^2)^{1/2} = T_{\text{lab}}$ . For consistency, one should verify that photons from  $B_1$  are seen blue-shifted by the factor  $(1 - w^2/c^2)^{-1/2}$  as seen at  $B_2$ . The transverse Doppler shift formula may not be used directly to find out how things are seen by  $B_2$  as it is in accelerated motion, but one may use either of two arguments to solve the problem. First, one may note that the shift already found is because clocks at  $B_2$  would run slower than those at  $B_1$  by a factor  $(1 - w^2/c^2)^{1/2}$ ; applying this in reverse, one finds the proper blue-shift of  $B_1$ 's spectrum as seen at  $B_2$ . Second, one can replace the special-relativistic discussion by one that depends on the equivalence principle, and use rotating co-ordinates in which  $B_2$  is still. Then the spectral shifts are gravitational in origin, because of the gravitational potential  $-\frac{1}{2} r^2 \omega^2$ .

This ensures reciprocity between the spectral shifts of each body as seen at the other. These shifts have been verified in the laboratory<sup>3</sup>, which verifies Einstein's law.

Landsberg's concern about the properties of bodies initially at rest and put into motion does not seem to fall within the scope of this discussion. The results depend on how they are set moving; does one keep the proper volume or the volume as seen in the laboratory constant during the acceleration? Furthermore, deformations must occur, since rigid bodies cannot accelerate, according to the special theory of relativity<sup>4</sup>. The transformation of temperature under consideration is valid only for different observers looking at the same body, or for finding  $T_{\text{lab}}$  for bodies the rest-temperature of which is known by other means, such as by knowledge of their volume, pressure and composition.

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## Temperature of a Moving Body

RECENTLY Landsberg<sup>1</sup> suggested that the "true" value of the temperature of a body moving at relativistic speeds will appear the same as the temperature measured by an observer moving with the body itself. This conclusion is reached by re-defining temperature in terms of entropy and internal energy.

Fremlin<sup>2</sup> suggests that some physical concept of temperature must be introduced before the mathematics becomes valid, and shows that if the temperature is defined on a kinetic model (that is relating the temperature to the random velocities of the molecules) then the body appears cooler to any observer relative to whom it has a uniform velocity. He states that the apparent component of velocity in the direction of motion is reduced by a factor  $\beta^2$ , where as usual

$$\beta = \left(1 - \frac{V^2}{c^2}\right)^{-1/2}$$

and  $V$  the velocity of the whole body relative to the observer, while the velocity components perpendicular to the direction of motion are reduced by a factor  $\beta$ .

The apparent reductions in the velocity components are, however, greater than those that Fremlin has calculated. Let the body have speed  $V$  relative to an observer  $A$ . If an observer at rest relative to the body,  $B$ , measures the velocity component of an average molecule in the direction

of relative motion as  $u$ , then the observer  $A$  measures this speed as

$$u' = \frac{u + V}{1 + \frac{uV}{c^2}}$$

(See, for example, McCrea<sup>3</sup> for a derivation of this.)

As the body has velocity  $V$ , observer  $A$  estimates the molecule speed relative to the body as

$$\frac{u + V}{1 + \frac{uV}{c^2}} - V = \frac{u}{\beta^2 \left(1 + \frac{uV}{c^2}\right)} \quad (1)$$

This expression reduces the apparent speed by the further factor

$$\left(1 + \frac{uV}{c^2}\right)$$

over what Fremlin obtained.

A velocity component  $v$  in a direction perpendicular to the motion of the body, as measured by  $B$ , is measured by  $A$  as

$$\frac{v}{\beta \left(1 + \frac{uV}{c^2}\right)} \quad (2)$$

This again is smaller than the Fremlin result by the same factor

$$\left(1 + \frac{uV}{c^2}\right)$$

Thus if the temperature is defined in terms of the random velocities of molecules, the temperature of a moving body will appear cooler. The amount of apparent cooling is, however, greater than deduced by Fremlin, though for most temperature ranges expressions (1) and (2) will reduce to those obtained by Fremlin as  $u$  will be much less than the velocity of light  $c$ .

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<sup>1</sup> Landsberg, P. T., *Nature*, **212**, 571 (1966).

<sup>2</sup> Fremlin, J. H., *Nature*, **213**, 277 (1967).

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## CHEMISTRY

### Hydrazine Synthesis in the Silent Electrical Discharge

THE synthesis of hydrazine from ammonia in the silent discharge was first reported by Besson<sup>1</sup>. Subsequent investigations by other workers on flowing systems<sup>2,3</sup> only led to both low conversion and very low hydrazine yields being obtained. The yields are normally expressed as grams of hydrazine per kilowatt hour of energy dissipated in the actual discharge. More recent work claims substantially increased yields by withdrawal from the discharge of the desired product in an absorbent<sup>4</sup>. A reasonable working explanation for the increase in yield reported in this case could well be as follows. The reactions in the discharge are undoubtedly of a very complex nature probably consisting of a series of competing formation and degradation reactions for any particular species in the discharge. The use of an absorbent is equivalent to reducing the residence time of the chemical species in the discharge, that is, it reduces the possibility of its decomposition by either further electron bombardment or other collision phenomena. Ideally, the absorbent would be selective only for the product and allow the activation reactions to take place virtually unhindered by its presence. Furthermore, if the effect of the absorbent is assumed to be entirely physical in nature, it follows that any method of reducing the residence time of the product

in the discharge would be expected to enhance yields. Other possible methods of achieving this same end are increased flow rate, pulsing of the discharge or removal of product by condensation, adsorption or chemical combination.

In order to determine whether such a mechanism is taking place, the hydrazine synthesis from ammonia has been studied in a concentric barrier discharge reactor using several of the suggested techniques for reducing the product residence time in the discharge. Provisional results are given in Figs. 1 and 2, where it can be seen first that yield increased considerably with decreasing residence time in the discharge. Actually, the results could not be extended into the more interesting region of very low residence times because of the pumping limitations set by the experimental apparatus used. This forcibly brought to notice the obvious economic limitation of this method of increasing yield which is dictated by the inherent pumping charges. One obvious way of avoiding this economic difficulty is to use a smaller discharge area. The yields were found to fall markedly with such changes in geometry, however, because of the increased influence of edge effects on the discharge characteristics. The problem could undoubtedly be partly overcome by use of venturi or centrifugal flow acceleration, but further perseverance with this method of reducing residence time in the discharge even with these modifications is not justified if the low percentage conversions are considered.

The use of an absorbent liquid (ethylene glycol) gives markedly increased yields (see Fig. 2). Endeavours to increase the yield still further by providing more intimate spray contact between the absorbent and discharge were not successful because of the dissimilar discharge geometries used for the two types of reactors. The spray reactor would have been expected to give slightly better yields over that of the film reactor. While the discharge gap distances were about the same in each reactor, however, the film reactor was of an annular design and the spray reactor was a wire in a concentric barrier. Thus, the geometry of the spray reactor resulted in the discharge intensity being higher in the centre of the discharge gap. As yield is inversely proportional to this parameter the spray reactor yields were a little lower than those of the film reactor with its more uniform discharge. Other modifications of the technique could be applied which would increase the absorbent discharge exchange, but the results appear to be only marginal.

Despite the use of the liquid absorbent the yield continues to increase with reduction in residence time (Fig. 1). This shows that there is still an appreciable quantity of hydrazine not being extracted by the absorbent. It seems more logical, therefore, to modify the discharge to ensure that hydrazine once formed is not degraded by

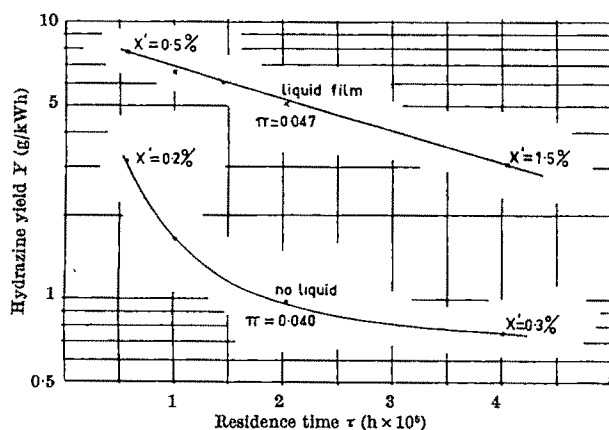


Fig. 1. The effect of residence time on hydrazine synthesis from ammonia in a silent electrical discharge. Operating pressure,  $P$ , 40 mm;  $\pi$ , discharge intensity kW/c.c.;  $x'$ , per cent conversion g hydrazine/g ammonia.

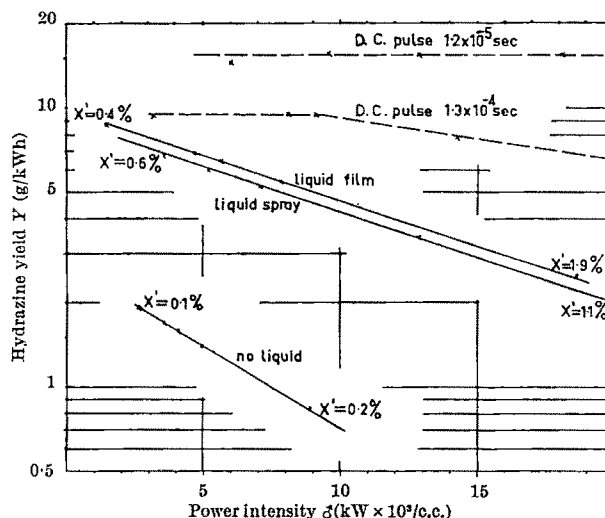


Fig. 2. The effect of liquid absorbent and discharge pulsing on hydrazine synthesis from ammonia in a silent electrical discharge. Operating pressure a.c. barrier reactor,  $P$ , 40 mm; d.c. pulsed discharge reactor  $P$ , 10 mm;  $x'$ , per cent conversion g hydrazine/g ammonia.

further electron bombardment which, incidentally, constitutes a waste in power<sup>4,5</sup>. A d.c. pulsed discharge with a variable on-period was used. Results (Fig. 2) show that the yield is increased considerably and that as the on-period is shortened the yield rises and becomes independent of power. It is therefore better to operate at the highest power possible as this will lead to a higher overall conversion from ammonia to hydrazine.

The best yield obtained so far with the d.c. pulsed discharge is about 15 g/kWh or 12.5 g/d. for a power charge of 1.2 d./kWh. As the economic price for large scale hydrazine manufacture and usage is close to this yield the silent electrical discharge route for its synthesis, even at this stage, looks promising. Further refinement of the pulsing technique is obviously required before the process becomes a commercial possibility, but from preliminary results an order of magnitude increase in yield might be possible in the foreseeable future.

There are several other rather obvious conclusions which can be drawn from these results. The observed increase in yield with both decreasing discharge power intensity and residence time, whether an absorbing film is used or not, shows almost conclusively that hydrazine is being degraded by the discharge after its formation. This confirms the working basis for these methods of increasing hydrazine yields. Second, the observed fall in conversion with decreasing intensity of discharge power and residence time implies that the methods used to reduce the residence time of the product in the discharge are not entirely physical, and appear to affect the reaction mechanism. Finally, the yield from the pulsed discharge reactor can never be greater than the equilibrium concentration set by the basic reaction mechanisms leading to hydrazine formation. This is not the case with the absorption technique where equilibrium is never reached because hydrazine is removed as it is formed. Therefore the conversion for the pulsed reactor is likely to be smaller than that obtained using the absorption technique.

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## Oxidation of Ethylene over Palladium and Palladium-Gold Alloys

UNTIL recently, nearly all successful catalysts used for the partial heterogeneous oxidation of ethylene contained silver as the active component<sup>1</sup>. In 1962, however, Kemball and Patterson<sup>2</sup> reported that ethylene could be oxidized to acetic anhydride and acetic acid over evaporated palladium films, and several applications have since then appeared in the patent literature<sup>3</sup>. Kemball<sup>2</sup> suggested that the partial oxidation products (which amounted to only about 3 per cent of the total) were formed by a path parallel to that for the complete oxidation to carbon dioxide. Acetaldehyde was proposed as the intermediate in the partial oxidation reaction, but none was detected, presumably because of its rapid oxidation to acetic anhydride. The purpose of the present work was to isolate and identify the proposed intermediate, and to determine whether higher selectivities might be obtained under more favourable reaction conditions.

A differential reactor was used in which the reactants were circulated rapidly over the catalyst at temperatures where the conversion in each pass was less than 1 per cent. The products were removed continuously from the gas phase by a U-tube trap maintained at  $-135^{\circ}\text{C}$ . In a typical experiment, 2 g of palladium sponge (99.95 per cent palladium, less than 10 p.p.m. silver, less than 15 p.p.m. chlorine; surface area,  $0.2\text{ m}^2/\text{g}$ ) were reduced in hydrogen at  $500^{\circ}\text{C}$  and then evacuated for 17 h at this temperature. A standard reactant mixture, containing 43 torr of ethylene and 100 torr of oxygen, was admitted to the reaction system and the gases circulated until about 30 per cent of the ethylene had reacted. The initial rates were close to zero order with respect to ethylene (in the range 10–47 torr) and 0.7 order with respect to oxygen (in the range 25–260 torr). The partial oxidation products, which represented 30–40 per cent of the ethylene oxidized, included acetaldehyde, acetic anhydride and acetic acid. The products were analysed by gas-liquid chromatography and identified by comparison with authentic compounds.

Acetaldehyde was confirmed by the following additional tests: (a) it had the correct retention time on a second (different) gas-liquid chromatography column (where a small quantity of ethylene oxide was also identified); (b) a positive test was obtained with fuchsin aldehyde reagent; (c) its mass spectrum corresponded to that of acetaldehyde. Depending on reaction conditions, acetaldehyde represented 1–20 per cent of the partial oxidation products.

The selectivity (percentage partial oxidation) was almost independent of temperature. In one series of experiments, it increased from 33 per cent at  $109^{\circ}\text{C}$  to 38 per cent at  $78^{\circ}\text{C}$ . These values, which are within our experimental error, indicate that the apparent activation energies of the two parallel reaction paths, if they exist, differ by less than 2 kcal/mole.

Unexpectedly, the selectivity was influenced by changes in oxygen pressure, increasing from 25 per cent to 43 per cent as the pressure was increased from 25 to 259 torr. This result can be explained if complete oxidation involves ethylene adsorbed associatively on adjacent metal atoms, while partial oxidation occurs on single sites. Alternatively, adjacent vacant metal atoms may effect complete oxidation by making possible the dissociative chemisorption of ethylene. The selectivity was not affected by changes in ethylene pressure between 10 and 47 torr.

The idea that the concentration of active sites for complete oxidation might be diminished by adsorption of oxygen led us to look for other ways of increasing the selectivity. Because gold was not a catalyst for this reaction, it was reasoned that the concentration of dual sites would be diluted in a palladium-gold alloy. This should favour partial oxidation. A poorly alloyed sample containing 18 atom per cent palladium was prepared by

reducing the mixed chlorides (obtained by evaporating a solution to dryness). Several experiments with this catalyst yielded selectivities as high as 70 per cent. Experiments with homogeneous alloys (verified by X-ray), prepared by reducing the metal salts with hydrazine, indicated that the selectivity reached a maximum at about 20 atom per cent palladium. Analyses showed, however, that samples prepared by reducing the metal salts with sodium borohydride were severely contaminated with sodium and boron. Our experience in this connexion makes us suspect the validity of using alloy catalysts prepared in this way to investigate the effects of alloying on catalytic properties, because these impurities drastically reduced both the rate and selectivity of the oxidation reaction. It is hoped that work now in progress will make it possible to interpret these results.

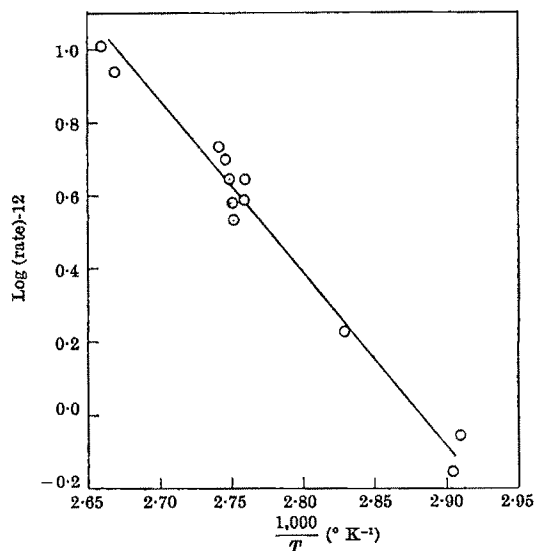


Fig. 1. Effect of temperature on rate.

The initial rates were measured over palladium as a function of temperature over the range  $71$ – $102^{\circ}\text{C}$  using the standard mixture of reactants. The Arrhenius plot is shown in Fig. 1. From the slope of this line, an apparent activation energy of 20 kcal/mole was calculated, and the initial rate could be expressed as

$$[d(\text{C}_2\text{H}_4)/dt]_{t=0} = 10^{24.6 \pm 0.1} \exp\left[-\frac{20,000 \pm 2,000}{RT}\right]$$

molecules/sec/cm<sup>2</sup>

The activation energy was about 6 kcal/mole greater than that found by Kemball and Patterson<sup>2</sup> for evaporated films. This discrepancy is outside our experimental error and may be attributed to the differences in catalyst preparations and reaction conditions. In particular, the reaction was first order in ethylene at the lower pressures used by Kemball and Patterson.

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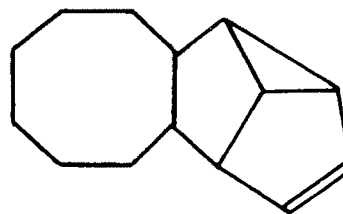
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## Versatile Constant Transmission Photochemical Reactor

THE ultra-violet irradiation of organic and organometallic compounds in the liquid phase is often complicated by the tendency for deposition of an opaque layer of polymeric materials and other reaction products on the cell walls. With the use of apparatus of conventional type this tendency can cause the observed rates of photochemical reactions to decrease rapidly with time, and thereby make it impossible to investigate some reactions beyond very low conversions of the starting materials. Accurate quantitative work on relative reaction rates and quantum yields becomes very difficult.

We now describe a photochemical reactor in which the transmission of the cell can be kept constant over long periods even under irradiation conditions where an inner transmitting surface would normally be rapidly occluded. This reactor is illustrated in Fig. 1 and has as its key feature an arrangement whereby the inner surface in contact with the photolysate is continuously rubbed by means of a mechanically rotated pad of fused silica fibre. Temperature control is achieved by the circulation of water through an outer jacket of fused silica and, if necessary, a thermostat device of conventional type. Various optical filter solutions can be used in place of water when it is desired to isolate a particular wavelength range. Under normal conditions of use, and with occasional reversal of the direction of rotation of the scrubber pad, the transmission of the cell remains effectively constant for periods of 20–40 h or even longer. Ordinary glass fibre has been used in place of silica fibre, but is slightly less durable. Any conventional external ultra-violet radiation source can be used, preferably with an aluminium reflector. We use up to three 500-W medium pressure Hanovia lamps for routine purposes. The design is an improved version of one previously reported from this laboratory<sup>1</sup>.

The following typical result illustrates the general utility. Irradiation of a solution of *cyclooctene* in benzene under nitrogen is known to produce a 1:1 adduct of structure (I)<sup>2</sup>. If the pad of fused silica fibre is not rotated,



(I)

the rate of formation of adduct (I) falls rapidly with time from the initial value (about 2 g/h with one 500-W medium pressure Hanovia lamp) and is virtually zero within about 9 h. Under similar conditions, but with operation of the scrubber as described above, the initial rate of formation of adduct (I) is readily maintained for over 24 h.

The constant-transmission principle of this reactor has an obvious extension to gas-phase investigations.

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<sup>1</sup> Blair, J. M., Bryce-Smith, D., and Pengilly, B. W., *J. Chem. Soc.*, 3174 (1959).

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## Spin Polarization in Atoms and $\pi$ -Radicals

IN certain radicals, sometimes referred to as  $\sigma$ -radicals<sup>1,2</sup>, the contribution to the orbital of the unpaired electron from one or more of the atoms involved includes a relatively high atomic  $s$  character, and this is manifested as a large isotropic hyperfine coupling. It is customary to estimate the  $s$  character by dividing this isotropic coupling by a calculated value ( $A^0$ ) for a spin density of unity in the appropriate orbital<sup>3</sup>. For  $\pi$ -radicals the isotropic hyperfine coupling constants of which are a consequence of spin-polarization or configuration interaction, however, the value is generally related, not to the atomic value,  $A^0$ , but to the value ( $Q$ ) that would have been obtained had the spin density in the  $p$  orbital of the atom concerned been unity.

Two correlations are revealed if these  $Q$  values are divided by the appropriate  $A^0$  value. (We have suggested that this ratio be given the symbol  $U$ .) The first is that when this is done for atoms such as nitrogen (normalized to one electron), the result is close to 0.2 per cent. The second is that for molecules having three  $\sigma$ -bonds to the atom in question the result is close to 4 per cent, and for all radicals tested it is never less than about 2 per cent. Although results for particular atoms and molecules have been discussed in great detail and refinement, this remarkable lack of sensitivity of the  $U$  values to the nature of the nucleus and bonding does not seem to have been recognized. Even more striking is the very large difference between results for atoms and those for molecules. This suggests to us that the dominating factor for molecules is polarization of the  $\sigma$ -bonding electrons and to a less extent non-bonding valence electrons having  $p$ - $s$  hybrid character, rather than of the core  $s$ -electrons. Perhaps the significant difference is the fact that for the molecules, the effective electrons are greatly attenuated away from the nucleus concerned and that this magnifies any spin-imbalance at this nucleus.

Details of these results will be published elsewhere. Here we wish to draw attention to the considerable utility of  $U$  values for radicals especially when considering the possibility of minor deviations from planarity<sup>3</sup>.

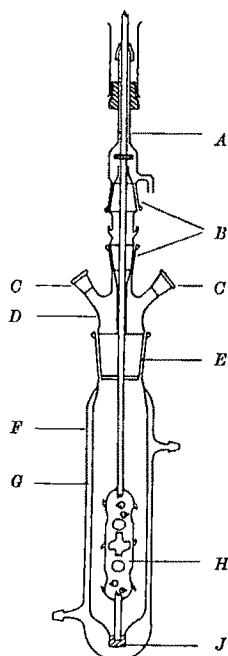


Fig. 1. A, Stirrer guide designed to avoid contamination with Nujol lubricant (ref. 3); B, 'B 19' joints; C, 'B 14' joints; D, borosilicate glass; E, 'B 50' joint with 'Teflon' sleeve; F, fused silica cell; overall length 35 cm, outside diameter 8 cm, working capacity about 400 ml.; G, annular gap of 1 cm or more for filter solution/coolant; H, hollow perforated borosilicate glass cylinder with hooks for attachment of fused silica fibre (the relative length can be greater than that indicated); J, bearing of 'Teflon' reinforced with glass fibre.

Also the remarkable insensitivity of the results to major changes in nuclei and bonding must be accommodated by any overall theory of the phenomenon.

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Received February 13, 1967.

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### Method for estimating the Molecular Weight of Linear Polyethylene

A METHOD for estimating the molecular weight of a long chain linear polyethylene filament is discussed. This method is based on the assumed chain configurations of the polymer before and after extrusion from a jet, and on the straightening of the chain of the filament in a load elongation experiment. This filament can be considered as partially oriented at extrusion (jet stretch). The degree of such orientation is largely related to the speed of extrusion and the speed of taking up the filament. A typical load-elongation curve of a high crystalline, linear polyethylene filament is shown in Fig. 1. "Necking" begins after a few per cent of elongation. The load is approximately a constant during necking. After the completion of necking the load increases with further extension until the filament breaks.

The high crystalline polymer is melted before extrusion and thus the chains are randomly coiled. Let  $N$  be the average number of the C—C bonds in the chain. Then the end to end distance of the randomly coiled chain, calculated from the principle of random flight<sup>1</sup>, is  $c(2N)^{1/2}$  where  $c$  is the C—C bond length. After extrusion the polymer molecules recrystallize (assuming that the ratio of amorphous part to crystalline part is very small) and the segments of a chain are straightened in the crystalline regions. Let  $n$  be the average number of the C—C bonds in a straight segment. The average length of the straight segment is  $n \cdot c \cdot \sin \theta$ , where  $2\theta$  is the C—C—C angle ( $2\theta = 112^\circ$ ).

We first assume that each chain passes through a crystalline region without chain folding. The end to end

distance of a chain in the filament (assuming random orientation for the crystallites) is then given by

$$n \cdot c \cdot \sin \theta \cdot (2N/n)^{1/2}$$

We may then define the per cent jet stretch  $e_j$  as

$$e_j = 100 \left[ \frac{nc \cdot \sin \theta \cdot (2N/n)^{1/2}}{c(2N)^{1/2}} - 1 \right] = 100 [n^{1/2} \sin \theta - 1] \quad (1)$$

At the completion of necking in a load-elongation experiment, a chain which passes through a number of crystalline regions in the filament is approximately straightened without intermolecular sliding. Let  $e_n$  be the per cent elongation at the completion of necking; we then have

$$e_n = 100 \left[ \frac{cN \sin \theta}{nc \cdot \sin \theta \cdot (2N/n)^{1/2}} - 1 \right] = 100 \left[ \left( \frac{N}{2n} \right)^{1/2} - 1 \right] \quad (2)$$

Eliminating  $n$  between equations (1) and (2) we obtain

$$e_j + e_n (1 + e_j/100) = 100 [(N/2)^{1/2} \sin \theta - 1] \quad (3)$$

Next we assume that the crystalline region is formed by chain folding. A single polymer chain in the filament can form a series of crystalline regions of random orientation. Let  $L$  be the average number of times of chain folding in a crystalline region. Assuming  $L < n$  we then have

$$e_j \approx 100 \left[ \frac{nc \cdot \sin \theta \cdot (2N/nL)^{1/2}}{c(2N)^{1/2}} - 1 \right] = 100 [(n/L)^{1/2} \sin \theta - 1] \quad (4)$$

and

$$e_n \approx 100 \left[ \frac{cN \sin \theta}{nc \cdot \sin \theta \cdot (2N/nL)^{1/2}} - 1 \right] = 100 [(NL/2n)^{1/2} - 1] \quad (5)$$

From equations (4) and (5), equation (3) is again obtained.

The equations can be used to calculate  $N$  from estimated values of  $e_j$  and  $e_n$ , or to calculate  $e_j$  and  $n$  (or the ratio  $n/L$ ) for a certain extrusion condition from known molecular weight of the polymer and estimated value of  $e_n$ . For the filament used to obtain Fig. 1, we estimate  $e_j \approx 300$  and  $e_n \approx 600$ . From equation (3) we obtain  $N \approx 2,300$ . The molecular weight of this sample is about 32,000.

Part of the work was carried out at the Research Laboratory, American Viscose Corporation, Marcus Hook, Pennsylvania.

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<sup>1</sup> Flory, P. J., *Principles of Polymer Chemistry*, 415 (Cornell University Press, Ithaca, 1953).

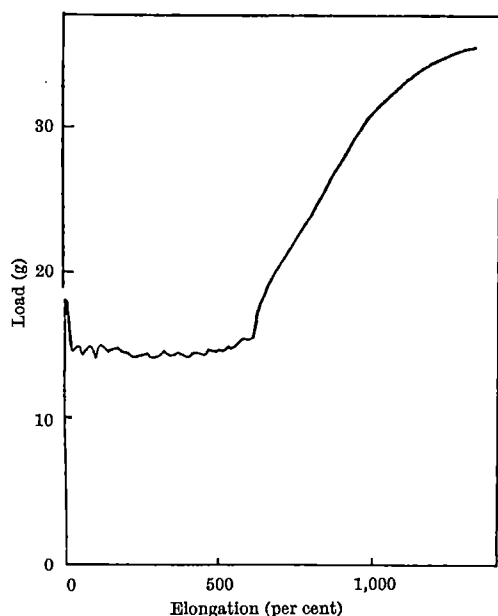


Fig. 1. Load-elongation curve of a linear polyethylene filament (linear density of the filament is 77 g per 9,000 m).

## BIOCHEMISTRY

### Biochemical Evidence for a Distinct Type of Primary Gout

IN recent years several reports<sup>1-3</sup> have called attention to a disorder occurring in the paediatric age group which is characterized by marked over-production of uric acid. The clinical features include haematuria and uric acid stone disease, and often, but not always, mental retardation, neurological signs, and self-mutilation. Intravenous injection of glycine-<sup>14</sup>C into such patients results in a rapid incorporation<sup>1</sup> of isotope into urinary uric acid,

signifying the presence of a shunt pathway whereby precursor is incorporated into uric acid without previous incorporation into nucleic acid purines.

Adult patients with gout characterized by over-production of uric acid show a similar but less marked pattern of incorporation of glycine into uric acid<sup>4,5</sup>. Recently, it has been shown that in the adult type of gout the shunt pathway can be abolished by administration of the synthetic purines azathioprine ('Imuran')<sup>6</sup> and allopurinol ('Zyloprim')<sup>6</sup>.

This communication reports on uric acid dynamics in a case of juvenile gout, and the failure of azathioprine and allopurinol to suppress *de novo* purine biosynthesis. The patient was a 13 year old boy who had had recurrent uric acid stone disease and haematuria since the age of 3. Except for mild choreiform movements in the outstretched arms and hands, and an area of self-induced alopecia, he presented no neurological signs.

The size of the miscible pool of uric acid and its rate of turnover were determined after intravenous injection of uric acid-2-<sup>14</sup>C (ref. 5). The incorporation of intravenously administered glycine-1-<sup>14</sup>C was determined three times: first during a period without treatment, and subsequently during treatment with azathioprine (4 mg/kg body weight per day) and allopurinol (400 mg/day). Glycine-1-<sup>14</sup>C was injected 96 h after treatment with azathioprine had begun, and on the twelfth day of allopurinol treatment.

Table 1 summarizes the results of the investigations. The daily production of uric acid was markedly enhanced to 2,396 mg/day, which, when corrected for body weight, represents a value of 42.8 mg/kg body weight. The normal endogenous production of uric acid is about 10 mg/kg daily. In contrast to the situation in adult gout with over-production of uric acid, azathioprine did not reduce either the plasma uric acid level or the urinary excretion of uric acid. Allopurinol produced a dramatic reduction in both plasma and urinary uric acid, but simultaneously there was an increase in the precursors of uric acid so that the total excretion of urinary oxypurines remained constant.

Table 1. SUMMARY OF DATA ON URIC ACID METABOLISM IN A CASE OF JUVENILE GOUT DURING A CONTROL PERIOD AND DURING PERIODS OF TREATMENT WITH AZATHIOPRINE AND ALLOPURINOL

	Control study	Azathioprine study	Allopurinol study
Miscible pool of uric acid (mg)	2,695		
Turnover of uric acid (mg/day)	2,397		
Mean plasma uric acid (mg per cent)	12.45	13.06	5.15
Mean urinary uric acid (mg/day)	1,741	1,778	344
Mean urinary uric acid formed from precursors (mg/day)	123		1,787
Mean urinary hypoxanthine (mg/day)	65		780
Mean urinary xanthine (mg/day)	38		787
Cumulative recovery of glycine- <sup>14</sup> C in urinary uric acid, 7 days (per cent of dose)	1.350	1.370	0.238

The strikingly accentuated incorporation of glycine into urinary uric acid may be compared with a normal incorporation of 0.1 to 0.15 per cent of the injected dose after seven days. The concentration of carbon-14 in urinary uric acid during the three glycine incorporation investigations is shown in Fig. 1. Although the maximum isotope concentration occurred later when azathioprine and allopurinol were given than in the control investigation, the shunt pathway was not suppressed by these compounds, and the cumulative recovery of carbon-14 in uric acid and its precursors was remarkably similar in all three investigations. With allopurinol, the cumulative recovery of carbon-14 in urinary uric acid was decreased, but when this figure was corrected for the simultaneous increase in hypoxanthine and xanthine the incorporation of glycine-<sup>14</sup>C into all oxypurines was comparable with the control value.

There is growing evidence for the view that purine biosynthesis is controlled by feedback inhibition. Wyngaarden *et al.*<sup>7</sup> have shown that glutamine phosphoribosylpyrophosphate amidotransferase, the synthesizing

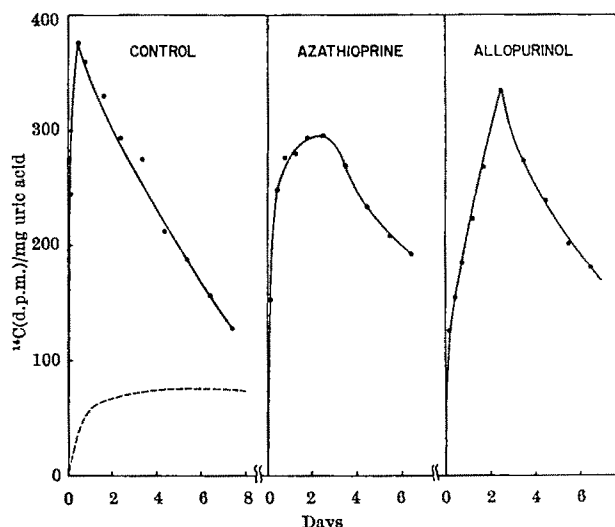


Fig. 1. Concentration of carbon-14 in urinary uric acid following intravenous injection of 100  $\mu$ c. of glycine-1-<sup>14</sup>C at zero time into a patient with primary juvenile gout, during a control period, and during treatment with azathioprine and allopurinol. The broken curve on the left depicts the normal glycine incorporation pattern.

enzyme that catalyses the first reaction in purine biosynthesis, has at least two separate regulatory sites, one for adenylyl and one for guanylyl ribonucleotides. The results of the investigations presented here indicate that there is a distinction between the metabolic pathways in juvenile and adult patients with over-production of uric acid. They imply that in juvenile gout glutamine phosphoribosylpyrophosphate amidotransferase is insensitive to its normal allosteric feedback inhibition; it is conceivable that this may be related to structural alterations in those sub-units of the enzyme concerned with the binding of normal inhibitors.

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### Gel Filtration using a Column packed with Elastin Fibres

THE molecular architecture of elastin as it exists in the water-swollen fibre is ill understood, but to account for the insolubility, rubber-like elasticity and swelling properties of the wet protein fibre its structure is regarded as a crosslinked gel. Chemical studies have shown that the peptide chains are organized as a three-dimensional network, crosslinked at intervals by covalent bonds<sup>1,2</sup>, and it has become usual to assume that between these links the chains are hydrated and in intimate contact with the swelling water. Studies of the rubber-like stress-strain characteristics of the wet fibres<sup>3</sup> indicate that there is no crystallinity, and suggest that the chains between the

crosslinks adopt a randomly crumpled conformation due to unrestricted thermal motion. In view of the large amounts of amino-acid with non-polar side-chains in elastin<sup>4</sup> it seems possible that sites of hydrophobic interaction of considerable size may occur in the network. Such a "corpuseular" model of elastin structure has recently been proposed to account for the behaviour of elastin with enzymes and for the course of fibrogenesis<sup>5,6</sup>. The distribution of the swelling water in the gel would be expected to have a marked influence on the behaviour of solute molecules diffusing in the water spaces, and the purpose of the present work was to examine the behaviour of such solutes using a chromatographic method.

Porath and Flodin<sup>7</sup> and others have shown that columns packed with crosslinked dextran gels are capable of sharp separations with solute molecules of different size. The dextran gels used in chromatographic gel filtration experiments have water regains in the range 7.5–20 g water/g dry gel, while the water content of the elastin gel is much less, about 2 g water/g dry protein. The elastin gel is therefore far more closely structured, but similar separation should be expected if the size of the test solutes is below that of the water-filled channels available for diffusion. In our experiments sugars, polyglycols and alcohols were chosen as uncharged solutes of a size suitable for separation using a column packed with elastin fibres.

The elastin fibres were prepared from the ligamentum nuchae of cattle 18 months to 2 years old, by extraction with 0.1 normal sodium hydroxide at 98° C for 45 min according to the method of Lansing *et al.*<sup>8</sup>. After extraction the fibre preparation was washed repeatedly with hot water until microscopic examination showed that the short smooth rod-like fibres (mean diameter 6.5 $\mu$ , length 0.15–0.50 mm) were well separated and the preparation contained no clumps.

The elastin fibres were suspended in 1 per cent v/v acetic acid and equilibrated by stirring gently for 48 h with repeated changes of solvent. The column (height 47.5 cm; internal cross section 2.27 cm<sup>2</sup>; volume 110.3 cm<sup>3</sup>) was poured in sections, using a thick slurry, and nitrogen under a pressure of 40 cm of mercury was applied to ensure rapid filtration and tight packing. Before applying the solutes, a large volume of degassed 1 per cent acetic acid was run through the column. The column was operated at 25° C with a rate of flow of 7 ml/h (nitrogen pressure 20 cm of mercury) and the fractions collected by means of a drop counter. The volume of the fractions was checked gravimetrically.

Fig. 1 shows the chromatogram obtained on applying a mixture of sugars (2 mg) and alcohols (40 mg each) together with cytochrome *c* (2 mg) to mark the void volume of the column. The mixture was applied in 1 ml. of 1

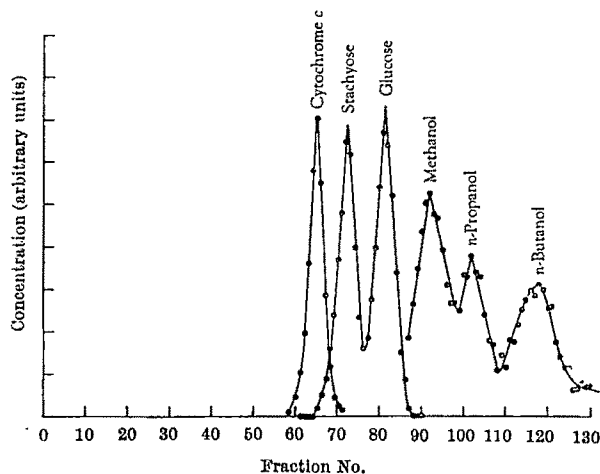


Fig. 1. The separation of a mixture of sugars and alcohols using a column packed with elastin fibres.

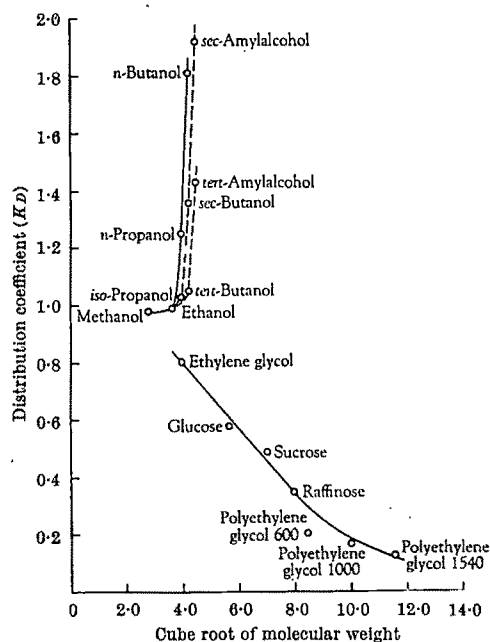


Fig. 2. The distribution coefficient ( $K_D$ ) between the water spaces in the elastin gel and the external water for a number of sugars, glycols and alcohols. Values for  $K_D$  greater than unity indicate adsorption on internal surfaces in the gel.

per cent v/v acetic acid and the column eluted with the same solvent. Cytochrome *c* with a molecular weight of 12,270 is not retarded under these conditions and shows no separation from bovine serum albumin, which has a weight of about 70,000. The sugars were estimated by the anthrone method<sup>9</sup>, cytochrome *c* by measuring extinction at 408 m $\mu$ , and the glycols and alcohols by permanganate oxidation. The mixture of solutes chromatographed in this experiment was chosen to display well separated peaks.

Chromatograms of the type shown in Fig. 1 can be used to calculate the distribution coefficients of the solutes between stationary phases and the flowing solvent phase. In the present experiments the relation of Gelotte<sup>10</sup>

$$K_D = \frac{V_e - V_o}{V_i}$$

was used to calculate the distribution coefficient ( $K_D$ ) of the solute between the water spaces in the swollen fibre and the external water. In this equation  $V_e$  and  $V_o$  are the elution volume of the solute and the void volume of the column respectively.  $V_i$ , the volume of solvent internal to the gel phase, was calculated from the measured water regain of elastin fibres in 1 per cent acetic acid at 25° C (55.8 per cent w/w = 63.1 per cent v/v). For the column used,  $V_i = 45.8 \times 0.631 = 28.9$  cm<sup>3</sup> and (from the position of the cytochrome peak)  $V_o = 64.5$  cm<sup>3</sup>.

Fig. 2 shows the distribution coefficient ( $K_D$ ) for a number of sugars, glycols and alcohols plotted against the cube root of the molecular weight of the solute. It will be seen that the sugars and glycols together fall into a uniform series, the distribution coefficients decreasing from values close to unity to very low values as the molecular weight increases. The polyglycols of very high molecular weight are almost entirely excluded from the water spaces in the gel, but a large part of the water in these spaces is available for the diffusion of ethylene glycol and sugars of low molecular weight.

The alcohols, on the other hand, fall into an entirely different series in which the lowest member (methanol) has a value for  $K_D$  just less than unity and the apparent distribution coefficient for the higher members is greater than unity. Thus for secondary amyl alcohol the apparent concentration in the stationary water phase is roughly



twice that in the surrounding solvent, indicating very marked absorption on internal surfaces in the protein gel. As would be expected, the adsorption of the alcohols depends greatly on the structure around the hydrophilic hydroxyl radical and the adsorption behaviour of the normal, secondary and tertiary homologues can clearly be distinguished. In all three homologous series adsorption increases with increasing molecular weight and increasing hydrophobic character. This suggests that the alcohols, unlike sugars and glycols, tend to be adsorbed at hydrophobic centres in the peptide fabric of the protein gel.

The results as a whole show that the wet elastin fibre contains water spaces of a size sufficient to allow the penetration of uncharged solutes with molecular weights up to about 1,500, and that while sugars and glycols are not adsorbed on the peptide fabric, even the lower alcohols show marked adsorptive effects. This adsorption is apparently brought about by hydrophobic interactions, and it may be presumed that the hydrocarbon side-chains of the non-polar amino-acids offer sites for adsorption.

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## Release of Enzymes from the Liver

DIFFERENT lesions of an organ lead to distinct enzyme patterns in the serum. Our knowledge about the mechanisms affecting the release of cellular enzymes from damaged tissues, however, is poor.

We studied the release of cellular enzymes in isolated perfused rat liver. By isolating the organ we were able to avoid the influence of extrahepatic factors and the superposition of enzyme patterns from various tissues. The rat livers were perfused by the method of Miller<sup>1</sup> as modified by Schimassek<sup>2</sup>. The cell free perfusion medium (a solution of physiological saline containing 25 g bovine albumin, 5.2 mmoles glucose, 1.3 mmoles lactate, and 0.1 mmoles pyruvate/l.<sup>2</sup>) was saturated with oxygen ( $pO_2$ , 300–400 torr.). The pH was adjusted to 7.30 ( $\pm 0.02$ ) and shifted during perfusion to 7.14 ( $\pm 0.02$ ). The temperature of the perfusion medium immediately after it entered the portal vein was 37° C ( $\pm 0.2^\circ$  C) and the flow was 1–3 ml./g liver/min.

The omission of erythrocytes from the perfusion medium caused a definite hypoxic lesion of the liver cells with a reduced consumption of oxygen (the gas analysis was carried out according to refs. 3 and 4). Simultaneously, leakage of enzymes from the liver was increased by lysing erythrocytes into the perfusion medium with a hypotonic solution. Histological sections and electron microscopy of normal liver under these conditions revealed that it was only slightly altered after 4 h of perfusion<sup>5</sup>. On average the loss of soluble protein (measured according to ref. 6) from the liver was 30 per cent. Within the 4 h of perfusion a remarkable increase in enzyme activity occurred in the circulating medium.

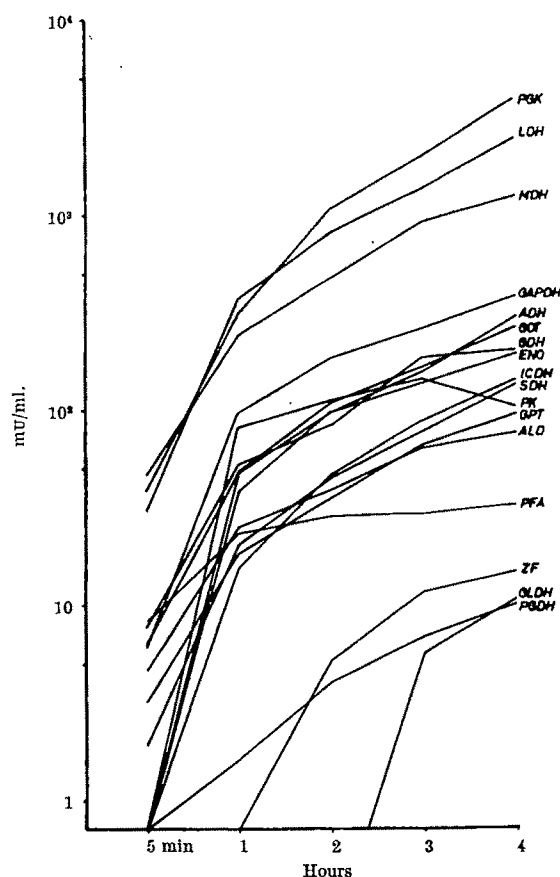


Fig. 1. Enzyme activities in the perfusion medium. (Ten perfusions; mean values.)

Fig. 1 shows the rise in activity of seventeen cellular enzymes in the extracellular space (the assays were performed according to refs. 7 and 8). In this figure, allowance has been made for the alterations which the enzyme activities undergo after release from the cells. These alterations consist of increases as well as decreases in activity. They depend on the composition of the perfusion medium, the dilution in the extracellular space, the temperature, the time, and are specific for each enzyme investigated<sup>9,10</sup>.

The enzyme pattern in the circulation system, as demonstrated in Fig. 1, is split into three groups of enzymes, the activities of which differ from each other by about an order of magnitude. It is evident from an analysis of this enzyme pattern that the rates of release of most of the enzymes investigated follow their respective concentration gradients between the liver and the circulation system: enzymes with high activities in the liver cell<sup>11,12</sup> reach high concentrations in the extracellular space, while those with low activities in the cell do not.

It can be seen from the upper part of Fig. 2 that the rate of release of most of the enzymes is inversely proportional to their respective molecular weights: the higher the molecular weight, the lower the percentage of intracellular activity found in the perfusion medium. GLDH, GOT and MDH\*, however, do not follow this rule. This indicates that the intracellular distribution delays the release from the cells: GLDH is solely in the mitochondria of the rat liver cell, which also contains more than 70 per cent of the GOT and 40 per cent of the MDH (refs. 12 and

\* Abbreviations: ADH, alcohol dehydrogenase; ALD, fructose-1,6-diphosphate aldolase; C, cytoplasmic; ENO, enolase; GAPDH, glyceraldehyde phosphate dehydrogenase; GDH,  $\alpha$ -glycerophosphate dehydrogenase; GLDH, glutamate dehydrogenase; GOT, glutamic oxalacetic transaminase; G-6-PDH, glucose-6-phosphate dehydrogenase (ZF); GPT, glutamic pyruvic transaminase; ICDH, isocitric dehydrogenase; LDH, lactic dehydrogenase; M, mitochondrial; MDH, malic dehydrogenase; PFA, fructose-1-phosphate aldolase; PGDH, 6-phosphogluconic dehydrogenase; PK, phosphoglycerate kinase; PK, pyruvate kinase; and SDH, sorbitol dehydrogenase.

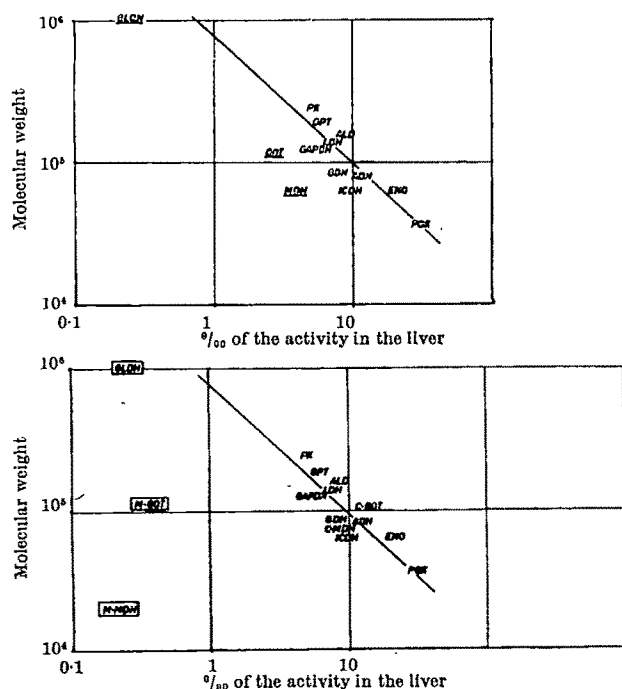


Fig. 2. Rates of release of enzyme activities into the perfusion medium after 4 h perfusion plotted against the molecular weights of enzyme proteins. (Ten perfusions; mean values.)

13). The other enzymes investigated originate from the cytoplasm of the cells. Most of the activities of MDH and GOT found in the perfusion medium are derived from their cytoplasmic isoenzymes, as has already been shown by column chromatography<sup>5</sup>.

In the lower part of Fig. 2 it can be seen that these cytoplasmic isoenzymes behave like the other cytoplasmic enzymes, depending on their concentration gradient and their molecular weight. The concentrations of the mitochondrial isoenzymes, and the G6PDH, however, do not show this correlation. Unfortunately, as yet we cannot be certain of the molecular weights of mitochondrial GOT and MDH.

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### Dependence of Uptake of Succinate by Mitochondria on Energy and its Relation to Potassium Retention

A LONG standing problem in mitochondrial metabolism is the origin of the inhibition of oxidation of anionic substrates which sets in on adding an excess of any agent which uncouples oxidative phosphorylation. It has been

noted that the inhibition is moved to higher uncoupler concentrations when the substrate concentration is raised<sup>1</sup>. This suggests that for some reason the permeation of substrate fails to keep up with the metabolic demand in presence of excess uncoupler. This supposition accords with observations that the respiratory carriers are left in an oxidized condition when inhibition supervenes<sup>2</sup>.

Parallel studies of the metabolic effects of agents which induce ion permeability, such as valinomycin, led to the finding that the maximal respiration attainable on adding an uncoupling agent could be increased by prior induction of a high permeability to potassium ions, with its attendant uptake of potassium, particularly at low substrate concentration (for example, 3 mmolar). Furthermore, the maximal rate of respiration attainable is consistently higher in a 150 mmolar potassium chloride medium than in a sucrose medium free of potassium ions. Release of potassium ions, when caused by addition of nigericin, has been shown to lead to inhibition of metabolism<sup>3</sup>.

Taken together, these facts suggested that the retention of potassium ions or the direction of the net movement of the ions is related to the ease of entry of certain substrate anions and thus to the maximal respiratory capability. We sought to test this; Fig. 1 shows records taken simultaneously of respiration, light scattering and the concentration of potassium ions in the medium during successive additions of valinomycin and the uncoupling agent trifluoromethoxycarbonylcyanidephenylhydrazide<sup>4</sup>. The valinomycin causes movement of potassium ions from the medium into the mitochondria and a swelling, shown by reduction of light scattering signal. A small dose of FCCP stops the uptake of potassium ions and induces a high respiratory rate while a further dose of FCCP leads to respiratory inhibition after a slight delay. The records show that potassium ions are lost from the particles, which shrink. A similar end result is obtained without the prior addition of valinomycin; respiratory inhibition sets in soon after loss of the endogenous potassium has started.

Measurements of the uptake by mitochondria of substrates labelled with carbon-14 have been made by the centrifugal method described previously<sup>5,6</sup>. Table 1 shows mean values of duplicate experiments run in parallel and allows comparison of the amounts of succinate plus metabolic products (fumarate and malate) in the mitochondria after brief (10 sec) incubations in media with or without additions of substances known to alter either energy production or potassium retention. Agents which uncouple oxidative phosphorylation when applied at levels sufficient to inhibit respiration (as found in other experiments) caused a marked diminution of uptake of labelled succinate (compare lines 2, 3 and 4 with line 1).

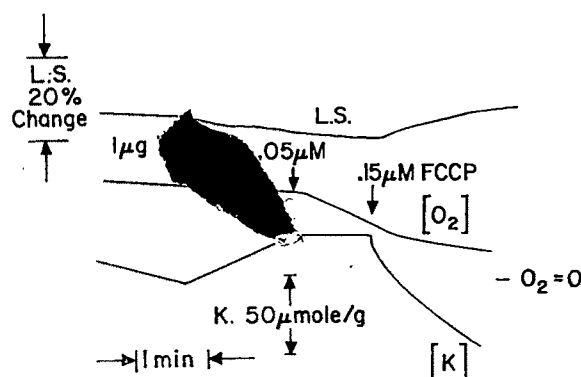


Fig. 1. Simultaneous recordings<sup>4</sup> of light scattering (L.S.), oxygen concentration ( $O_2$ ) and potassium concentration (K) were made from a suspension of rat liver mitochondria (2 mg/ml), in a medium containing: 250 mmolar sucrose; 20 mmolar tris chloride pH 7.4; 2 mmolar potassium chloride; 3 mmolar tris-glutamate; 3 mmolar tris malate. Further additions of valinomycin (Val) and trifluoromethoxycarbonylcyanidephenylhydrazide (FCCP) were made as indicated. Swelling is indicated by a downward deflexion of the light scattering trace.

Table 1

Addition	Experiment 1	Experiment 2	Experiment 3	Experiment 4
1. —	2.4	3.4	2.2	1.8
2. 10 $\mu$ molar TTFB	0.8	—	—	—
3. 200 $\mu$ molar dicumarol	—	1.2	—	1.2
4. 3 $\mu$ molar FCCP	—	—	1.6	—
5. 0.2 $\mu$ g/g valinomycin	—	—	3.4	—
6. 0.3 $\mu$ g/g nigericin	—	—	1.2	—
7. 0.6 $\mu$ g/g antimycin A	—	—	—	1.9
8. 0.25 $\mu$ g/g oligomycin + 0.25 $\mu$ g/g antimycin A	—	—	—	0.8

Concentration ratios of succinate- $^{14}$ C between total water of rat liver mitochondria and medium after approximately 10 sec exposure time at 22° C. Reaction was carried out by centrifugation of the mitochondria through a medium containing a 1 mmolar succinate- $^{14}$ C (3 mmolar in experiment 3), 250 mmolar sucrose, 20 mmolar *tris* chloride pH 7.4, and 5 mmolar potassium chloride and 0.5  $\mu$ g/ml. rotenone, collecting the mitochondria in acid and by measuring the amount of succinate- $^{14}$ C taken up with a liquid scintillation counter.

There was no effect on uptake if these agents were applied at levels at which respiration was stimulated. In experiments like that illustrated in Fig. 1 the high level of uncoupling agent leads to potassium discharge; a discharge of potassium ions is also induced by nigericin and it too inhibits substrate entry (line 6). On the other hand, addition of valinomycin, which promotes potassium uptake (as seen in the first part of Fig. 1), stimulates uptake of the succinate (line 5). The addition of antimycin, which inhibits respiration, failed to decrease the uptake of succinate (line 7); this result is important for two reasons: (1) it shows that succinate itself can be accumulated because the material labelled with carbon-14 will not be oxidized; and (2) it shows that the endogenous adenosine triphosphate (ATP) can provide energy for substrate transport in the absence of respiration. The latter point is confirmed by the very small entry (line 8) obtained when oligomycin is used to block the utilization of ATP in presence of antimycin which has been used to block respiration.

Unambiguous examples of accumulation of an anion in conditions in which it is not oxidized have also been obtained using labelled acetate or citrate in the presence of rotenone, with succinate as energy source.

Because the data obtained to date do not allow us to decide in which part of the mitochondrion the anions are accumulated, the concentration ratios given in Table 1 are computed for the total mitochondrial water. The actual gradient of concentration may be much greater, however, if the substrate is accumulated in the sucrose impermeable space which comprises only approximately one-third of the mitochondrial water. Conditions which give rise to potassium loss are associated with reduced uptake of substrate, while stimulated potassium uptake is accompanied by an increase in substrate uptake. This agrees with the conclusion of Lynn and Brown<sup>7</sup>. It is difficult to decide at present whether there is a common energy dependence of the two parallel processes or whether active uptake of one ionic species is a prerequisite for movement of the other species.

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## Acetate Repression of Chlorophyll Synthesis in *Euglena gracilis*

THE photosynthetic apparatus of *Euglena gracilis* is exceptionally labile and can be bleached by a variety of chemical and physical agents. During a recent series of experiments on streptomycin<sup>1</sup>, heat<sup>2</sup> and hydroxyurea<sup>3</sup> inhibition of chlorophyll synthesis in *Euglena*, it was noted that cells grown in the presence of excess sodium acetate contained less chlorophyll than cells grown with smaller amounts of acetate. This observation led to the present experiments.

Stock cultures of *Euglena gracilis*, strain Z, were grown at room temperature in a medium containing the following per litre: 2.5 g proteose-peptone, 2.5 g bactotryptone, 0.1 g yeast extract, 0.1 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 1 g each of glucose, potassium dihydrogen phosphate, potassium monohydrogen phosphate and sodium acetate. Subsequent greening experiments with various amounts of acetate were carried out at room temperature with incandescent light (100 W) placed above the culture flasks. Cell counts were made with a microscope with a Whipple disk and Sedgwick-Rafter counting chamber. Cells were concentrated by centrifugation and chlorophyll was extracted with 80 per cent acetone for 2 h in the dark. The optical density of the extract was measured at 663 and 645 m $\mu$ . Chlorophyll concentration was calculated according to Arnon<sup>4</sup>.

Fig. 1 shows the effect on growth and chlorophyll synthesis of *Euglena* of raising the concentration of acetate from 11.5 mmoles/l., the usual amount, to 112 mmoles/l. Both growth and chlorophyll synthesis were inhibited, the latter being the more sensitive. By 120 h, chlorophyll content per millilitre of the high acetate culture was only 15 per cent of that found in the low acetate culture.

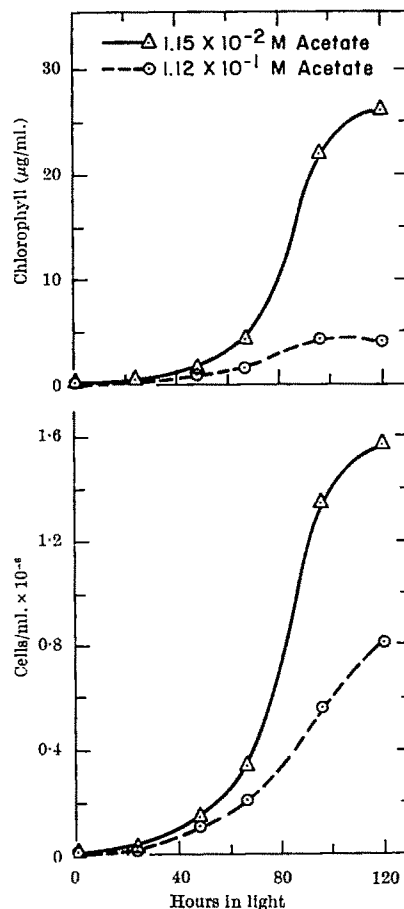


Fig. 1. Growth and chlorophyll synthesis of *E. gracilis* in the presence of low (Δ) and high acetate (○). Initial chlorophyll/ml. was 0.15  $\mu$ g and initial cell number/ml. was 7,200.

Furthermore, no increase in chlorophyll/ml. was observed in the high acetate culture after 96 h. Thus, because the number of cells in this culture increased between 96 and 120 h, the amount of chlorophyll present actually decreased from 7.7  $\mu\text{g}/\text{cell}$  at 96 h to 4.9  $\mu\text{g}/\text{cell}$  at 120 h. Low acetate cells contained 16.6  $\mu\text{g}$  chlorophyll/cell at 120 h. On the basis of these results, it was decided to run a series of acetate concentrations together with an added control culture lacking acetate. Table 1 shows that the culture lacking acetate synthesized the most chlorophyll per cell in 96 h. Cultures were initially inoculated with 9,000 cells/ml. and all cultures were in the late log phase or early stationary phase of growth at this time. The usual growth condition (11.5 mmolar acetate) was inhibited 33 per cent. Inhibition of chlorophyll synthesis reached 73 per cent with 45.7 mmolar acetate, but the inhibition did not increase with higher concentrations of acetate. It was further observed that cells grown in 23 mmolar or higher concentrations of acetate were densely packed with paramylon granules, the carbohydrate storage product of *Euglena*. This observation agrees with that of Pringsheim and Pringsheim<sup>5</sup>, who noted an increased content of paramylon granules in *E. gracilis* grown with 14.7 mmoles/l. or more acetate. The inhibition of the synthesis of chlorophyll, however, imposed by 112 mmolar acetate, for example, was readily reversed. Under sterile conditions, 10 ml. of cells from the 96 h culture (Table 1) was concentrated by centrifugation at 4° C, washed once with 0.9 per cent sodium chloride, resuspended in normal medium (11.5 mmolar acetate) and placed within 16 in. of the 100 W light. By 48 h the chlorophyll content/10<sup>7</sup> cells was 154  $\mu\text{g}$ , and by 74 h it was 181  $\mu\text{g}$ .

Table 1. INFLUENCE OF ACETATE ON CHLOROPHYLL CONTENT OF *E. gracilis*

Acetate (mmoles/l.)	Chlorophyll ( $\mu\text{g}/10^7$ cells)
0	181
11.5	123
23.0	85
45.7	49
90.0	44
112.0	49

Cultures were grown for 96 h at room temperature and then sampled for chlorophyll determination.

The early observation of Hall<sup>6</sup> showed that the growth of *E. stellata* on complex medium was somewhat inhibited at concentrations of acetate greater than about 24.4 mmoles/l. Ternetz<sup>7</sup> even earlier had noted a fading of the green colour of *E. gracilis* var. *bacillaris* when it was inoculated into a rich organic medium. App and Jagendorf<sup>8</sup> showed that non-multiplying *Euglena* incubated in phosphate buffer with 8.1 mmolar acetate contained only 38 per cent and 77 per cent as much chlorophyll as control cells in buffer alone at 18 and 36 h respectively. The present investigation further shows that excess acetate not only represses the synthesis of chlorophyll in multiplying *Euglena*, but also leads to a concomitant increased synthesis of paramylon. Such an effect presumably has adaptive significance because ultimately the cells must use storage material for the synthesis of chloroplasts and basal metabolism when external acetate is used up. Support for this interpretation is found in the recent work of Cook<sup>9</sup> which shows that carbon fixed in photosynthesis in *Euglena* is routed almost exclusively into protein when acetate is present, whereas the acetate incorporated mainly enters lipid and polysaccharide.

Magasanik<sup>10</sup> showed that, in addition to glucose, any carbon compound efficiently used as a source of intermediary metabolites and of energy by a micro-organism can repress certain inducible enzymes. Unlike the effect of excess glucose on *Chlorella*<sup>11</sup>, the repression of chlorophyll synthesis in *Euglena* by acetate is not complete. Increasing amounts of acetate increasingly inhibit chlorophyll synthesis, but a maximum inhibition of about 75 per cent occurs when the concentration of acetate is 45.7 mmoles/l. and greater (Table 1). It would seem that

in *Euglena* acetate can strongly repress certain enzymes used during the final maturation of the chloroplast. The present investigation, however, indicates the existence of cellular control mechanisms which prevent total repression by acetate of chlorophyll synthesis in *Euglena*.

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### Isoamylases of Human Parotid Saliva

COLUMN electrophoresis with a polyacrylamide gel was used by Muus and Vneuchak<sup>1</sup> to separate from crystalline human saliva amylase four protein staining bands which suggested four different molecular forms of amylase. The staining evidence was corroborated with the finding of four areas of amylase activity from gel section eluates. A more direct method with greater resolving power for detecting the multiple molecular forms of amylase has been developed for use with polyacrylamide gel columns. Preliminary studies show that there are generic differences in salivary amylase and that a polymorphism exists among human parotid salivas.

Parotid saliva was bilaterally collected from selected individuals by using modified Carlson and Crittenden cups. The salivary flow was stimulated by having the patient taste *ad libitum* a commercial, natural strength, reconstituted lemon juice<sup>2</sup>. Whenever practicable, electrophoresis was performed on the day of collection; otherwise, the samples were lyophilized and stored in a freezer. These samples were reconstituted in the electrophoresis buffer just before use.

Crystalline preparations of both the pooled and individual saliva samples were made by the method of Fischer and Stein<sup>3</sup>. Electrophoretically purified salivary amylase was prepared by paper electrophoresis, removing the amylase section and recovering the amylase by eluting in water and lyophilizing. The Durrum type electrophoresis cell was used with veronal buffer, pH 8.6, ionic strength of 0.05, 8 pre-cut S and S 2043 A mgl strips per run for 23 h at 200 V of constant voltage. For a comparative study rat parotid saliva was collected by the method of Wolf, Kakehashi, and Taylor<sup>4</sup>.

Fifteen  $\mu\text{l}$  of parotid fluid were subjected to electrophoresis at 10 m.amp per gel for 60 min at 2°–5° C on a polyacrylamide gel column by the method of Ornstein and Davis<sup>5</sup>. Following electrophoresis the acrylamide gel column was placed on a starch-agar coated glass slide. 2 ml. of the following mixture had been previously pipetted on to a microscope slide and allowed to dry at room temperature: 1 g noble agar, 0.25 g starch (hydrolysed), 0.25 g amylopectin and 10 ml. of 0.1 M, pH 6.9, phosphate buffer also containing 0.1 M sodium chloride diluted to 100 ml. The column was then compressed to about 3 mm by another glass slide and incubated for 15 min at room temperature. Amylase position was indicated by clear zones on a purple field of starch iodine complex after the incubated starch-agar gel slide had been developed in an iodine-potassium iodine solution (0.01 M iodine and 0.01 M potassium iodine) for 15 sec.

The zymogram patterns of Fig. 1 illustrate the effect of preparation and sampling. Seven bands are seen in Fig. 1a, and five bands are seen in Fig. 1b. The seven bands of the zymogram Fig. 1a were obtained when testing amylase crystals from a pooled human parotid saliva sample. The five bands of Fig. 1b were obtained when amylase crystals from the parotid saliva of one person were subjected to electrophoresis. The finding of more bands with the pooled sample crystals than with the crystals from a single source indicated that the pooled sample had components which the non-pooled sample did not. This finding was taken to indicate the existence of a polymorphism and corroborating evidence will be given later in this paper. Using a comparable method of separation but testing a crystalline pooled whole human saliva sample and using a different detection method, Muus found four areas of amylase activity. She also speculated that there may be "... differences among the amylases secreted by different individuals"<sup>1</sup>. We found variation in the intensity of the bands and this feature may prove to be a distinguishing characteristic as has been found with LDH (ref. 6) and animal amylase studies<sup>7-9</sup>. Trailing was found to be negligible because the weaker bands migrate farther and the strongest was usually next to the slowest band.

The parotid saliva amylase zymograms of Fig. 1a, b and c were products of samples collected at different times and prepared by different methods. Those samples receiving less preparative treatment appeared to be similar. The effect of sampling time variation was negligible because repeated samples from individuals yielded identical amylase zymograms. After the more drastic treatment of crystallization, the zymogram Fig. 1b (5 bands) showed less resemblance to the zymogram of Fig. 1d (7 bands) which was produced from an untreated parotid saliva sample. The sample which was purified by paper electrophoresis, Fig. 1c, and the untreated parotid saliva sample, Fig. 1d, yielded zymograms with seven bands similar both in relative density and banding order. A greater difference was found between the crystallized sample and untreated

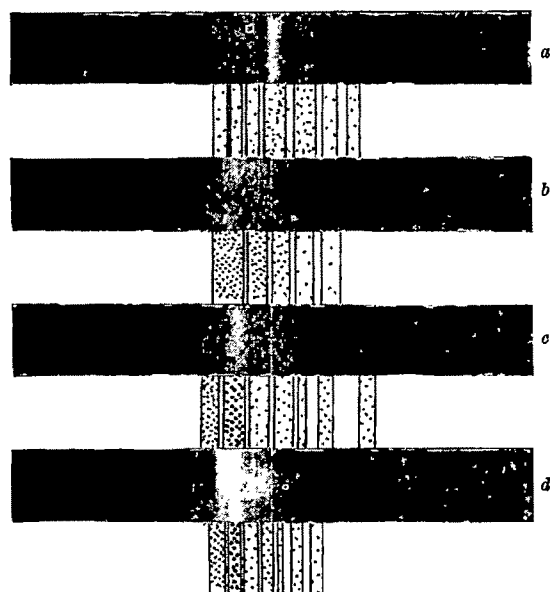


Fig. 1. Zymograms of human parotid salivary isoamylase illustrating variation by preparation. Electrophoresis was on 7.5 per cent polyacrylamide gel for 1 h at 4 ma/gel. The buffer was a 0.025 M tris (hydroxymethyl) aminomethane and 0.192 M glycine of pH 8.3. The spacer gel was at the top of the gel columns (left side of illustration) and migration toward the anode. (a) From amylase crystals of pooled parotid saliva (8 individuals). (b) From amylase crystals of one person; one of the authors (R.O.W.). (c) From paper electrophoretically purified amylase of parotid saliva from R.O.W. (d) From neat parotid saliva of R.O.W.

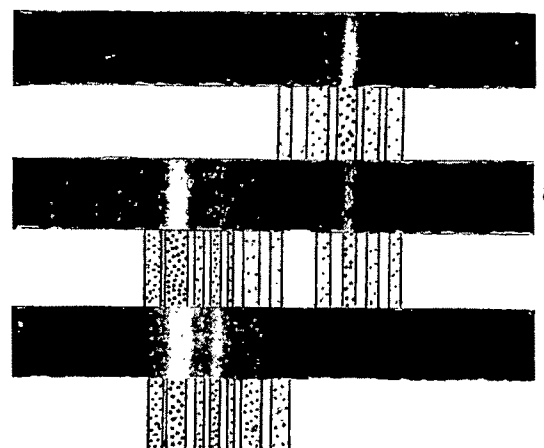


Fig. 2. Zymograms illustrating parotid saliva amylase species differences. (a) Rat parotid saliva. (b) Human and rat parotid saliva. (c) Human parotid saliva.

sample than was found between the sample purified by paper electrophoresis and the untreated sample. This illustrates the effect of treatment. Muus<sup>1</sup> as well as Kaplan<sup>10</sup> recognized the possibility of purification procedures introducing artefacts by changing the properties of isoenzymes.

The zymogram of the electrophoretically purified amylase (Fig. 1c) illustrates the resolving power of the polyacrylamide gel column electrophoresis compared with that of paper strip electrophoresis. Where the one amylase band was recovered from paper electrophoresis and then subjected to electrophoresis on the acrylamide gel system, seven bands were detected. It was concluded that the isoenzymes of amylase are separated as one band during paper electrophoresis at pH 8.6.

The results of mixing parotid saliva of human and rat are shown in Fig. 2. The amylases of these two species are different with respect to the rate of electrophoretic migration and they maintain a high degree of autonomy when mixed, suggesting amylase differences among genera.

Table 1. NUMBER OF PAROTID SALIVA ISOAMYLASE BANDS FOUND IN ELEVEN INDIVIDUALS

No. of bands	Individual frequency
5	3
6	6
7	1
8	1

By using the same quantity of saliva for each electrophoresis we have found that a polymorphism exists among the population tested. The number of parotid saliva isoamylase bands found in eleven individuals is given in Table 1.

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### Radioimmunoassay of Bradykinin

In the past few years highly sensitive and specific radioimmunoassay methods have been developed for the determination of many polypeptide hormones<sup>1</sup>. Radioimmunoassays are usually based on the competitive reaction of a measured amount of polypeptide hormone labelled with iodine-125 and iodine-131 and varying amounts of unlabelled hormone with hormone antiserum. This report describes a radioimmunoassay technique for bradykinin, a nonapeptide of considerable physiological and pathological importance. Bradykinin differs from other polypeptide hormones, which have been assayed by this method, in its lack of immunogenicity and inaccessibility to iodination. Formation of antibody against this peptide was therefore induced by immunizing goats with a bradykinin-haemocyanin conjugate<sup>2</sup>. An isotopic label was introduced into the molecule by acetylating its terminal amino-group with <sup>14</sup>C-acetic anhydride<sup>3</sup>. This approach makes it possible to use commercially available bradykinin and renders an isotopic synthesis of the peptide unnecessary. Antibody which binds <sup>14</sup>C-acetyl bradykinin was demonstrated in antiserum by a gel filtration procedure which separates antibody-bound from unbound peptide. Partial to complete displacement of the labelled acetyl peptide from its antibody-complex was observed on addition of increasing amounts of unlabelled bradykinin or acetyl bradykinin to the incubation mixture of a fixed amount of <sup>14</sup>C-acetyl bradykinin and antiserum. Unlabelled bradykinin was found to be about 10–20 times less effective in this reaction than unlabelled acetyl bradykinin.

Haemocyanin of molecular weight  $7 \times 10^6$ , apparently homogeneous by sedimentation velocity and electron microscopy, was isolated from keyhole limpets and kept at  $-20^\circ\text{C}$  until used. The conjugate was prepared as follows: 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (825 mg) was dissolved in 2 ml. of saline and added dropwise with stirring to a solution of 55 mg of haemocyanin and 100 mg of bradykinin triacetate in 3 ml. of saline. The mixture deposited a gelatinous precipitate and was allowed to stand at room temperature for 30 min. It was then dialysed against two changes of 2,000 ml. saline for 60 h in the cold room. Amino-acid analysis of a hydrolysed sample indicated that approximately 15  $\mu\text{moles}$  of bradykinin had been incorporated in each 100  $\mu\text{g}$  of haemocyanin. Two goats were immunized by subcutaneous and intramuscular injection of the conjugate suspended in Freund's complete adjuvant. Each goat received 100 mg of the conjugate over a period of 3 months. The animals were bled 3 weeks after the last injection and were then given further immunizations over a period of 6 months with periodic bleedings. Pre- and post-immunization sera were stored at  $-20^\circ\text{C}$  for immunoassay.

Bradykinin triacetate was dissolved in a minimum of acetic acid and allowed to react with a 20 molar excess of acetic-<sup>14</sup>C-anhydride at room temperature for 3 days. Excess acetic acid and anhydride was removed *in vacuo*. The O-acetyl group (serine) was hydrolysed by treatment of the residue with 0.1 molar *tris* buffer at pH 8.7. After acidification and removal of acetic acid and solvent *in vacuo* the product was purified by ion-exchange on an 'Amberlite CG-50' column<sup>5</sup>. The purified <sup>14</sup>C-acetyl bradykinin was checked by paper chromatography against a sample of unlabelled acetyl bradykinin. Both samples had  $R_F$  values of 0.42 (*n*-butanol-acetic acid-water 90:10:25) and gave a positive Sakaguchi stain but a negative ninhydrin reaction. All radioactivity was located in the area of the Sakaguchi stain. <sup>14</sup>C-acetyl bradykinin was found to possess half the biological activity of bradykinin in the rat uterus assay<sup>6</sup>. Pre-immunization serum or antiserum (1 ml.) was incubated for 30 min at  $5^\circ\text{C}$  with a fixed amount (0.36  $\mu\text{g}$ ) of <sup>14</sup>C-acetyl bradykinin in 0.5

ml. of borate buffer (0.01 molar, pH 7.5) made up in 0.9 per cent saline. The buffer also contained bacitracin (0.5 mg/ml.) or lysozyme (1 mg/ml.) to prevent adsorption of the peptide to glass, and arginine (0.46 mg/ml.), an inhibitor of bradykininase<sup>7</sup>. The concentration of peptide present in the test solutions was checked by amino-acid analysis of hydrolysed samples. Incubation mixtures were applied to a  $0.9 \times 40$  cm column of 'Sephadex G-50' and developed with the borate buffer at  $5^\circ\text{C}$ . The effluent was collected in 1 ml. fractions and its radioactivity determined in a scintillation counter<sup>8</sup>. Binding of <sup>14</sup>C-acetyl bradykinin by antiserum was demonstrated by the emergence of radioactivity in the void volume (peak 1, Fig. 1). The percentage of labelled hapten bound by antibody is represented by A in the first peak and unbound radioactive hapten by A in the second peak. When varying quantities of unlabelled peptides (B, 0.33  $\mu\text{g}$  unlabelled acetyl bradykinin; C, 5  $\mu\text{g}$  unlabelled bradykinin; and D, 20  $\mu\text{g}$  unlabelled bradykinin) were added to the incubation mixture, corresponding decreases of radioactivity in peak 1 and increases in peak 2 were observed, indicating partial to complete displacement of labelled acetyl bradykinin by unlabelled peptides. When pre-immunization control serum was used all radioactivity was found in peak 2.

The unexpected finding that antiserum binds acetyl bradykinin much more strongly than unacetylated bradykinin (Fig. 1) may be explained by the fact that at least part of the antibody formed is directed against a conjugate in which bradykinin is acylated by carboxyl groups of haemocyanin. Such a derivative is chemically and immunologically more closely related to acetyl bradykinin than the unacetylated peptide.

The sensitivity of this assay can be increased greatly by using tritiated acetyl bradykinin of higher specific activity (<sup>3</sup>H-acetic anhydride, 3,000 mc./mmole, available from Nuclear Chicago Corp.). This would permit detection of less than 0.005  $\mu\text{g}$  of bradykinin and suffice for the determination of blood levels in patients with carcinoid syndrome. Concentrations of 0.39–1.2  $\mu\text{g}$  bradykinin/ml. have recently been reported in this condition<sup>9</sup>. For the assay of much smaller quantities in greatly reduced volumes of blood a bradykinin derivative of still higher specific activity might be prepared by acylation with an group such as pipsyl bearing iodine-125 or iodine-131. Work to this end is progressing in our laboratories.

As this paper was being submitted for publication an article appeared which describes a similar radioimmunoassay<sup>10</sup>. Antibody against bradykinin was obtained by immunization with a polylysine-bradykinin co-polymer and labelled hapten was produced by acetylation with tritiated acetic anhydride.

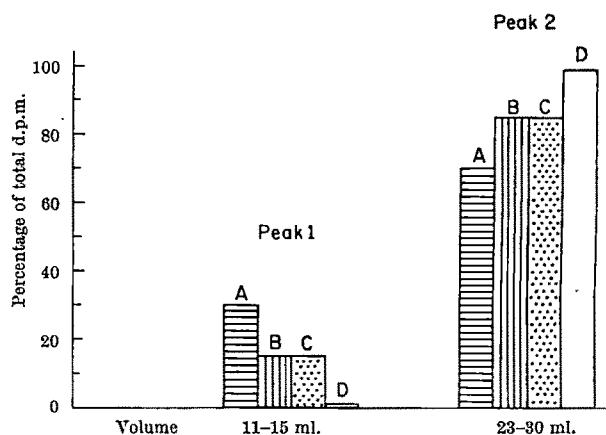


Fig. 1. A constant system of antiserum (1 ml.) and <sup>14</sup>C-acetyl bradykinin (0.36  $\mu\text{g}$ ) was incubated with: A, no addition; B, 0.33  $\mu\text{g}$  unlabelled acetyl bradykinin; C, 5  $\mu\text{g}$  unlabelled bradykinin; D, 20  $\mu\text{g}$  unlabelled bradykinin; d.p.m., disintegrations per minute. Bars of peak 1 represent pooled eluate collected in tubes 11–15 of individual experiments; peak 2, pooled eluate collected in tubes 23–30.

\* While this work was in progress two reports appeared in the literature describing similar approaches in the development of radioimmunoassays for angiotensin and growth hormone<sup>11,12</sup>.

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### Membrane Filtration for determining Protein in the Presence of Interfering Substances

SEVERAL substances that are present in biological materials and some compounds which are added during protein purification procedures interfere with protein determinations by the biuret and Folin-phenol procedures<sup>1</sup>. For example, substances with two or more peptide bonds and ammonium salts give a positive biuret reaction and aromatic amino-acids, uric acid, guanine, and xanthine cause similar difficulties in the Folin-phenol method. Reducing agents such as 2-mercaptoethanol interfere with both procedures. The effects of these substances can be minimized if protein samples are analysed after being precipitated, centrifuged and further washed with trichloroacetic acid (TCA). If, however, the initial protein solution is very dilute and large portions must be taken for analysis, or if only a small amount of protein is to be assayed, problems arise in recovering and washing the precipitated protein free of interfering substances. Micromethods have been devised<sup>2</sup> to surmount these problems, but for routine analysis a rapid and effective procedure is needed for removing these undesir-

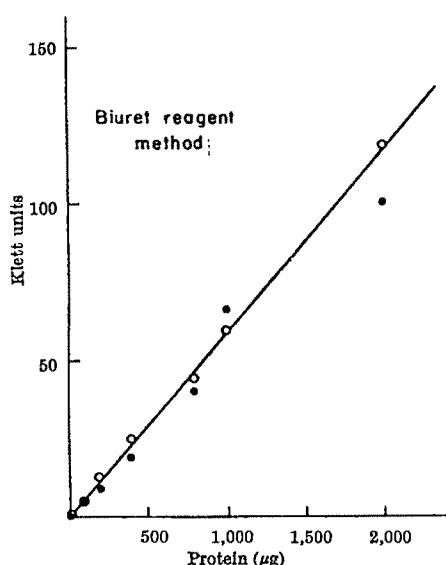


Fig. 1. Biuret determination of bovine serum albumin by a standard procedure (○) and by the membrane filter method after precipitation with trichloroacetic acid (●).

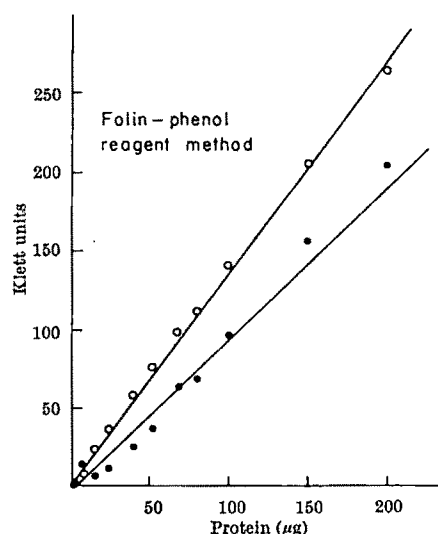


Fig. 2. Folin-phenol procedure for determination of bovine serum albumin by a standard procedure (○) and by the membrane filter method after precipitation with trichloroacetic acid (●).

able substances and determining protein. These requirements appear to be fulfilled in the method reported here. This method involves the analysis of protein precipitated by TCA and then separated from interfering substances by membrane filtration on 'Millipore' filters.

Both the biuret<sup>3</sup> and Folin-phenol<sup>2</sup> reagent procedures have been adapted for the membrane filtration method. In the biuret and also in the Folin-phenol procedure, the protein is precipitated with TCA (5–7 per cent final concentration), collected on a 'Millipore' filter and washed with 5–10 ml. of TCA. Filters (25 mm diameter) with pore diameter of 0.45, 3.0, 5.0 and 8.0  $\mu$  were equally effective. While the filter is still in position on the filter holder, it is cut into quarters with a razor blade and the sections transferred to a 1.5  $\times$  10 cm test-tube.

In the biuret method, 0.1 ml. sodium hydroxide (0.1 normal) is added to the protein filter sample and the mixture vibrated or "buzzed", then allowed to remain at room temperature for 15 min, with occasional "buzzing". Prolonged exposure to sodium hydroxide and a higher concentration of sodium hydroxide should be avoided because the filters will dissolve and liberate phenolic substances that interfere with the protein determination. 0.4 ml. of water is then added and the reaction mixture "buzzed". Biuret reagent<sup>3</sup> (2 ml.) is added with "buzzing" and the mixture is then allowed to stand for 30 min at room temperature. The optical density (Klett units) at 540 m $\mu$  is determined against a blank consisting of a 'Millipore' filter carried through the entire procedure. In Fig. 1 representative data for this method are compared with data for a bovine serum albumin standard that was not precipitated.

For the Folin-phenol procedure, 0.5 ml. of 0.1 normal sodium hydroxide is added to the protein-filter sample, the mixture "buzzed" several times during a 15 min period, and then 0.5 ml. of water is added with mixing. 5.0 ml. of reagent D (ref. 2) (carbonate-copper solution) are added to the sample, mixed, and after 15 min 0.5 ml. of reagent E (Folin-phenol reagent)<sup>2</sup> is added. The mixture is "buzzed" and the optical density at 660 m $\mu$  is determined after an additional 30 min. A 'Millipore' filter carried through the entire procedure is used as a blank. Fig. 2 compares the determination of protein by the filter method with non-precipitated standards.

As shown in Table 1, high concentrations of several interfering agents did not affect protein determinations when the membrane filtration method was used. It is essential, however, that this type of information be

Table 1. EFFECT OF INTERFERING SUBSTANCES ON PROTEIN DETERMINATION BY THE MEMBRANE FILTRATION TECHNIQUE

Interfering substance added	Klett units	
	Biuret reagent	Folin-phenol reagent
None	59	52
Ammonium sulphate, 450 mmolar	56	49
2-Mercaptoethanol, 45 mmolar	55	48
Glutathione, 45 mmolar	55	56
Tyrosine, 4.5 mmolar	61	50
Xanthine, 9.0 mmolar	58	—

Each protein sample contained the compound at the final concentration indicated in a total volume of 1.0 ml. 1.0 mg protein was used in the biuret assay; 0.05 mg in the Folin-phenol. Blanks, that is, no protein, were found, for each addition, to be identical to a standard 'Millipore' filter blank.

obtained for an interfering compound under consideration because some materials are adsorbed to membrane filters.

The optical densities of samples determined by the biuret-filter method (Fig. 1) were almost identical to standard protein determinations without precipitation. The values, however, obtained by the Folin-filter method (Fig. 2), for reasons not yet determined, were consistently lower than with protein standards that had not been precipitated. This fact makes it necessary to establish standard curves by precipitating known amounts of protein. Although the membrane filtration method is less sensitive for small amounts of protein, it is, nevertheless, as effective as standard methods over a wide range of protein content (40–2,000 µg). The important feature of the filter procedure is that it provides a rapid method for protein determinations on dilute solutions of protein containing substances which interfere with the standard biuret and Folin-phenol procedures.

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## Urinary Excretion Patterns of Individual Acid Mucopolysaccharides

DURING recent years several authors have shown that certain hereditary dysostoses are caused by an abnormal deposition in bone and various parenchymatous organs of acid mucopolysaccharides (AMP). Maroteaux and Lamy<sup>1</sup> subdivided these mucopolysaccharidoses into five different types according to their characteristic excretion patterns of AMP. In this communication we present our results of a comparable investigation of urinary excretion of individual AMP in normal persons and patients with different types of mucopolysaccharidoses. Through the introduction of column chromatography the determination of AMP pattern in the urine has become an especially valuable tool in the biochemical identification and classification of atypical cases of hereditary dysplasias of bone.

Acid mucopolysaccharides in the urine of eight normal individuals of both sexes and various ages, four patients with gargoylism (Pfaundler-Hurler) and one patient with polydystrophic oligophrenia (type Sanfilippo) were fractionated. The procedure included precipitation of urinary AMP material with cetyltrimethylammonium bromide and subsequent column chromatography on 'Dowex 1 X 2' (ref. 2). The hexuronic acid content of each fraction was determined by the carbazole reaction<sup>3</sup> and the anthrone reaction<sup>4</sup>, respectively. Details of the method used have been reported elsewhere<sup>5</sup>.

Normal persons of various ages have a typical excretion pattern of urinary AMP. In Table 1 the values are compiled of four AMP excreted by healthy individuals. The data are expressed in terms of percentages of the total carbazole material determined after column chromatography. The main compound corresponds to chondroitin

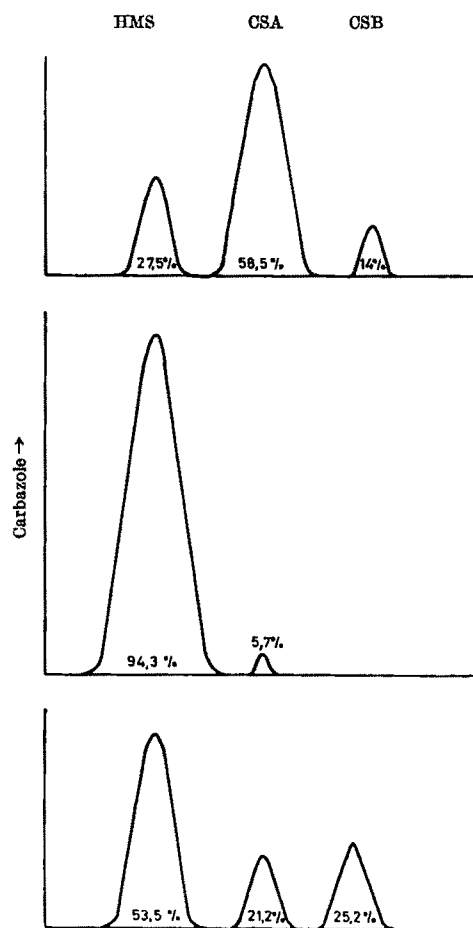


Fig. 1. Patterns of urinary acid mucopolysaccharides in normal persons (top,  $n=8$ ) and patients with mucopolysaccharidoses. Middle, polydystrophic oligophrenia (type Sanfilippo) ( $n=1$ ); bottom, gargoylism ( $n=4$ ).

sulphate A (CSA) (mean value, 58.5 per cent), while chondroitin sulphate B (CSB) (mean value, 14 per cent) and heparitin sulphate (HMS) (mean value, 27.5 per cent) are excreted in smaller amounts. It should be borne in mind, however, that the chromogenicity in the carbazole reaction of CSB is about one third that of CSA. Similar results of AMP excretion determined by different methods in normal persons have been reported in the literature<sup>6</sup>.

Table 2 contains the percentages of urinary AMP in four patients with gargoylism and one patient with polydystrophic oligophrenia (type Sanfilippo). The two disease states reveal a distinctly abnormal AMP excretion. The different patterns, in fact, seem to be pathognomonic of the respective mucopolysaccharidoses. In typical gargoylism HMS (53.6 per cent) and CSB (25.2 per cent) are the main urinary AMP, while polydystrophic oligophrenia is characteristically associated with a predominant HMS excretion (94.3 per cent). Fig. 1 shows a schematic drawing of the different AMP excretion patterns in normal

Table 1. URINARY EXCRETION OF ACID MUCOPOLYSACCHARIDES IN NORMAL PERSONS

The values are given in percentages of total hexuronic acid eluted from the columns and determined by the carbazole reaction.

Carbazole	1	2	3	4
	per cent HMS	per cent CSA	per cent CSB	KS
V. F. J., ♂, 9½ yr	24.8	60.6	14.6	trace
E. H., ♀, 11 yr	36.9	54.2	9.2	trace
H. B., ♂, 22 yr	23.1	56.7	20.2	trace
H. A., ♂, 8½ yr	28.7	61.5	9.8	trace
B. E., ♀, 6 yr	26.8	61.5	11.7	trace
Z. G., ♀, 10 yr	23.0	57.6	19.4	
K. P., ♀, 8 yr	30.4	57.5	12.1	trace
R. A., ♀, 3 yr	28.0	57.0	15.0	trace
Mean values	27.5	58.5	14.0	trace

Table 2. URINARY EXCRETION OF ACID MUCOPOLYSACCHARIDES IN PATIENTS WITH DIFFERENT MUCOPOLYSACCHARIDOSES

The values are expressed in percentages of total hexuronic acid eluted from the columns and determined by the carbazole reaction.

Carbazole	1 per cent HMS	2 per cent CSA	3 per cent CSB	4 KS
GARGOYLISM				
E. M., ♂, 10 yr	52.5	17.5	30.0	
D. K., ♂, 6 yr	55.3	22.2	22.5	
R. F., ♀, 8 yr	54.0	22.0	24.0	
L. B., ♀, 9½ yr	52.5	23.0	24.5	
Mean values	53.6	21.2	25.2	
POLYDYSTROPHIC OLIGOPHRENIA				
S. C., ♀, 9 yr	94.3	5.7	—	

individuals and patients with mucopolysaccharidoses. According to our present experience the urinary AMP pattern is of valuable assistance in the diagnosis of some hereditary bone dysplasias. In addition it affords certain clues as to their basic biochemical defect.

Fractionation of urinary mucopolysaccharides was achieved by the following procedure: dialysis of the urine; precipitation with cetyltrimethylammonium bromide; washing of the precipitate with 95 per cent ethanol; column chromatography on 'Dowex 1 X 2'; and colorimetric determination of hexuronic acid and/or neutral sugar content of each fraction.

Eight normal persons of various ages revealed the following mean excretion of acid mucopolysaccharides: heparitin sulphate, 27.5 per cent; chondroitin sulphate A, 58.5 per cent; chondroitin sulphate B, 14 per cent; keratan sulphate, trace. The values are expressed in percentages of total carbazole and anthrone material eluted from the columns. In four patients with gargoylism the mean excretion was as follows: heparitin sulphate, 53.6 per cent; chondroitin sulphate A, 21.2 per cent; chondroitin sulphate B, 25.2 per cent. One patient with polydystrophic oligophrenia excreted heparitin sulphate almost exclusively (94.3 per cent).

I think that the urinary mucopolysaccharide pattern is pathognomonic of certain hereditary bone diseases and may serve as an aid in their diagnosis.

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### Disk Electrophoresis of Acid Mucopolysaccharides

WITHIN the past four years the application of disk electrophoresis for the separation of serum proteins<sup>1</sup> and lipoproteins (S. Lakshmanan, personal communication, 1966) has become more prevalent. The present report deals with the application of disk electrophoresis to the separation of acid mucopolysaccharides, in particular chondroitin sulphate B (CSB) and heparitin monosulphate (HMS).

Purified CSB and HMS which were homogeneous to paper chromatography and moving boundary electrophoresis were applied to gel columns separately and as a mixture. Acid mucopolysaccharides obtained from the urine of Hurler's syndrome patients were also investigated. Paper chromatography, moving boundary electrophoresis and uronic acid determination revealed the polysaccharide

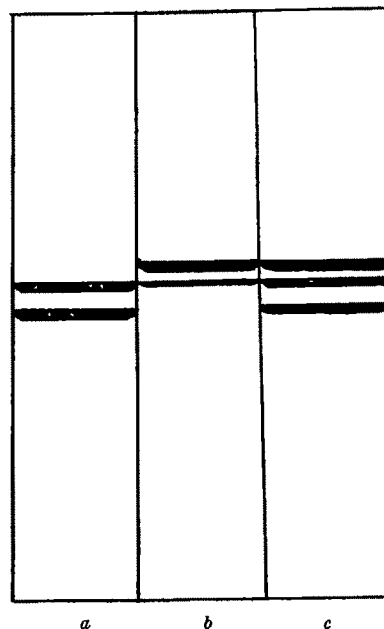


Fig. 1. Disk electrophoresis of acid mucopolysaccharides. a, CSB; b, HMS; c, Hurler's AMPS, 60 per cent CSB, 40 per cent HMS.

from one of these patients to consist of 60 per cent CSB and 40 per cent HMS.

A 'Canalco' disk electrophoresis apparatus was used. The polyacrylamide gel was prepared in the conventional fashion. The buffer used consisted of: *tris*-3.0 g, glycine—14.4 g, diluted to 1 l. The electrophoresis was run at a current of 5 m.amp/column, and no interference resulted from the use of tracking dye. The disks were identified by staining with a solution of 1 per cent toluidine blue in 3.5 per cent acetic acid for 1 h, and subsequent destaining.

The electrophoresis of homogeneous CSB resolves two bands as shown in Fig. 1a. A slow moving major component (CSB-2) and a fast moving component (CSB-1) are separated. Similar results are obtained with HMS. In this case the major component is fast (HMS-1) and the minor component (HMS-2) is slow. Calculation of the  $R_F$  indicates that HMS-2 and CSB-1 have the same rate of migration in the buffer system employed. Electrophoresis of a mixture of CSB and HMS results in the resolution of three bands. The same result is obtained from the electrophoresis of mucopolysaccharide from a Hurler's patient (Fig. 1c).

These data, as well as others conducted in this laboratory, indicate that this is not an artefact caused by the procedure. Cifonelli and Dorfman have demonstrated that small amounts of iduronic acid may be found in HMS<sup>2</sup>. If the additional bands demonstrated by the method are not impurities it is possible that the component common to CSB and HMS contains iduronic acid. One is tempted to speculate further that the region of the glycopeptide linkage of mucopolysaccharides may be found in this common component.

The samples of CSB and HMS were given by Dr. A. Stone. I wish to thank Dr. S. Lakshmanan for his encouragement and great interest.

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# Maturation of Low Molecular Weight RNA in Tumour Cells

INVESTIGATIONS carried out *in vivo* on the methylation of RNA in Krebs II ascites tumour cells using  $^{14}\text{C}$ (methyl)-L-methionine as donor of methyl groups have demonstrated that only nascent RNA is methylated<sup>1</sup>. Of the high molecular weight RNA species, both ribosomal components as well as the 35S ribosomal precursor species are extensively methylated<sup>1</sup>. In this investigation, gel filtration was employed to separate the small molecular weight RNA components, and the results demonstrate the possibility that several steps are involved in the biogenesis of low molecular weight RNA.

Krebs II ascites tumour cells were suspended in Hanks medium, supplemented with horse serum, as described previously<sup>1</sup>. After incubation with appropriate additions, the cells were removed from the medium, the RNA was extracted using a cold phenol procedure (our unpublished work), and subjected to gel filtration using 'Sephadex G-100' following the method of Galibert *et al.*<sup>2</sup>, so that a separation of ribosomal RNA (30S and 19S), which is excluded from the column, from the 5S (ribosomal) and 4S (transfer) RNA species was achieved (Fig. 1). Other investigations<sup>3,4</sup> appear to rule out the possibility that the 5S RNA component is a precursor of 4S RNA, although it has a reasonably similar base composition to 4S RNA and is devoid of pseudouridine residues and any of the methyl bases.

The tumour cells were incubated for various times with  $^3\text{H}$ -uridine and  $^{14}\text{C}$ (methyl)-L-methionine in the presence of large amounts of non-radioactive cytidine (to

prevent the conversion of  $^3\text{H}$ -uridine to  $^3\text{H}$ -cytidine derivatives within the cell) and formate (to prevent the equilibration of the methyl group of the input L-methionine with the one-carbon unit pool<sup>5</sup>). When RNA was extracted after a short period of incubation, such as 5 min, radioactivity derived from tritiated uridine was found in positions corresponding to ribosomal, 5S and 4S RNA. In addition there were peaks of relatively high activity eluting in positions between 5S and 4S RNA (peaks C and D in Fig. 1a). By comparison, the tritium radioactivity corresponding more closely to the 4S material (for example, peaks E, F and G in Fig. 1a) was relatively low. From Fig. 1a, it can also be seen that practically no incorporation of methyl groups from labelled (methyl)-L-methionine could be found in the 5S region although some could be found in the regions of peaks D, E, F and G. At this stage, however, the ratio of RNA methylation to RNA synthesis (ratio of carbon-14 radioactivity to tritium radioactivity) is low.

When the time of incubation was increased to 20 min more tritium radioactivity was present in all fractions, especially those corresponding to 4S material (regions E, F and G of Fig. 1b), and there was greater methylation of these RNA species. After 45 min the tritium radioactivity associated closely with the main RNA species (Fig. 1c) and while negligible methylation of 5S RNA could be detected, the number of methyl groups per unit length of newly synthesized RNA in the 4S region is now considerable.

It is possible that the peaks of tritium radioactivity which occur between the 5S and 4S RNA species in short-term labelling experiments represent "methyl-deficient"

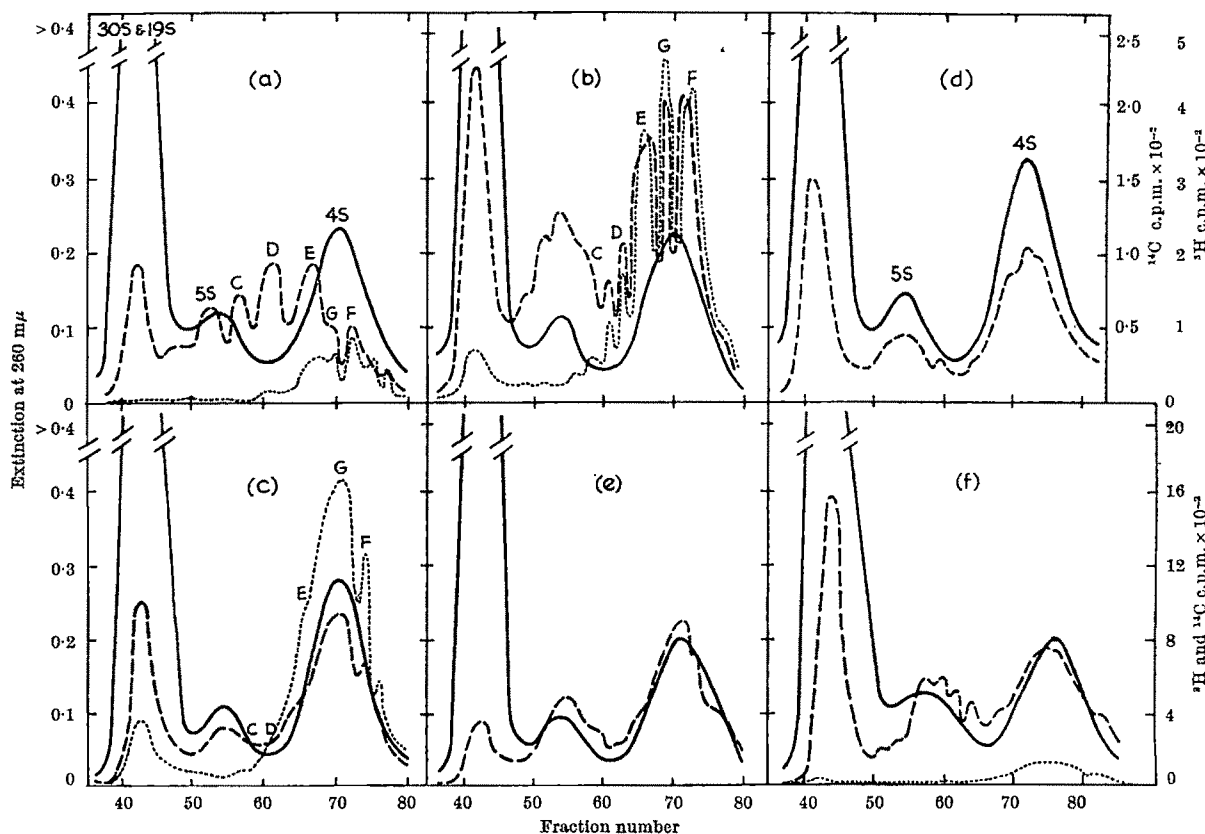


Fig. 1. Elution profiles of Krebs II ascites tumour cell RNA from 'Sephadex G-100' in 0.05 molar ammonium acetate pH 5.1, after labelling the cells with tritiated uridine, 3.3 c./mmole (10  $\mu\text{C}$ /ml.) and  $^{14}\text{C}$ (methyl)-L-methionine, 29.5 mc./mmole (1  $\mu\text{C}$ /ml.) at 37° C in the presence of 1 mmolar of cytidine and 20 mmolar of sodium formate for (a) 5 min, (b) 20 min, (c) 45 min, (d) 5 min followed by the "actinomycin-pulse" and "chase" described in the text, (e) 60 min in the presence of 50  $\mu\text{g}$ /ml. of puromycin, (f) 45 min after an initial preincubation with DL-methionine (10 mg/ml.) for 30 min. Each fraction was first assayed for extinction at 260 m $\mu$ , the pH adjusted with *tris* buffer to pH 8.5, incubated at 37° C for 30 min to hydrolyse the amino acyl ester bond of any  $^{14}\text{C}$ -methionyl-SRNA present<sup>1</sup>, acidified with 5 per cent trichloroacetic acid, the acid insoluble radioactivity collected on 'Millipore' filters and assayed using a liquid scintillation counter. Extinction at 260 m $\mu$ , —; tritium radioactivity, ---; carbon-14 radioactivity, ....



intermediates in a process leading to the formation of 4S RNA, which, by virtue of a looser secondary structure, are less effectively retained by columns of 'Sephadex G-100'. To evaluate this, a "chase" type of experiment was carried out. The tumour cells were labelled for 5 min, treated with actinomycin D (50 µg/ml.) for 15 min to inhibit synthesis of 5S and 4S RNA, washed twice and finally suspended in fresh medium containing large amounts of non-radioactive uridine (0.1 mmolar), L-methionine (0.1 mmolar), cytidine (1 mmolar) and sodium formate (20 mmolar) and incubated for a further 1 h. This "actinomycin-pulse" before the "chase" procedure is required with these tumour cells to obtain maximal blocking of RNA synthesis, because it is difficult to dilute the radioactivity in the nucleotide precursor pools sufficiently quickly. The result of this type of experiment is shown in Fig. 1d, and demonstrates that the level of tritium radioactivity eluting in the region corresponding to 4S RNA after the "chase" correlates well with a redistribution of the radioactivity which originally appeared, for example, in the C and D regions after 5 min of incubation (Fig. 1a).

If the additional components (peaks C and D) represent intermediate steps of a process leading to the "maturation" of 4S RNA, the question arises as to whether or not a puromycin-sensitive step is involved, as seems to be the case in the process leading to the synthesis of ribosomal RNA from precursor RNA (refs. 6 and 7). When the incubation of the tumour cells was carried out in the presence of puromycin the "maturation" process appeared to be unaffected (Fig. 1a), although the radioactivity in the excluded ribosomal peak was reduced.

It seemed also of interest to determine to what extent the process depended on methylation of RNA. Previous treatment of the tumour cells with DL-ethionine was sufficient to depress, by about 75 per cent, the subsequent incorporation of labelled methyl groups from labelled (methyl)-L-methionine into RNA. Presumably this was because enough S-adenosylethionine<sup>8,9</sup> was formed within the cells to impair either the formation, or functioning, of S-adenosyl-L-methionine, the donor of methyl groups to RNA. The pattern of incorporation of label from tritiated uridine added after the preincubation period with DL-ethionine indicates that the "maturation" process was only slightly impaired, which suggests that, although possibly a number of steps are involved in the biogenesis of 4S RNA in Krebs II ascites tumour cells, insertion of methyl groups appears to be a refinement rather than an obligatory step, although there is some evidence from bacterial systems that "methyl-deficient" transfer RNA has altered physical properties<sup>10</sup>.

One of us (B. M. L.) is a Colombo Plan Fellow.

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## IMMUNOLOGY

### Action of Disinfectants on Experimental Mouse Scrapie

Mouse adapted scrapie, a progressive degenerative disease of the central nervous system, appears to be unusually resistant to heat and exposure to formalin. In view of this, it was decided to attempt to kill the experimental scrapie infectious agent by treating with some disinfectants, such as alcohol, a hypochlorite, a surface-active agent, and the agent was also boiled for 60 min. A 1:10 w/v suspension of mouse brain infected with scrapie was mixed with an equal volume of the suitably diluted disinfectant and the mixtures were kept overnight at +4° C before female mice (18–20 g) were inoculated by the subcutaneous route. Each 0.5 ml. dose of the mixture contained 0.25 ml. of the infective material. Controls were inoculated with disinfectants and scrapie suspension. An additional control group was inoculated with 0.03 ml. of scrapie intracerebrally to give an early indication of the pathogenicity of the scrapie agent; the incubation period in mice was considerably shorter by this route<sup>1</sup>.

Table 1

Material inoculated	Total No. of mice inoculated	Total No. of survivors	Percentage of survivors at 9 months
Scrapie and 2 per cent surface-active agent	16	5	31
Control	12	12	100
Scrapie and 50 per cent alcohol	16	0	0
Control	11*	9	82
Scrapie and 2 per cent hypochlorite	16	15	94
Control	16	13	81
Scrapie boiled 1 h	15*	14	93
Scrapie only, intracerebral route	6	0	0
Subcutaneous route	28	7	25

\* Denotes one mouse died within a few days of inoculation and was not included in the result.

The mice were kept for 9 months and all mice dying with an illness typical of scrapie within the average incubation period for the disease were considered to have died from scrapie. A random brain sample was examined histologically to confirm the diagnosis.

A summary (Table 1) of the results of two experiments using either BSVS or MRCTO mice showed that a 2 per cent hypochlorite solution killed the infectious agent, and so did boiling for 60 min. The surface-active agent and alcohol, however, were not effective in the concentrations used.

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### Specific Antigenicity of Thymocytes

THYMOCYTES (thymic lymphocytes) are believed to differ from lymphoid cells of other organs. They show a different response to a variety of physical, chemical and biological stimuli<sup>1</sup>, such as irradiation<sup>2</sup> and phytohaemagglutinin<sup>3</sup>, and it has been claimed that they have an epithelial rather than mesenchymal origin<sup>4</sup>. In the mouse, autoantibodies<sup>5</sup> and isoantibodies<sup>6,7</sup> have been demonstrated with a selective toxic effect on thymocytes. The present investigation with a heterologous thymus-specific antiserum reveals rat thymocytes differing antigenically from the lymphoid cells of lymphatic circulation, lymph node and spleen.

Thymuses of 5-7 week Lister hooded rats were homogenized and fractionated to obtain microsomal material<sup>8</sup>. This was then identified by electron microscopy, whereby the presence of endoplasmic reticulum vesicles and ribosomes was confirmed while mitochondrial and nuclear contamination were not detected. The preparation was incubated at 37° C because autolysis had previously been shown to facilitate solution of microsomal antigens<sup>9</sup>. The yield from 15 g of thymus tissue (seventy-five rats) was 90 mg of microsomal protein, the concentration of which was adjusted with physiological saline to 2 mg of protein/ml.

Because the microsomal antigen was in such short supply, mice (*BALB/c*) were chosen as the source of antisera. Six mice were immunized over a period of 5 weeks by weekly intraperitoneal injections of 0.25 ml. of the microsomal preparation (that is, 0.5 mg of protein). One week later they were given a subcutaneous injection of the same dose of antigen and, to stimulate ascites as an abundant source of antibody, 0.5 ml. intraperitoneally of Ehrlich ascites tumour, also from *BALB/c* mice. Ten days later the peritoneal exudate was tapped, centrifuged to remove the clot, and the globulin-rich supernatant ("antiserum") stored frozen. The antiserum was found to be immunologically active by immunofluorescence, but it gave no precipitin lines when tested by gel diffusion against the thymus antigen. Absorptions of the antiserum were carried out by shaking with a half volume of rat organ homogenate for 2 h at room temperature followed by centrifuging.

Immunofluorescent staining with general procedures and controls described elsewhere<sup>10</sup> was effected by the "sandwich" method with fluorescein-labelled horse anti-mouse globulin absorbed with rat tissue homogenates. Preparations of rat thymus for immunohistological examination included unfixed frozen sections, smears and impression films, of which the latter gave the most satisfactory results. They were made by touching a chemically clean microscope slide with the cut surface of a fresh thymus and drying overnight with a fan (at 2° C). Before staining they were washed by gentle shaking for 15 min with phosphate-buffered saline (0.1 molar phosphate, pH 7.1), drained, and then treated with a drop of "antiserum" for 30 min. After rinsing and washing for 10 min in two changes of buffered saline, they were stained with the conjugate, again washed and mounted in buffered glycerol. Microscopical preparations were examined not more than 2 h after mounting, because staining deteriorated rapidly, being largely dissipated after standing overnight.

The unabsorbed antiserum gave diffuse staining of rat connective tissue and parenchymal organs, and the reaction was intense with all the lymphoid organs; skeletal muscle fibres did not stain. After absorption by rat liver homogenate, the serum stained only the lymphoid organs (Table 1). Further absorption with circulating lymphocyte microsomes or lymph node or spleen homogenates made the serum fully thymus-specific, when it stained the cytoplasmic rim of thymocytes only (Fig. 1). The staining often had a distinct granular pattern. It was not affected by serum absorption with rat skeletal muscle or

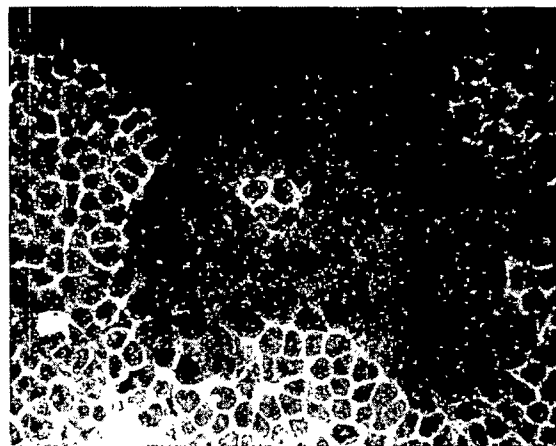


Fig. 1. Unfixed impression film of thymus treated first with mouse anti-rat thymus microsome serum absorbed with rat liver and lymph node homogenates, and second, with fluorescein-conjugated horse anti-mouse globulin. Many thymocytes show fluorescent staining limited to the cytoplasmic rim. Other cells recognizable by autofluorescence only. ( $\times 470$ .)

brain homogenates, but it was completely inhibited after absorption with thymus homogenate.

The histological distribution of thymus-specific staining could not be assessed with certainty in the impression films, but it was largely located in the cortical thymocytes. Some thymocytes failed to stain as could be judged by combined fluorescence and phase-contrast microscopy and no specifically stained non-lymphoid cells could be identified. Hassall's corpuscles gave bright non-specific staining with the conjugated antiglobulin serum alone; there was no evidence of specificity of reaction with the anti-thymus serum.

Our findings indicate that there are at least two special antigens in the cytoplasm of the majority of thymocytes. One, common to all lymphocytes, could be demonstrated in frozen sections, films and smears, and its corresponding antibody could be neutralized by absorption with circulating lymphocytes, lymph node or spleen. The second, more soluble, could be detected only in whole thymocytes either in films or smears and its corresponding antibody could be neutralized by absorption with thymus homogenate only. The cells containing both antigens were morphologically indistinguishable from those with only the common lymphoid antigen. Further work is required to determine whether these two cell types represent original and immigrant<sup>11</sup> populations of the thymus. The possibility that the antigenic difference reflects immunological commitment should also be explored.

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Table 1. IMMUNOFLOUORESCENT STAINING OF RAT LYMPHOID TISSUES WITH ANTI-THYMUS SERUM

Serum*	Absorption by rat tissues	Staining of			
		Thymus	Circulating lymphocytes	Lymph node	Spleen
Anti-thymus	Liver	++	+	+	+
Anti-thymus	Liver + circulating lymphocyte microsomes	++	...	-	-
Anti-thymus	Liver + spleen	+(+)	-	-	-
Anti-thymus	Liver + lymph node	+(+)	-	-	-
Anti-thymus	Liver + thymus	-	-	-	-
None-immune	Liver	-	-	-	-

+ (+) = Staining intensity intermediate between + and ++.

\* Obtained from mouse ascitic fluid.

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## Correlation between "Methicillin Resistance" and Serotype in Staphylococcus

"METHICILLIN resistance" has been found in a number of strains of staphylococcus isolated in French hospitals. Chabbert and Baudens<sup>1</sup> found in 1961 that ten of eighty-two strains investigated (12 per cent) were resistant to methicillin. In 1962 they found twenty-one resistant strains out of 127 (17 per cent), and in 1963 twenty-six out of 134 (19 per cent)<sup>2,3</sup>. Courtieu *et al.*<sup>4</sup> found a similar picture in their investigation of hospitals in other parts of France. In 1964 they found thirty-one resistant strains out of 216 (14 per cent). The frequency found in France seems to be different from that found among strains isolated in England and the United States<sup>5-8</sup>.

Serological typing of staphylococcus has been carried out in France by the slide-agglutination technique since 1950<sup>9-12</sup>. Using this technique, Pillet *et al.* have developed the work of Cowan<sup>13</sup>, Christie and Keogh<sup>14</sup> and Hobbs<sup>15</sup> to show that the predominant serotype of staphylococcus in French hospitals before 1960 was serotype III. They then began to find a new type of hospital staphylococcus emerging, namely, type 14. Since 1963/64 this type is replaced by yet another, type 18, which is now prevalent. Although it is easy to obtain specific monovalent sera of types III, 14 and 18, there are antigenic relationships between strains of type III and 14 and also between strains of type 14 and 18. Furthermore, staphylococci belonging to serotypes III, 14 and 18 are usually lysed by group III phages.

We have therefore attempted to determine the correlation between agglutination serotype and methicillin resistance for various strains of staphylococcus.

We have investigated the serotype of ninety-nine strains isolated in 1965 from ten hospitals in Paris. Twenty-one of these strains belonged to serotypes I and II, twenty-eight to serotype III, seven to serotype 14, and forty-three to serotype 18.

The minimum inhibitory concentrations were determined using the agar streak method, using an inoculum consisting of one-hundredth of an overnight culture, on trypticase soy agar with 5 per cent added sodium chloride. This proportion of sodium chloride allows the phenotypic expression of methicillin resistance<sup>16</sup>.

The results obtained with methicillin are shown in Table 1 and those with cephaloridin in Table 2. Both tables clearly show that twenty out of twenty-one strains of serotypes I and II and seven out of seven of serotype 14 are inhibited in these conditions by 8 µg/ml. or less of methicillin and by 2 µg/ml. or less of cephaloridin; these strains are sensitive. Conversely, the forty-three strains of serotype 18 are inhibited by 16 µg/ml. or more of methicillin and 4 µg/ml. or more of cephaloridin. These strains can be classified as resistant. The twenty-eight strains of serotype III are inhibited by various concentrations.

These results show clearly that all the strains belonging to serotype 18 are "methicillin resistant". But the resistant strains may also belong to serotype III. This probably means that there is no linkage between the

genes responsible for serotype and resistance. A greater frequency of mutants resistant to β-lactam ring antibiotics (penicillins and cephalosporins) would be observed in this serotype. In any case, "methicillin resistant" staphylococcus serotype 18 has shown a particular ability to be selected in French hospitals. It would be interesting to investigate its prevalence in other countries.

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## MICROBIOLOGY

### Mutation of Bacterial Cells by Controlled Desiccation

PREVIOUS work has established reasonably well that the reorientation or removal of water molecules bound to macromolecules may result in the loss of viability and infectivity of bacterial cells and virus particles<sup>1</sup>. During the above studies when auxotrophic cells were held at certain levels of relative humidity (RH), prototrophic mutants were found, apparently produced as a direct result of desiccation. Later the mutation of bacterial cells by ultra-violet light was found to be more easily achieved if the cells were partially desiccated at 40 per cent RH (ref. 2), indicating that bound water molecules played some part in the mechanisms responsible for mutation. Cells or virus particles held at RH levels between 80 and 30 per cent contain from 35 g water/100 g of cell solids (35 per cent water) to 3 per cent water<sup>1</sup>. Because 30–40 g water/100 g of solid is just sufficient to hydrate fully protein RNA and DNA, desiccation below 80 per cent RH affects only the quantity of bound water in the cell. The apparent mutation of cells from desiccation suggested that water molecules were, in part, responsible for maintaining the biological integrity of DNA, and therefore further studies were warranted. It is the purpose of this paper to present the results of preliminary investigations.

Cells of *Escherichia coli* B were grown at 37° C in 'Bacto' brain-heart infusion broth, supplemented with 0.5 per cent yeast extract. After 12 h or 48 h of growth the cells were gathered by centrifugation, washed once in de-ionized water and resuspended in 20 ml. of water or 5 per cent w/v *D*-inositol. The latter compound was used because it had been found previously to prevent the death of cells by desiccation<sup>1</sup>. Oxygen free nitrogen gas was bubbled through the cell suspension for 1 h and the cells were then atomized with nitrogen in a rotating steel drum which contained nitrogen. In the drum the temperature was maintained at 25° C and the relative humidity was preset by spraying water free of oxygen into the drum,

Table 1. NUMBER OF STRAINS INHIBITED BY DIFFERENT CONCENTRATIONS OF METHICILLIN

Serotype	No.	Methicillin Minimum inhibitory concentrations (µg/ml.)							
		2	4	8	16	32	64	128	256
III	21	3	16	1	1				
III	28		7	4	3		3	6	5
14	7		3	4					
18	43				2	2	15	15	9

Table 2. NUMBER OF STRAINS INHIBITED BY DIFFERENT CONCENTRATIONS OF CEPHALORIDIN

Serotype	No.	Cephaloridin Minimum inhibitory concentrations (µg/ml.)								
		0.12	0.25	0.5	1	2	4	8	16	32
I	21	7	5	5	1	2			1	
III	28	4	1	7	2				9	
14	7		1	4	2					
18	43						2	9	31	1

and measuring the relative humidity with a 'Serdex' membrane RH meter. The spray device generates droplets of 10  $\mu$  mean mass diameter which evaporate within 0.2 sec (ref. 3). The atomized cells were held for 60 min and then 10<sup>7</sup> cells/ml. were collected in a liquid impinger containing 8.0 ml. of 1.0 per cent glucose in 0.85 per cent chloride sodium. Immediately after collection, 2.0 ml. of double-strength yeast extract was added and the cells incubated at 37° C for 90 min. The cells were then sedimented by centrifugation, washed once in saline, and then starved for 30 min in 0.1 molar phosphate buffer, pH 6.8. After starvation the cells were resuspended in 10 ml. of a minimal salts medium<sup>4</sup>, 1.0 ml. was removed, serial ten-fold dilutions in saline were made and viable cell counts determined by plating 0.2 ml. of each dilution on to agar with yeast extract. The cells in the remaining 9.0 ml. were sedimented, resuspended in 9.0 ml. of the minimal salts medium containing 0.6 molar sucrose and 0.01 molar magnesium sulphate, and incubated at 37° C on a shaker for 90 min<sup>5,6</sup>. Then 0.1 ml. of a solution containing 100,000 units of penicillin/ml. was added and the cells incubated for a further 90 min without shaking. The treated cells were sedimented, washed and resuspended in 9 ml. of sterile water. Aliquots of 0.2 ml. were then spread on to an outgrowth minimal medium agar supplemented with 1.0  $\mu$ g/ml. of twelve amino-acids and 0.5  $\mu$ g/ml. of the five nucleic acid bases and six vitamins. Auxotrophic cells on such a medium tend to produce very small colonies and surviving prototrophic cells large ones. After 36 h at 37° C, the colonies were picked off and the cells suspended in 5 ml. of sterile saline. The cells in each suspension were spotted on to minimal agar and three types of enriched minimal agar each containing one of the following: (a) 0.5 per cent amino-acids free of vitamin; (b) 100  $\mu$ g/ml. of the five nucleic acid bases; and (c) 20  $\mu$ g/ml. of six vitamins. After 36 h of growth, cells forming colonies on one of the three enriched media but not on the minimal medium were considered to be auxotrophic mutants. To serve as controls, a dilution of the cells remaining in the spray bottle after atomization containing approximately  $1 \times 10^7$  cells/ml. was made in glucose-saline and treated in the same way as the desiccated ones. Three experiments at 30, 40, 55 and 75 per cent RH with both log phase and stationary phase cells and two with inositol at 40 and 55 per cent RH were conducted. Results were calculated on the basis of number of mutant colonies/ $1 \times 10^6$  viable cells.

The number of large colonies formed on the outgrowth medium accounted for less than 1.0 per cent of the total and all except one contained prototrophic cells; also, of the smaller colonies, 32 per cent were found subsequently to be prototrophic. From the results shown in Tables 1 and 2, for stationary phase and log phase cells respectively, it is clear that auxotrophic mutants were more plentiful in the cells surviving desiccation at RH levels below 55 per cent RH. With stationary phase cells the maximum number occurred at 40 per cent RH and with log phase cells at 55 per cent RH. Moreover, when inositol was used to protect the cells against loss of viability, the number of mutants was considerably reduced, particularly with cells in the stationary phase of growth.

It has been known for a number of years that sometimes when freeze-dried or dried stock cultures are rehydrated the culture possesses different characteristics from those of the original strain and it has been argued that mutant cells may be more resistant to the treatment and, therefore, are selected. While investigations to date do not disprove this theory, in order to explain the presented results in the same terms, it is necessary to postulate that (a) auxotrophic cells are more resistant to desiccation at 55 per cent RH and below than the parent cells, and (b) inositol is not as efficient a stabilizer of mutants as it is of the parent cell. Both notions appear unlikely because prototrophic cells are produced from auxotrophic ones during desiccation below 55 per cent

RH, and in previous work carried out over the past 10 yr the ability of inositol to protect cells has been found to be independent of the strain of organism used. The pronounced dependence of mutant numbers on the RH at which the cells were held suggests that the removal or reorientation of bound water molecules is mutagenic and there appear to be two possible explanations. One is that the removal of water allows the irreversible attachment of proteins to certain gene sites and the other that changes in DNA structure occur as a result of partial desiccation. The former suggestion cannot be discounted, but seems improbable because there is no experimental evidence to support it at this time. The latter hypothesis is favoured because the structure of DNA is known to rely on bound water<sup>5</sup> and because at 55 per cent RH the water lattice of DNA is broken and water bound to  $=P=O$  or  $=C=O$  only is present<sup>5</sup>. It therefore seems reasonable to suggest that interactions between the desiccated  $-N$ ,  $-NH$ ,  $NH_2$  and  $-OH$  groups and the partially hydrated  $=P=O$  and  $=C=O$  groups will occur. In addition, water orientated by hydrophobic groups which normally assist in maintaining the structure of macromolecules will be absent. Such interactions will distort the DNA molecule and could result in irreversible changes, especially if the DNA is replicating and in the open stranded state. The higher incidence of mutations in log phase cells is considered, therefore, to be caused by a greater sensitivity of the DNA to desiccation when it is replicating, and the

Table 1. PRODUCTION OF AUXOTROPHIC MUTANTS BY CONTROLLED DESICCATION

Relative humidity		Mutant type		
		Amino-acid dependent	Base dependent	Vitamin dependent
30 per cent water	1	114	18	6
	2	106	7	3
	3	92	16	4
40 per cent water	1	301	44	16
	2	322	36	11
	3	304	49	14
55 per cent water	1	255	22	11
	2	203	14	8
	3	268	36	4
75 per cent water	1	2	0	0
	2	3	1	0
	3	0	0	0
40 per cent water + inositol	1	4	1	2
	2	5	3	0
55 per cent water + inositol	1	8	0	3
	2	10	3	1
Control	1	1	0	0
	2	0	0	0
	3	3	1	0
	4	1	0	1

Cells grown for 48 h in yeast extract broth, washed in water and desiccated for 60 min in nitrogen.  
1, 2, 3, 4 are the results from four independent experiments.

Table 2. PRODUCTION OF AUXOTROPHIC MUTANTS BY CONTROLLED DESICCATION

Relative humidity		Mutant type		
		Amino-acid dependent	Base dependent	Vitamin dependent
30 per cent water	1	124	12	10
	2	177	16	8
	3	111	5	3
40 per cent water	1	342	32	12
	2	316	31	16
	3	298	41	10
55 per cent water	1	408	66	22
	2	516	48	35
	3	564	57	48
75 per cent water	1	10	3	3
	2	4	0	8
	3	3	4	1
40 per cent water + inositol	1	42	10	4
	2	26	6	0
55 per cent water + inositol	1	53	21	10
	2	72	18	12
Control	1	0	1	0
	2	0	0	2
	3	2	0	1
	4	0	2	0

Cells grown for 12 h in yeast extract broth, washed in water and desiccated for 60 min in nitrogen.  
1, 2, 3, 4 are the results from four independent experiments.

reduced incidence at 30 per cent is thought to reflect a more severe damage leading to cell death rather than to mutation. Further evidence to support the water reorientation hypothesis comes from work in which inositol has been shown to prevent X-ray and ultraviolet damage to semi-dried cells by apparently taking on the role of the bound water removed by desiccation<sup>1</sup>, and desiccation alone to induce the prophage in lysogenic cells<sup>2</sup>. The latter finding strongly indicates that desiccation does affect the biological integrity of DNA.

In order to test the validity of the hypotheses presented, the desiccation sensitivity of some twenty mutants is now being determined. To date, the mutant cell appears to have either the same sensitivity or to be more sensitive than the parent cell. It will, however, be some time before all the strains have been examined, but if mutagenesis by desiccation can be substantiated it could be a useful tool in experimental genetics.

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### Ectotrophic Mycorrhizae as Deterrents to Pathogenic Root Infections

MANY investigators have demonstrated the importance of ectotrophic mycorrhizae in the growth of trees. The role of these structures is physiological: mycorrhizae increase the absorbing surface area of roots; they exert a more selective ion absorption and accumulation, and they make very slightly soluble substances in soil available to their host. Another function has been postulated by Zak<sup>1</sup>, who suggested that ectotrophic mycorrhizal roots may be less susceptible than non-mycorrhizal roots to infection by root pathogens. He proposed several mechanisms by which these structures could be resistant to pathogenic infections.

We have found evidence to support this hypothesis. Mycorrhizal and non-mycorrhizal roots attached to potted shortleaf pine (*Pinus echinata* Mill.) seedlings grown in finely divided shortleaf pine humus were enclosed in small glass cells and inoculated with zoospores (20,000/ml.) of *Phytophthora cinnamomi* Rands. After incubation for 10 days in a growth chamber the roots were examined histologically. Results indicated that mature mycorrhizae were resistant to infection, whereas non-mycorrhizal short roots and lateral root tips were highly susceptible (Table 1).

Table 1. INFECTION AFTER 10 DAYS BY *Phytophthora cinnamomi* OF ROOTS OF SHORTLEAF PINE SEEDLINGS GROWN IN HUMUS IN GREENHOUSE POT CULTURE

Series	Mycorrhizal roots		Non-mycorrhizal short roots		Non-mycorrhizal lateral root tips	
	No.	Per cent	No.	Per cent	No.	Per cent
Inoculated	58		27		12	
Infected		0		100		100
Non-inoculated	38		16		9	
Infected		0		0		0

Similar results were obtained using detached mycorrhizae on lateral root segments of shortleaf pine seedlings, and intact mycorrhizae synthesized aseptically with different mycorrhizal fungi on roots of seedlings of shortleaf and loblolly pine (*Pinus taeda* L.). We concluded that ectotrophic mycorrhizae on roots of shortleaf and loblolly pine are protective barriers to pathogenic

infection by *P. cinnamomi*. In field conditions, one could expect trees with abundant development of mycorrhizae to have less susceptible root tissue exposed to *P. cinnamomi*, and perhaps other root pathogens, than trees with few or no mycorrhizae. This work is being extended and will be published in detail later.

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## PATHOLOGY

### Aetiological Significance of Rod-shaped Bodies in Rheumatoid Synovia

IN recent years there has been renewed interest in the old rejected notion that rheumatoid arthritis may be caused by an infectious agent<sup>1</sup>. Highton *et al.*<sup>2,3</sup> have reported rod-shaped bodies in the synovial vessels of four out of seven rheumatoid patients and two out of six patients with Reiter's disease. No such bodies were found in seven normal or near-normal synovia. These authors have therefore suggested that "these intracellular rod-shaped inclusion bodies found in endothelial cells and pericytes of synovial blood vessels may be phases of an infective agent".

During the course of our investigations of the ultrastructure of normal and pathological synovia<sup>4,5</sup> we have frequently seen these rod-shaped bodies in vascular



Fig. 1. Rod-shaped bodies (R) in synovial vascular endothelial cell from a case of traumatic arthritis. (×48,000.)



endothelium. Since the publication of the paper<sup>2</sup> by Highton *et al.*, we have re-examined some of our material and have found these bodies in the synovial vessels of five out of six cases of rheumatoid arthritis, six out of six cases of osteoarthritis, five out of six cases of traumatic arthritis and three out of four normal or near-normal human synovial membrane. We have also seen these bodies in five out of six normal rat synovia.

Our observations obviously reveal a state of affairs quite different from that described by Highton, and one must ask whether we are all investigating the same structure.

Weibel and Palade<sup>6</sup> described rod-shaped bodies in the vascular endothelia of various tissues of man and rat (synovium was not investigated). Highton *et al.*<sup>2</sup> considered that the rod-shaped bodies which they had observed in the capillaries of rheumatoid synovia were different from those seen by Weibel and Palade<sup>6</sup>. We cannot accept this view, for the bodies seen by us, by Highton and by Palade are of similar morphological appearance and size. Thus the rod-shaped bodies (Fig. 1) in our specimens measure approximately 0.1  $\mu$  in diameter, as also do the bodies described by Highton and Palade. Further, in all these investigations these bodies are seen to be bounded by single membranes and to contain tube-like structures of approximately 150 Å in diameter.

Thus it is obvious that we are all referring to the same structure and that it is widely distributed in a variety of normal and pathological tissues. The nature and significance of this structure are, however, obscure. It remains for future research to determine whether this is a normal organelle found in the vascular endothelium of some species or a structure of exogenous origin which is or is not a factor in the production of some disease process in man.

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### Unstable Rapidly Labelled RNA in Chronic Lymphocytic Leukaemic Cells

THE relative amounts of the various forms of RNA—namely messenger RNA, transfer RNA and ribosomal RNA—in different cells and in different phases of the cell cycle would seem to be of the utmost importance and may provide a clue to the nature of pre-mitotic events in the cell<sup>1</sup>. Information along these lines is rapidly accumulating. Various attempts have been made to characterize these RNA patterns in several mammalian cell lines, namely, the blast cells derived from lymphocytes stimulated by phytohaemagglutinin (PHA)<sup>2</sup>, the HeLa cell<sup>3</sup> and cells from a variety of pathological processes<sup>4</sup>.

Recently, Storti and Torelli<sup>5</sup>, using autoradiographic techniques and performing actinomycin D chase experiments on normal myeloid cells and myeloblastic leukaemic cells, have determined the relative amount of rapidly labelled unstable RNA (messenger RNA?) in these cells. There appeared to be no unstable RNA in the myeloblastic leukaemic cells compared with substantial amounts in the normal myeloid cells. This is a provocative finding because investigations of other mammalian cells have shown that some unstable rapidly labelled RNA is always

present. It seemed to us that it would be of interest to repeat these observations on other forms of leukaemia in man.

We chose to study the cells from patients with chronic lymphocytic leukaemia (CLL) because they are easily obtained from patients and because recent studies<sup>6</sup> have shown that there is good reason to believe that the response of the chronic lymphocytic leukaemic cell population to PHA is a measure of the proportion of abnormal cells to normal cells. Each sample can therefore be tested by exposing it to PHA and the proportion of PHA blast cells that result in 72 h ascertained. If the conversion is very low, it can reasonably be assumed that the population of abnormal cells obtained is relatively homogeneous. A further reason for our choice was that we and others<sup>7</sup> had found that RNA synthesis in CLL cells occurs in a fairly brisk fashion as judged by the uptake of tritiated uridine when cells are flash labelled. Moreover, we chose to obtain these quantitative data by liquid scintillation counting methods combined with autoradiography.

Cells from patients with chronic lymphocytic leukaemia were obtained from their peripheral blood by collecting it in dextran and heparin and allowing the erythrocytes to settle. The lymphocytes from the supernatant were suspended in tissue culture medium T.C. 199 (Glaxo) and normal AB serum at a concentration of 25 per cent (2–3,000 cells/mm<sup>3</sup>). A total of seven patients were chosen on the basis that their lymphocytes showed less than 15 per cent (one case 27 per cent) blast cell conversion in the presence of phytohaemagglutinin after culture for 72 h (Table 1).

Table 1

Patient and time cells in culture before study (h)	Peripheral blood WBC $\times 10^3$ mm <sup>3</sup> at time of study	Percentage of blast cells after 72 h culture with PHA	Treatment at time of study
B.H. 0	11.5	4	Chlorambucil
L.W. 0	15.8	15	Chlorambucil
A.H.* 0	16.4	13	No treatment for 6 months
72			
S.F. 0	9.0	14	No treatment for 1 month
J.W. 0	11.5	27	No treatment for 4 months
72			
A.H.* 0	10.5	15	Chlorambucil
72			
H.G. 0	225.6	6	Chlorambucil
72			

\* Same patient sampled on two separate occasions.

Peripheral blood from normal human donors was obtained and mixed with heparin and PHA (Difco), 0.1 ml./5 ml. of blood. The erythrocytes were allowed to settle and the supernatant rich in white cells was diluted in T.C. 199 and AB serum (75 : 25) (cell concentration of 1–2,000 cells/mm<sup>3</sup>). Additional PHA M was added at a concentration of 0.1 ml./5 ml. of culture. The cells were collected after 72 h and treated in the same manner as the cells from the chronic lymphocytic leukaemic patients.

The CLL cells without PHA and the 72 h PHA blast cells were studied in the following manner: The cells were exposed to uridine-5-<sup>3</sup>H (specific activity 5,000 mc./mmole) at a concentration of 2–10  $\mu$ Ci/ml. of culture for 1 h. The cells were either washed once and resuspended in normal media with "cold" uridine at a concentration of 1 mmolar and actinomycin D (10  $\mu$ g/ml., kindly supplied by Merck, Sharp and Dohme) or actinomycin D 10  $\mu$ g/ml. was added directly to the cell culture without washing. The former will be referred to as a washed chase and the latter as an unwashed chase. At the time of addition of actinomycin D and at subsequent intervals thereafter, duplicate samples were taken for extraction of RNA and liquid scintillation counting as well as for the preparation of autoradiographs.

One millilitre of culture material was immediately placed in an ice bath. This was done in a duplicate fashion at each appropriate interval. The duplicate

samples were centrifuged for 10 min at 2,500 r.p.m. and 1 ml. of 0.5 normal perchloric acid<sup>8</sup> was added to the cell button. This was centrifuged for 10 min at 2,500 r.p.m. and washed with 0.5 normal perchloric acid. The cell precipitate was collected by centrifuging and to it was added 1 ml. of 0.3 normal sodium hydroxide. The precipitate was agitated and dissolved in the sodium hydroxide, and then incubated at 37° C for 1 h. It was then removed, placed in an ice bath, and 2 ml. of 0.5 normal perchloric acid added. The supernatant containing the RNA fraction was obtained after separation by centrifuging for 10 min at 2,500 r.p.m. This was then stored at 4° C until it was counted in the liquid scintillator.

Autoradiographs were prepared at the same times by smearing cells on an ordinary slide, staining by the periodic acid-Schiff reaction, then immersing the slides in Kodak 'NTB-2' emulsion, exposing them for 1, 6, 14 and 30 days, then developing and counterstaining with aqueous haematoxylin.

Grain counts were performed on fifty cells in each slide. The percentage of 500 cells labelled was ascertained and the total grain count was calculated by allowing for the background and correcting for the percentage of cells labelled.

The supernatant containing RNA was adjusted to a pH of 9 and 0.5 ml. was mixed with 10 ml. of liquid scintillator. The latter was a mixture of 2,5-diphenyl-oxazole/naphthalene/dimethyl 2-*p*-phenylenebis 4-methyl, 5-phenyloxazole in dioxan. The mixture was then placed in the refrigerator at 4° C overnight and the next morning counted in a Nuclear Chicago counter. Efficiency was determined by the multiple channels ratio method and varied between 6 and 11 per cent. All samples were extracted in duplicate and each duplicate was counted in duplicate in the counter. Results for both autoradiographic and liquid scintillation counting data are expressed as a percentage of the 1 h sample for the appropriate technique.

The PHA blast cell in the washed chase showed a 50 per cent drop in counts 1 h after addition of actinomycin D on the basis of two experiments. Four experiments with these cells in unwashed chases showed a 40 per cent drop in counts both by autoradiographic and liquid scintillation counting data. The standard deviations of the liquid scintillation counting data are shown in Fig. 1*a* and *b*. The standard deviations of the autoradiographic values ranged from  $\pm 16$  per cent to  $\pm 9.1$  per cent. The presence of cold uridine following a wash most certainly decreases continued uptake of uridine-5-<sup>3</sup>H compared with the unwashed chases<sup>9</sup>.

Four washed chase experiments were performed on CLL cells. The autoradiographic and liquid scintillation data agree remarkably and by 2 h after addition of actinomycin D reveal a 70–75 per cent drop in counts.

Unwashed chase experiments were completed on seven sets of CLL cells (three were 72 h samples). The autoradiographic data reveal a 73 per cent drop in the grain count 45 min after addition of actinomycin D (see Fig. 2*a* and *b*). The standard deviation of the autoradiographic data varied from  $\pm 22$  per cent to  $\pm 7.5$  per cent with a mean of  $\pm 15.4$  per cent. The unstable RNA appeared to be entirely nuclear in origin as determined by nuclear and cytoplasmic grain counts.

Two aspects of this investigation deserve comment. It is gratifying to see that autoradiography can be relied on to give some quantitative estimate of the metabolism of rapidly labelled cellular RNA when certain technical features are considered: namely, the adjustment of the exposure times on the autoradiographs to ensure that the number of grains is not so great as to render counting inaccurate.

Autoradiography enables us to ascertain whether the quantitative data obtained by RNA extraction procedures and liquid scintillation counting pertain to a certain type of cell identified by morphology or whether they reflect the

behaviour of the entire cell population. In this investigation, the latter appeared to be the case as virtually all CLL cells were labelled with only the occasional cell showing extremely high grain counts or none at all.

The second and more important aspect is that there appears to be a greater proportion of unstable rapidly labelled RNA in CLL cells compared with the 72 h PHA blast cell or the 1 h PHA incubated lymphocyte which has approximately 30 per cent unstable RNA (ref. 10). The populations of CLL cells studied all showed less than 0.1 per cent incorporation of tritiated thymidine and were obviously non-proliferating; whereas the PHA blast cell is rapidly proliferating. Are these differences related to phases of the cell cycle? We assume that the CLL cells are arrested in the  $G_1$  phase<sup>11</sup> or perhaps in the  $G_0$  phase, out of cycle<sup>12</sup>, and therefore differences between these cells and the 72 h PHA blast cells which are entering the S phase at a rapid rate may reflect basic differences in the RNA pattern at these various phases of cell life.

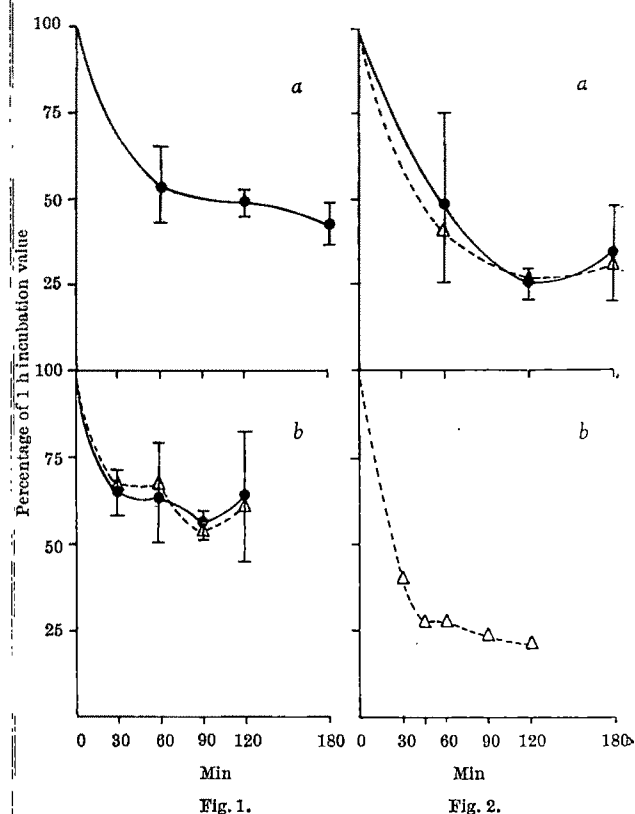


Fig. 1. Results for washed (*a*) and unwashed (*b*) chase data for 72 h PHA blast cells expressed as a percentage of the 1 h incubation value.

Fig. 2. Results for washed (*a*) and unwashed (*b*) chase for CLL cells. In all experiments, actinomycin D was added to cells after incubation for 1 h with uridine-5-<sup>3</sup>H. Time zero indicates addition of actinomycin D. ●—●, Liquid scintillation counting values with standard deviation; Δ—Δ, autoradiographic values (see text for S.D.).

These findings are dissimilar to those of Storti and Torelli working with acute myeloblastic leukaemia cells. Where we found 75 per cent unstable rapidly labelled RNA in CLL cells, they found none in the myeloblastic cells. This may reflect a basic difference in the two types of cells or again may be related to differences in phases of cell cycle. The question may be answered by the characterization of this unstable RNA according to molecular-weight before one can assume that it represents messenger-RNA, and the correlation of these changes in a more precise fashion to phases of the cell cycle.

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## RADIOBIOLOGY

### Effects of Irradiation and Antigenic Stimulation on Circulating Haemopoietic Stem Cells of the Mouse

WHOLE body irradiation causes depression of haemopoiesis, and a sufficiently large dose of X-rays in an exposure of a few minutes can be effectively 100 per cent lethal. Thus, in *CBA/H* mice a dose of 1,000 rads results in aplastic anaemia, lethal within 2 weeks. Nevertheless, successful therapy is possible with intravenous injections of certain cell suspensions, some components of which act as seed for the recolonization of myeloid and lymphoid tissues<sup>1,2</sup>. The various cell lines, erythrocytic, granulocytic, thrombocytic and lymphocytic, may be restored by their respective precursors (polyphyletic hypothesis) or be derived by differentiation from a common multipotent cell (monophyletic hypothesis). In either case the precursor can be termed a stem cell.

McCulloch and Till<sup>3</sup> have made the important observation that, after the administration of such suspensions with cell numbers suboptimal for clinical recovery, examination of the spleen on about the tenth day reveals colonies which histologically are of erythropoietic or granulopoietic or thrombopoietic type: at a late stage they may be mixed<sup>4</sup>. Cytological evidence suggests that each colony represents a clone formed from a stem cell<sup>5</sup> which itself may have reproduced other stem cells<sup>6</sup>. Of the total stem cells administered only a fraction (about one-sixth<sup>6</sup>) wends its way to the spleen. These particular stem cells or colony forming units (CFU) are present in but low concentration in the effective cell-suspensions, for example, about 1/10,000 nucleated cells in normal murine bone marrow<sup>7</sup>.

Similar stem cells are present in circulating blood of normal mice, for their leucocytes in sufficiently large number can recolonize lethally irradiated mice<sup>7</sup> and produce spleen colonies<sup>8</sup>. The concentration of colony forming units, however, in peripheral blood is still lower (about 10<sup>-6</sup>) than in bone marrow.

The present note is concerned with these circulating colony forming units in mice under special circumstances.

Blood was withdrawn with a heparinized syringe by cardiac puncture from comparable mice, deeply anaesthetized with chloroform, and then pooled. After the

nucleated cells had been counted in a Bürker haemocytometer, intravenous injections of measured volumes of the blood, whole or diluted with Tyrode solution, were made into lethally irradiated syngeneic *CBA/H* mice. Each dose was given to a batch of 5–10 mice, and when practicable a range of doses in descending order from  $5 \times 10^6$ – $10^5$  nucleated cells was employed.

The recipient mice were killed after 10–11 days, the spleens were fixed in Bouin's solution and the colonies visible to the eye counted later. Mice dying between the eighth and eleventh days were also accepted if fresh and the counts in the fixed spleen used.

Table 1 shows the ratio CFU/10<sup>5</sup> nucleated cells given, and CFU/cm<sup>3</sup> of blood given, in a representative experiment involving ninety-six recipient normal mice on five different mornings. There is a variation of estimate from 0.09–0.33 (mean  $\sim 0.2$ ) in CFU ratio and from 3–17 (mean  $\sim 10$ ) in CFU/cm<sup>3</sup>.

Table 1. CFU RATIO AND CFU/CM<sup>3</sup> IN NORMAL BLOOD

Nucleated cells injected	CFU ratio					CFU/cm <sup>3</sup>				
$5 \times 10^6$	> 0.17	—	(0.14)*	0.09	0.13	> 10	—	(7)	4	9
$2 \times 10^6$	—	0.12	—	—	—	—	3	—	—	—
$1 \times 10^6$	0.23	(0.18)	(0.32)	0.12	0.12	14	(5)	(16)	6	8
$5 \times 10^5$	(0.20)	(0.36)	(0.33)	0.16	(0.20)	(12)	(9)	(17)	8	(13)
$1 \times 10^4$	(0.20)	—	—	—	(0.20)	(15)	—	—	—	(13)

\* Figures in parentheses are derived wholly or predominantly from mice dead after 8 days.

In blood, taken from irradiated mice 1, 3, 7, 10, 21, 43 and 63 days (experiment A) after receipt of a dose of 350 or 700 rads of X-rays to the whole body, the concentrations of colony forming units are compared in Table 2 with those of normal mice killed at the same time. Colony forming units detectable by the method disappear after X-irradiation and return 7–10 days later when the myeloid tissues are known to be regenerating. At that time the CFU ratio may be more than ten times greater than in normal blood, for example, values of 4.9 and 2.7 on the tenth day following 700 and 350 rads respectively compared with the average normal of 0.2 and the specific control value of 0.14. This high ratio, however, is caused by a decrease in the denominator, that is, the leucopenia, and the value of CFU/cm<sup>3</sup> of blood is around the normal 10. One high value of 46 was found later at 3 weeks after 700 rads. In a replicate experiment B, blood was taken at 7, 10 and 14 days. The results were substantially the same in controls and mice given 350 rads. In the mice given 700 rads, though no colonies were found at 10 days, there was an increase in both CFU ratio and CFU/cm<sup>3</sup> at 14 days.

Table 2. CFU RATIO AND CFU/CM<sup>3</sup> IN BLOOD AFTER X-IRRADIATION

Days after irradiation		700 rads		350 rads		0 rads	
Exp. A	Exp. B	Ratio/cm <sup>3</sup>		Ratio/cm <sup>3</sup>		Ratio/cm <sup>3</sup>	
1		(0)	(0)	0	0	0.08	4
3		(0)	(0)	(0)	(0)	0.15	7
7		(0)	(0)	0.07	2	0.22	10
	7	0	0	(0)	(0)	0.11	6
10		4.9	11	(2.7)	(10)	0.14	8
	10	(0)	(0)	0.93	13	(0.15)	(8)
	14	> 3.5	> 30	0.52	15	0.15	8
21		0.9	> 46	0.33	12	0.10	7
43		0.26	10	0.21	10	0.17	11
63		0.23	11	0.19	6	0.24	14

\* Figures in parentheses are derived wholly or predominantly from mice dead after 8 days.

Observations in this laboratory on leucocytes from immunized mice (Mayrhofer, unpublished) indicate an increase in colony forming units 7 days after an administration of pertussis vaccine with alum-precipitated calf serum by intraperitoneal and subcutaneous routes. The time-course of this response over 3 weeks is given in Table 3. (The data in Table 1 are the specific control normal animals for this experiment.) The CFU ratio is increased some ten-fold as early as the third day, is about the same at the seventh day and declines to normal values by 3 weeks. Because these values are associated with leuco-

Table 3. CFU RATIO (R) AND CFU/CM<sup>3</sup> IN BLOOD AFTER IMMUNIZATION

Nucleated cells injected	Days after immunizing injection									
	1	3	7	10	21					
	cm <sup>-3</sup>	cm <sup>-3</sup>	cm <sup>-3</sup>	cm <sup>-3</sup>	cm <sup>-3</sup>					
5 × 10 <sup>4</sup>	—	—	—	—	—	0.15	9			
2-2.6 × 10 <sup>4</sup> (0.10)	(5)	++	++	++	++	0.16	10			
1 × 10 <sup>4</sup>	(0.09)	(5)	0.95	41	++	0.67	51	(0.17)	(10)	
5 × 10 <sup>3</sup>	(0.13)	(8)	1.5	63	1.7	150	0.70	53	(0.14)	(9)
1 × 10 <sup>3</sup>	(0.10)	(5)	1.85	80	(2.4)	(200)	(0.30)	(23)	—	—

\* + signs indicate that too many colonies were present to be counted accurately.

cytosis, the CFU/cm<sup>3</sup> may be raised more than ten-fold in the peak at 7 days.

The origin of stem cells in the peripheral blood has been attributed to myeloid tissue<sup>9</sup>, because in the normal adult mouse the highest concentration of colony forming units is found in bone marrow.

The results reported here from irradiated mice support this interpretation. Extensive damage to the postulated source cut off the supply for a week. If myeloid tissue had been a site of concentration of stem cells imported via the blood stream rather than an exporting source, an increase might have been expected in the blood before regeneration of the marrow, which occurs in the first two weeks<sup>7</sup>. On the occasions after 2-3 weeks when an increased concentration of colony forming units was found per cm<sup>3</sup> of blood, the marrow (and splenic red pulp) would classically show histological hyperplasia.

The results which followed a powerful antigenic stimulus were less expected. This stimulus is known to produce, in the peripheral blood of mice, cells capable of undergoing mitosis *in vitro* under the influence of phytohaemagglutinin (Breckon and Ford, unpublished). Observations indicate that histologically at the relevant time the bone marrow is hyperplastic and predominantly granulopoietic and with this is associated a granulocytic leucocytosis in the peripheral blood. The spleen is also hyperplastic: there is a brisk response to the antigen by the Malpighian bodies and the red pulp is hyperplastic and unduly myeloid, but mainly in the direction of erythropoiesis and megakaryocytopoiesis.

Micklem<sup>10</sup> has shown an increase of 30-100 per cent in CFU/cm<sup>3</sup> in the peripheral blood 3 days after intravenous injection of phytohaemagglutinin (0.3 ml.). This treatment entails a shock to the system (with leucopenia at this time) so severe that some of the mice died within 16 h.

The concentration of colony forming units in the blood is high in late foetal and early neonatal life<sup>9</sup>.

Previously<sup>11</sup> it was postulated that stem cells left the marrow and were transported by the blood to the thymus and perhaps other lymphatic tissue for committal to lymphoid successors. The possibly obligatory role of the thymus has now been excluded by examining thymectomized animals<sup>12</sup>. Micklem *et al.*<sup>13</sup>, however, could not decide whether the thymic precursors left the marrow as multipotent stem cells or as cells already committed to lymphoid development.

The stem cells have not yet been identified morphologically, despite claims that some small cells in the bone marrow are stem cells though similar looking cells in the lymph node and thymus are not<sup>14</sup>. Until stem cells are identified and marked, it will scarcely be possible to interpret whether increased concentrations in blood as reported here indicate an increased intake into or a decreased extraction from the blood stream. Nevertheless, for the moment it may be assumed that in the four cases cited here an increased intake is the more likely. In each case the time factors are such that there is an increased need for lymphoid cells. Restoration of the lymphoid tissues from damage by radiation is slow and succeeds regeneration of myeloid tissue while immune response demands an early hyperplasia of lymphoid tissue. Phytohaemagglutinin may have acted as an antigen or, as *in vitro*, as an immediate stimulant to lymphoid cells while the lymphatic tissue is emerging under the direction of the thymus<sup>15</sup>.

Because of the correlation so far between the concentration in blood of stem cells, identified as colony forming units, and the need for lymphocytes, we incline to the view that the lymphoid precursors leave the marrow as multipotent stem cells most of which find their way to lymphoid tissue where they are committed. Under the conditions of the present experiments these stem cells were taken from their primary hosts and installed in irradiated secondary hosts where there were other and more pressing requirements for granulocytes, platelets and later red corpuscles, so that the multipotent cells developed along these lines rather than to lymphocytes.

On this hypothesis we would predict that where there is a reduced demand for lymphocytes, as in the germ-free state, colony forming units should be in reduced concentration or absent from the peripheral blood, whereas if circulating lymphocyte precursors are committed cells their concentration might well be independent of that of colony forming units.

In summary, we found that colony forming units, present in normal circulating blood of mice in concentrations of about 10/cm<sup>3</sup>, were not detected after sub-lethal doses of X-rays (350 and 700 rads) until about 10 days later. Then they were present in normal, and in two instances increased, concentration. In other mice given a single immunizing injection of a mixture of pertussis vaccine and calf serum the concentration of colony forming units in blood was increased temporarily with a peak value of more than ten-fold after 7 days but returned to normal within 3 weeks. It is suggested from this and other observations that these colony forming units may be multipotent stem cells exported from myeloid tissue to colonize lymphoid tissue where they are committed to lymphoid differentiation.

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## PHYSIOLOGY

### Dependence on Temperature of the Conduction Velocity of the Action Potential of the Squid Giant Axon

SEVERAL investigations of the effects of temperature on the squid giant axon have been made, but as yet the changes of the conduction velocity associated with altering the temperature have not been accurately determined<sup>1-3</sup>.

The conduction velocity of the action potential of the isolated giant axon has been measured over the temperature range -1° to +40° C. The action potentials were recorded by two glass microelectrodes filled with 3 molar potassium chloride (resistance 10-20 MΩ) inserted into the axon so that they were separated by 2 cm of

axon. Action potentials were evoked at 10/sec by brief currents applied through two silver wire electrodes at one end of the axon. The temperature was reduced by circulating pre-cooled sea water through the experimental chamber and measured by a small bulb mercury thermometer placed close to the axon. The sea water supply was then stopped and the conduction velocity and the temperature of the sea water were continually measured as the temperature gradually increased from  $-1^{\circ}$  or  $0^{\circ}$  C towards room temperature ( $25^{\circ}$  C). Adequate mixing of the sea water was achieved by vigorous aeration through the floor of the experimental chamber on either side of the axon. A similar procedure using sea water pre-warmed to  $40^{\circ}$  C was used to obtain temperatures above room temperature.

The distance between the two microelectrodes was measured with an eyepiece micrometer. The conduction time was measured from midpoint to midpoint of the rising phase of the action potentials.

Huxley<sup>4</sup> derived an expression for the conduction velocity based on a dimensional argument. It is

$$\theta^2 = \frac{a}{2R_2\gamma C_m} \varphi f(\eta/\varphi) \quad (1)$$

where  $\theta$  is the conduction velocity;  $a$  is the radius of the axon;  $C_m$  is the membrane capacity;  $R_2$  is the specific resistance of the axoplasm;  $\varphi$  is the factor by which the permeability changes are accelerated by a rise in temperature;  $\eta$  is the factor by which the absolute values of all the ionic currents are altered by temperature;  $\gamma$  is the factor by which the membrane capacity is changed by temperature.

The experimental and theoretical relations between conduction velocity and temperature are expressed for convenience as the logarithm of the change of the velocity relative to that at the standard temperature of  $6.3^{\circ}$  C (mean value  $9.15$  m/sec for the experimental velocity). The line (solid and dashed) in Fig. 1 has been computed by substituting  $Q_{10}$  values of 3.0 for  $\varphi$  (see ref. 5) and 1.3 for  $\frac{1}{R_2}$ , and a linear increase of 4 per cent/ $^{\circ}$ C of the  $15^{\circ}$  C value of  $\eta$  (see ref. 2), into the equation, and assuming that there was no change in the membrane capacity ( $\gamma = 1$ ) or the axon radius, and by obtaining the values for  $f(\eta/\varphi)$  from Fig. 20 in ref. 4, which is derived from the Hodgkin-Huxley equations. It is unlikely that  $\varphi$  and  $\eta$  can be reliably extrapolated beyond the range of temperature in which

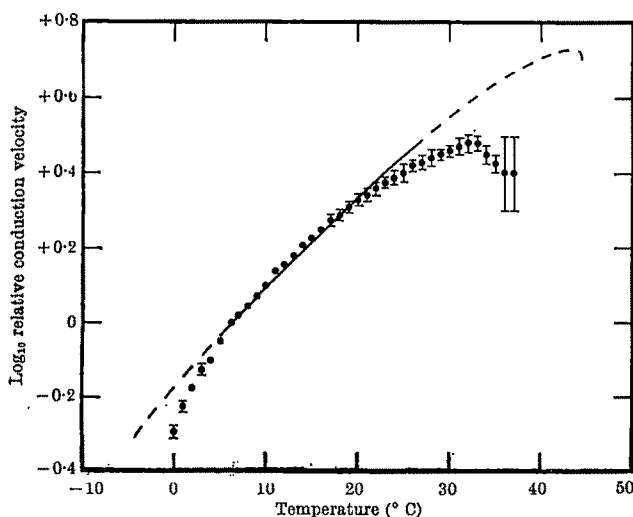


Fig. 1. The effect of temperature on the conduction velocity (mean values) of ten squid giant axons (filled circles) and on the computed conduction velocity (solid and dashed line). In each case the velocity was taken as unity at the standard temperature of  $6.3^{\circ}$  C. The dashed lines represent the computed relative velocity in the temperature ranges where the predictions of the equation are likely to be unreliable (see text). The vertical bars represent  $\pm$  the standard deviation of the mean where this is larger than the filled circle.

they were originally determined ( $5^{\circ}$ – $25^{\circ}$  C); therefore the computed relationship of the conduction velocity at temperatures outside this range is shown as a broken line in Fig. 1.

Good agreement between the observed and the computed relationships is obtained in the temperature range where the predictions of the equation are probably most reliable, that is,  $5^{\circ}$ – $25^{\circ}$  C. At temperatures less than  $5^{\circ}$  C the conduction velocity shows a progressively greater dependence than predicted by equation (1). This divergence is presumably related to the striking increase in the thermal dependence of the duration of the action potential and of the threshold described by Spyropoulos<sup>3</sup> at temperatures near  $0^{\circ}$  C. In the giant axon of *Loligo vulgaris* the conduction of the action potential is reversibly blocked in the region of  $-0.5^{\circ}$  C; associated with the development of this cold block is a sudden reversible reduction of the resting potential by 15–30 mV. It may be, however, that the conduction block is not directly caused by this depolarization because action potentials can still be evoked under space clamp conditions<sup>4</sup>. At temperatures greater than  $25^{\circ}$  C the resting and action potentials are progressively reduced until conduction fails reversibly between  $35^{\circ}$  and  $37^{\circ}$  C, while the computed temperature for conduction failure is  $44.5^{\circ}$  C. Part of this discrepancy is related to the progressive reduction of the resting potential which occurs as the temperature is raised beyond  $25^{\circ}$  C, as these effects are not included in the equation.

The changes of the action potential with temperature, amplitude, maximum rate of rise and maximum rate of fall, were very similar to those reported by Hodgkin and Katz<sup>1</sup>, while the  $Q_{10}$  values for the conduction velocity were:  $0^{\circ}$ – $10^{\circ}$  C, 2.21 s.d. 0.27;  $10^{\circ}$ – $20^{\circ}$  C, 1.70 s.d. 0.03;  $20^{\circ}$ – $30^{\circ}$  C, 1.40 s.d. 0.01; which are close to those reported for other nerve fibres.

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### Responses of Muscle Spindles in the Lizard

In the past 30 years many experiments have been carried out on the structure and function of muscle spindles in both amphibia and mammals. There have been no accounts of experiments concerned with the function of muscle spindles in reptiles although their structure has been described<sup>1,2</sup>.

The muscle spindles of both snakes and lizards usually contain a single intrafusal muscle fibre. A large afferent nerve fibre enters the central capsular region of the spindle, and then bifurcates with each of the two branches running in opposite directions along the surface of the muscle fibre. Spindles commonly occur in a tandem arrangement<sup>3</sup>, with motor endings between the adjacent sensory regions. In lizards the intrafusal muscle fibres are innervated by branches of motor fibres that also supply extrafusal muscle fibres<sup>1</sup>. The number of motor endings on the spindle varies, but most commonly one occurs near each pole of its capsule.

All the anatomical and physiological findings presented here were made with the pubotibialis muscle of the southern blue tongue lizard *Tiliqua nigrolutea*. The nerve supply to this muscle was isolated and the severed end of the sciatic nerve was split into fine filaments from which



responses were recorded. Changes in tension produced in the muscle were recorded by a tension transducer attached to the tendon of the muscle.

In fifteen experiments a total of sixty-five units was examined. Forty-seven units were randomly selected and, of these, thirty-two showed a resting discharge with the muscle slack while the other fifteen were silent unless some tension was applied. The frequency of the resting discharge varied between 3 and 20/sec, with the maximum discharge rate of 180–300/sec. At appropriate stimulus strengths all units showed "in parallel" behaviour, that is, the discharge was interrupted during the rising phase of the contraction and accelerated during the falling phase. The low mechanical threshold and "in parallel" behaviour were the criteria used to classify these responses as coming from muscle spindles<sup>4</sup>. At a critical stimulus strength, bursts of afferent discharge appeared in all or nothing fashion during the phase of increasing tension. Such an apparent "in series" response occurred for all the units examined. No responses of a "tendon organ" pattern were ever observed, that is, where the response discharge increases with increasing twitch tensions. By contrast the discharge of all the above units was reduced in intensity as larger portions of the muscle contracted. Fig. 1 illustrates some typical responses; the upper traces represent the afferent discharge while the lower trace is a recording of the tension changes in the muscle. *A* represents the resting discharge with no tension on the muscle. A weak shock to the muscle (*B*) results in a small contraction which interrupts the afferent discharge during the rising phase of the contraction and accelerates the response during the falling phase. In *C* the stimulus strength to the muscle has been only slightly increased, but this has brought in a burst of action potentials during the rise of tension. This

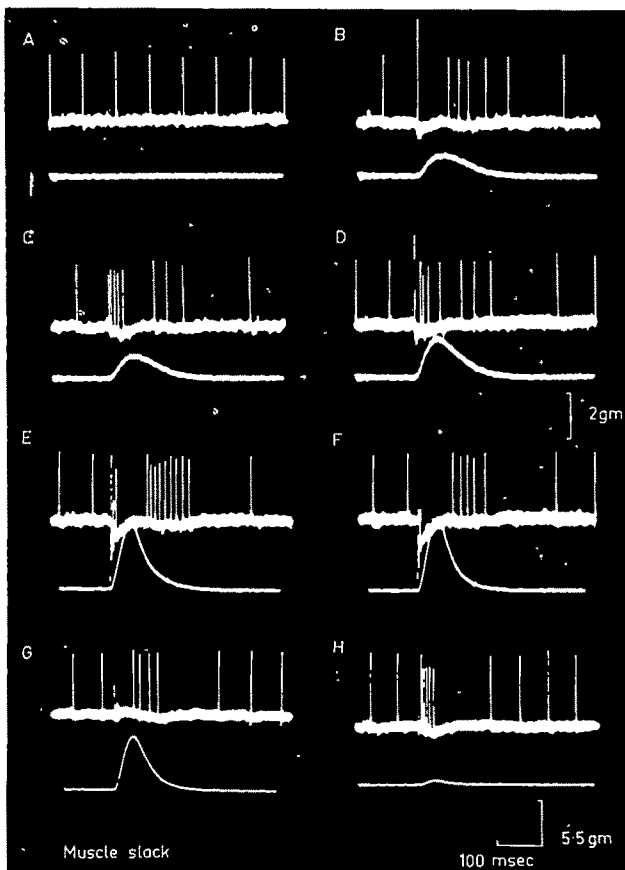


Fig. 1. Stretch receptor discharges from the pubotibialis muscle recorded from fine filaments of the dissected sciatic nerve. The upper trace in each record represents the nerve discharge while tension is recorded on the lower trace. For explanation see text.

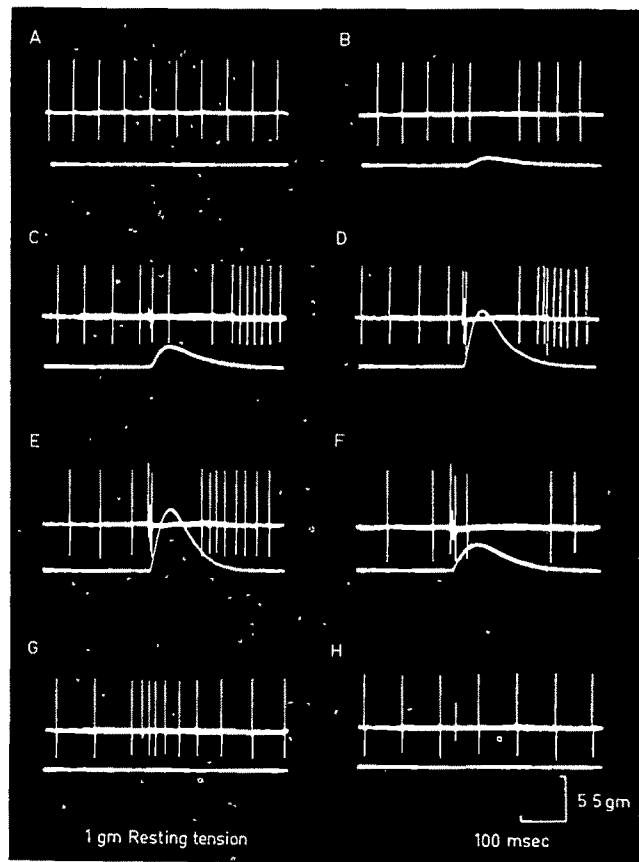


Fig. 2. Stretch receptor discharges from the pubotibialis muscle at a resting tension of 1 g. For explanation see text.

burst is reduced for higher twitch tensions (*D* and *E*) while in *F*, using a supramaximal shock, it completely disappears. With a greater resting tension of the muscle, the number and frequency of the "in series" discharges increased and were no longer completely removed at maximal twitch tensions.

A spike discharge on the rising phase of the contraction can be attributed to stray extrafusal muscle fibre pulls acting on the spindle<sup>6</sup>. If the motor supply to the muscle is reduced so that only single motor units are stimulated, then the possible effect of stray pulls will be minimized. This technique has been applied to the muscle of lizards. While recording from one filament the rest of the nerve was split and small filaments stimulated. Stimulation of the majority of filaments did not alter the afferent discharge. A few filaments caused the unit to behave in an "in parallel" fashion (Fig. 1*G*) while one or occasionally two filaments (which appeared to behave as single motor units) resulted in a powerful burst discharge on the rising phase of the contraction (Fig. 1*H*). The specificity of this response to the minimal motor stimulus makes it unlikely that it results from extrafusal stray pulls.

In the frog the intrafusal motor endings are less sensitive to curare than extrafusal endings<sup>8</sup>. This enables extrafusal contractions to be blocked without affecting intrafusal junctions: this differential neuromuscular block was also achieved in lizards using the drug 'Flaxedil' (gallamine triethiodide). Fig. 2 *A-D* shows responses from another unit. Here the discharge on the rising phase of the twitch consists of two spikes which are reduced to one at higher twitch tensions. 'Flaxedil' (2 mg) was then introduced intravenously. Fig. 2 *E-H* shows that as the twitch size gets smaller, the discharge on the rising phase of the contraction is unmasked, and persists even though there is no tension being registered. If the responses on the rising phase of the contraction were caused either by stray

extrafusal pulls or by ephaptic stimulation from extrafusal muscle action potentials then they would have been expected to disappear on application of the extrafusal neuromuscular block. The persistence of this response can be taken as positive evidence that it arises from a specific intrafusal contraction.

From these findings, it is suggested that large motor axons branch to supply both intrafusal and extrafusal muscle fibres. This was confirmed by examination of a number of histological preparations and appears to be a common feature. Whether this pattern of innervation occurs exclusively or whether occasionally the intrafusal fibre is innervated by unbranched motor axons is difficult to determine, but histological observations suggest that the latter occur rarely. The apparent absence of a specific motor supply to the lizard muscle spindle distinguishes this group from mammals and relates it to the amphibians, although the reptilian receptor is unique in the simplicity of its construction. The functional significance of the differences in structure remains as yet unknown.

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### Release of Factor VIII (Antihæmophilic Factor) from Perfused Organs and Tissues

It has been known for many years that patients with classic hæmophilia bleed because they lack a plasma factor required for normal blood coagulation. This factor, factor VIII (antihæmophilic factor) of normal plasma, has been extensively investigated, but its exact site of origin is unknown. Recent developments in organ transplantation have stimulated us to look for the specific organ source of this procogulant. If the site of synthesis of factor VIII could be identified, permanent replacement of the deficient factor in hæmophilia might be possible. In the past, a variety of experimental approaches has been used to investigate the body's mechanisms for maintaining hæmostatic levels of factor VIII: organ ablation<sup>1</sup>, total body irradiation<sup>2</sup>, administration of hepatotoxins<sup>3,4</sup>, reticulo-endothelial blockade<sup>5</sup>, physical exercise<sup>6</sup>, hormone administration<sup>7</sup>, and thromboplastin injections<sup>8</sup>. Several recent investigations have suggested that the spleen may play a part in the regulation of concentrations of factor VIII in the plasma of normal individuals. Weaver *et al.* demonstrated that factor VIII is maintained at normal concentrations when intact normal dogs are cross-circulated with hæmophilic dogs, but that the concentrations decrease if a splenectomy is first performed on the normal animals<sup>9</sup>. In humans, Libre *et al.*<sup>10</sup> have observed that splenectomy abolishes the rise in factor VIII that is known to follow injection of adrenaline into normal subjects<sup>10</sup>. Pool was able to recover antihæmophilic activity in extracts of splenic tissue, but not from a variety of other tissues<sup>11</sup>.

The availability of both hæmophilic and normal dogs for experimental use has made it possible for us to explore the problem of factor VIII production by perfusing isolated organs with hæmophilic blood. A systematic investigation of major organs has now added direct evidence that the spleen is one of the sources of factor VIII and that, therefore, it may play an important part in

maintaining physiological plasma concentrations of this factor. Some of these perfusion experiments are reported.

Organs and tissues to be perfused were obtained from normal mongrel dogs or from dogs hemizygous or heterozygous for classic hæmophilia<sup>12</sup>. The dogs weighed about 20 kg. The animals were anaesthetized with sodium pentobarbital, and major vessels of the part to be perfused were isolated and cannulated with vinyl plastic tubing. Prompt irrigation with citrated saline (4° C) was started and was continued during and after excision of the organ; removal of retained blood usually required about 4–6 l. of citrated saline. Perfusion was then performed by recycling citrated hæmophilic whole blood (37° C) through the organ. The blood was propelled by a 'Sigma' motor pump and was oxygenated in a paediatric aerator at an oxygen flow rate of 1–2 l./min. Blood samples were withdrawn at intervals, centrifuged and the plasma stored at –20° C until assayed for factor VIII activity<sup>13</sup>.

Sample data are presented in Table 1. In each experiment, no factor VIII could be detected in the effluent at the conclusion of saline irrigation and at the time whole blood perfusion was begun. There was then a lag period of at least 30 min before appreciable factor VIII activity appeared, and then no activity could be obtained from normal lung or kidney or from a hæmophilic spleen. High concentrations of antihæmophilic activity consistently appeared only with perfusion of normal and heterozygous spleens. At periods later than those tabulated, values as high as 110 per cent were obtained. Less activity was recovered from an isolated hind limb and from the liver of normal dogs.

All the organs selected from these perfusion investigations were highly vascular structures. Thus, the possibility of blood being sequestered within closed areas of the microcirculation must be considered. Relatively complete removal of blood seems to have been accomplished, however, for we failed to detect any activity of factor VIII after irrigation with saline. In one experiment, an additional attempt to exhaust the spleen of pooled blood was made by repeated injections of adrenaline chloride into the irrigation medium. This resulted in splenic contraction and expression of additional blood, but did not prevent the later increase in factor VIII concentrations. Further, in another experiment, saturation of a hæmophilic spleen with normal blood was first accomplished by cross-circulation of a hæmophilic dog with a normal dog for 60 min. The accumulated factor VIII was easily exhausted from the isolated spleen by irrigation and no further factor VIII appeared on perfusion, even when adrenaline was introduced. Furthermore, the kidney and lung are also highly vascular viscera and offer opportunity for pooling of blood in the microcirculation. These organs, however, failed to yield detectable factor VIII when they were perfused. It is of interest that the isolated kidney is not inert in similar circumstances; it will release plasminogen activator when perfused and stimulated with vasoactive drugs<sup>14</sup>.

A second common structural denominator between the organs in which activity of factor VIII was found is that each is relatively rich in reticulo-endothelial elements. It is therefore interesting to recall the experiments that Pool and Spaet performed 13 years ago that tended to point to the reticulo-endothelial cells as the site of factor VIII

Table 1. RELEASE OF FACTOR VIII WITH PERFUSION

Organ perfused	No. of experiments	Mean factor VIII after 90 min*	Range*
Normal hind limb	4	3.4	<1–9
Normal liver	1	9.5	—
Normal lung	2	<1	<1
Normal kidney	3	<1	<1
Normal spleen	7	49	27–65
Hæmophilic spleen	1	<1	—
Heterozygous spleen	1	43	—

\* Values are expressed as percentage of activity of a normal canine plasma control.

production<sup>17</sup>. Penick *et al.* were able to depress partially the levels of circulating factor VIII by damaging the liver with chloroform<sup>4</sup>. More recently, Straub *et al.* have shown that factor VIII concentrations can be elevated in the presence of lethal liver necrosis<sup>15</sup>. In earlier experiments<sup>2</sup>, destruction of much of the splenic parenchyma by total body irradiation had failed to depress factor VIII, but the reticular cells in these animals appeared to be viable histologically. Although lung macrophages are often included as a component of the reticulo-endothelial system, the lung does not appear to release significant amounts of factor VIII.

It was initially feared that thromboplastin might be released from blood cells during the extracorporeal circulation and masquerade as factor VIII. No factor VIII activity was detected, however, in the perfusates from the lung, kidney and the haemophilic spleen, even though appreciable haemolysis was evident during the terminal stages of each experiment. Neither did the perfusate shorten the recalcification time of platelet-free normal plasma. In fact, it may be that the detected concentrations of factor VIII were erroneously small, for it has been shown that extracorporeal circulation and aeration of blood can cause some consumption of factor VIII (ref. 16).

Organ perfusion appears to offer positive and direct evidence that organs containing reticulo-endothelial elements, and especially the spleen, are capable of releasing factor VIII into the circulation. In all experiments so far, it is difficult to decide whether the role of the spleen is one of storage or production. Furthermore, it is well established that asplenic humans and animals do not develop a permanent deficiency of factor VIII (refs. 8 and 9). Thus, it is especially interesting that we were able to demonstrate some factor VIII in perfusates from the liver and leg. It is conceivable that the liver and bone marrow could be able to compensate for a removed spleen in its role of releasing factor VIII.

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## Antagonism of Intra-arterial Acetylcholine Induced Contraction of Skeletal Muscle by Sea Snake Venom

Marsden and Reid<sup>1</sup> in their investigations on victims of sea snake (*Enhydryna schistosa* Daudin) poisoning found evidence of progressive muscular weakness and myonecrosis. Carey and Wright<sup>2</sup>, however, concluded that the venom had a neuromuscular blocking action when tested on the isolated rat phrenic nerve-diaphragm preparation.

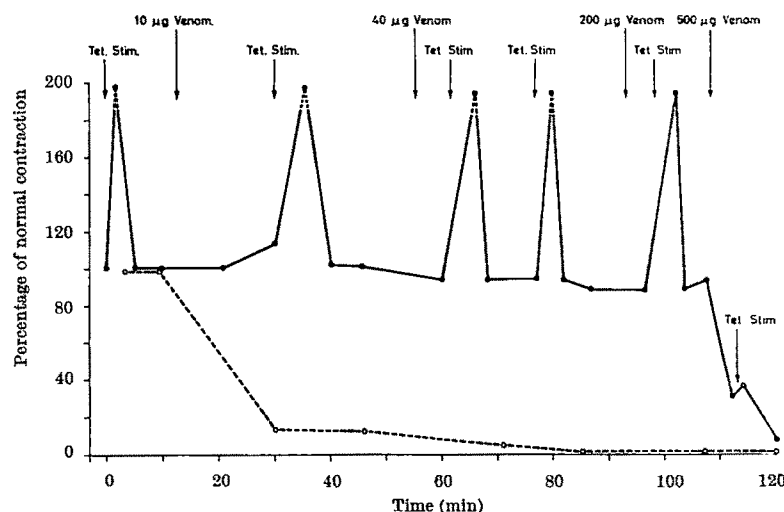


Fig. 1. Comparison of height of muscle contraction caused by indirect stimulation of anterior tibial nerve (●—●) and that caused by intra-arterial acetylcholine (○—○) after *E. schistosa* venom in a cat weighing 2.0 kg.

Our investigations of the pharmacological properties of the venom confirmed the development of muscle weakness and respiratory paralysis in the intact guinea-pig, although in animals which acutely succumbed to subcutaneous injection of the venom there was no histological evidence of muscle necrosis. Evidence obtained from the isolated phrenic nerve diaphragm and sciatic nerve gastrocnemius preparation of the rat suggested a neuromuscular blocking action.

In the cat tibialis anterior muscle preparation, however, it was possible to demonstrate repeatedly that small doses of venom could abolish twitches caused by intra-arterial acetylcholine without interfering with muscle contraction resulting from a supramaximal electrical stimulus applied to the anterior tibial nerve<sup>3</sup>. Neither was the post-tetanic facilitation modified. Only with comparatively large doses was there gradual and progressive diminution in neuromuscular transmission (Fig. 1).

This abolition of muscular twitch from intra-arterial acetylcholine without any apparent change in neuromuscular transmission is contrary to traditional concepts of acetylcholine as the transmitter substance at the neuromuscular junction<sup>4</sup>. Furthermore, we have been unable to detect any atropine-like or cholinesterase activity in the venom. It is difficult to reconcile this interesting finding without postulating that acetylcholine may not be the actual substance affecting the sub-neural membrane of the motor endplate as the transmitter substance. There is evidence<sup>5</sup> which suggests that acetylcholine may act on the motor nerve terminal to trigger off an unknown mechanism for muscle contraction<sup>6</sup>. Iontophoresis<sup>7</sup>, though fine enough to localize acetylcholine action to the motor endplate region, is, nevertheless, unable to discriminate between the different components of the motor endplate thus affected. The action of sea snake venom can apparently differentiate between cholinceptive sites which cause the muscle twitch from acetylcholine and the receptor sites responsible for contraction of indirectly stimulated muscle.

The exact location of acetylcholine action is difficult to resolve. There is evidence for action at the motor nerve terminal<sup>5</sup> and a possibility of action on receptors on the muscle membrane outside the motor endplate region<sup>8,9</sup>. Either or both could account for our observation, but we feel the former is a more likely explanation because the latter has been shown to be a phenomenon peculiar to chronic denervation.

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## GENETICS

### Presence of the Duffy Blood Group Gene *Fy<sup>b</sup>* demonstrated in Melanesians

THE Duffy (*Fy*) blood group discovered in 1950 was the eighth of the principal blood group systems to be identified. The first antigen found was called *Fy<sup>a</sup>* and its corresponding antibody anti-*Fy<sup>a</sup>*. The antithetical antigen *Fy<sup>b</sup>* and its antibody anti-*Fy<sup>b</sup>* was found in 1951. These antibodies occur as natural antibodies or as the result of immunization by blood transfusion or by pregnancy, and it is known that anti-*Fy<sup>a</sup>* may cause haemolytic blood transfusion reactions and also haemolytic disease of the newborn. Two alleles, however, *Fy<sup>a</sup>* and *Fy<sup>b</sup>*, have not been sufficient to explain the Duffy reactions found in inheritance, and a third allele, *Fy*, occurring frequently in Negroes and occasionally in Caucasians, has been postulated although no anti-*Fy* antibody has been found. The distribution of both *Fy<sup>a</sup>* and *Fy<sup>b</sup>* in Caucasians and in Negroes has been thoroughly investigated, while Japanese and Koreans tested with only anti-*Fy<sup>a</sup>* have been found to be almost 100 per cent anti-*Fy<sup>a</sup>* positive<sup>10</sup>. During 1953-61 blood group research workers published findings on the distribution of the Duffy *Fy<sup>a</sup>* blood group gene in the Pacific peoples, and these investigations have shown that, with the exception of the Polynesians<sup>1</sup>, other Pacific peoples including Melanesians are, with few exceptions, 100 per cent *Fy(a+)*, that is, all blood samples tested have reacted with anti-*Fy<sup>a</sup>* testing reagent.

The present communication deals exclusively with investigations carried out on various groups of people within the Melanesian population during 1965, and the new data presented demonstrate that the Duffy *Fy<sup>b</sup>* gene is also present in Melanesians, but its distribution is limited to certain linguistic groups living in different geographical locations.

The recent investigations were made possible by the identification in Melbourne in 1964 of a potent saline agglutinating anti-*Fy<sup>a</sup>* antiserum by one of us (R. T. S.), and as a result many blood grouping laboratories in different countries have received this serum for special work. Only a few examples of anti-*Fy<sup>b</sup>* antisera have been found since its discovery<sup>2</sup>, and at no time have supplies of this important testing reagent been available for extensive investigations in racial blood group genetics. Table 1 summarizes the results of anti-*Fy<sup>a</sup>* testing recorded in the years 1953-61 for various linguistic groups in the

Melanesian populations of New Britain, Papua-New Guinea, West New Guinea and the Solomon Islands (B.S.I.P.). In all, 2,392 blood samples out of 2,397 (99.8 per cent) were found to be *Fy(a+)*. In New Britain all of the 132 samples from five linguistic groups (100 per cent) were all *Fy(a+)*, in New Guinea 1,410 out of 1,411 (99.9 per cent) were *Fy(a+)*, in West New Guinea 712 out of 715 (99.6 per cent) were *Fy(a+)* and in the Solomon Islands 138 out of 139 (99.3 per cent) were *Fy(a+)*. It was uncertain whether the one negative recorded in New Guinea, the three negatives in West New Guinea and the one negative found in the Solomon Islands were truly negative, or whether they had failed to react because of technical difficulties, or because of the age of the blood samples when tested. It seemed reasonable to conclude that the latter was possibly the case in samples from West New Guinea which were 6-9 months old when tested, after storage at 5° C in glucose-citrate solution. Thus, the unanswered question was whether the *Fy<sup>a</sup>* frequency in Melanesians was 100 per cent, or whether there was another Duffy gene also present to explain the five negatives recorded in the 2,397 tests made.

Investigations carried out in 1965 with both anti-*Fy<sup>a</sup>* and anti-*Fy<sup>b</sup>* saline agglutinating sera versus 20 per cent red cell suspensions in glucose-citrate solution<sup>18</sup> on slides kept in moist-chambers, and read after 60 min at 22° C, gave the results of Table 2. The anti-*Fy<sup>a</sup>* agglutinating serum has been in use in Melbourne since its identification in 1957.

Throughout the year samples were flown to Melbourne for blood group genetic investigations from New Guinea, from Manus Island in the Admiralty Islands, the Solomon Islands, the New Hebrides and, in December 1965, from New Britain. In all, 892 out of 892 (100 per cent) of the New Guinea samples were *Fy(a+b-)*, 124 out of 124 (100 per cent) of the Manus Island samples were *Fy(a+b-)*, and 142 out of 142 (100 per cent) of the New Hebrides samples were *Fy(a+b-)*. In tests on the forty-seven Guadalcanal (Solomon Islands) samples, forty-six were *Fy(a+b-)* and one was *Fy(a+b+)*. Previously (Table 1) one sample in 139 from Guadalcanal was *Fy(a-)*. It was in New Britain, however, that tests on peoples of the Tolai and Sulka linguistic groups

Table 1. SUMMARY OF DUFFY *Fy<sup>a</sup>* INVESTIGATIONS IN MELANESIANS, 1953-1961

Population	Location or administrative district	Linguistic group	Proportion <i>Fy(a+)</i>	Percentage <i>Fy(a+)</i>
New Britain*	Gazelle Peninsula Central area	Bainings <sup>4</sup>	22/22	100
		West Nakanai <sup>5</sup>	46/46	100
		Kakuna Mamusi <sup>6</sup>	19/19	100
		Kisiluvu Mamusi <sup>6</sup>	15/15	100
		Central Nakanai <sup>6</sup>	14/14	100
		West Nakanai <sup>6</sup>	16/16	100
	Total		132/132	100
Papua-New Guinea†	Port Moresby Mt. Hagen, Goroka Nondugl N.G. Natives Okapa	Unclassified <sup>7</sup>	252/252	100
		Unclassified <sup>8</sup>	70/70	100
		Unclassified <sup>9</sup>	380/381	99.7
		Fore (kuru victims) <sup>10</sup>	119/119	100
	E. Highlands	Fore (normals) <sup>11</sup>	121/121	100
		Unclassified <sup>11</sup>	183/183	100
		Unclassified <sup>11</sup>	214/214	100
	W. Highlands Papua	Enga <sup>11</sup>	49/49	100
		Orokaiva <sup>11</sup>	22/22	100
	Total		1,410/1,411	99.9
West New Guinea	Biak Sorong Wissel Lakes ‡ South Coast Lake Sentani Central area	Unclassified <sup>12</sup>	183/183	100
		Unclassified <sup>13</sup>	25/25	100
		Kapaukoe <sup>13</sup>	27/30	90
		Asmat <sup>14</sup>	100/100	100
		Sentani <sup>16</sup>	166/166	100
		Dani <sup>18</sup>	173/173	100
	Total	Dani-Mullia <sup>11</sup>	38/38	100
			712/715	99.6
Solomon Islands	Guadalcanal (B.S.I.P.) Honiara	Unclassified <sup>17</sup>	138/139	99.3
	Grand total		2,392/2,397	99.8

\* Locations of linguistic groups in New Britain have been presented by Simmons *et al.*<sup>2</sup> in 1964.

† The Administrative Districts of New Guinea have been presented by Simmons *et al.*<sup>11</sup> in 1961.

‡ The blood samples were 6-9 months old when tested with anti-*Fy<sup>a</sup>* serum.

Table 2. DUFFY  $Fy^a$  AND  $Fy^b$  BLOOD GROUP GENES AS FOUND IN MELANESIANS IN 1965

Population	Location or administrative district	Linguistic group	No. tested	$Fy(a+b-)$	Duffy blood types $Fy(a+b+)$	$Fy(a-b+)$
New Britain	Gazelle Peninsula	Tolai	100	88	11	1
		Sulka	36	32	4	0
Total			136*	120	15	1
Admiralty Islands	Manus Island	Several	124	124	0	0
New Guinea	Sepik District	Several	260	260	0	0
	Eastern Highlands	Several	338	338	0	0
	Eastern Highlands (kuru patients)	Fore	35	35	0	0
Total	Morobe District	Kukukuku	259	259	0	0
			892	892	0	0
Solomon Islands	Guadalcanal	.	47	46	1	0
New Hebrides	Emai Island	.	95	95	0	0
	Emau Island	.	47	47	0	0
Total			142	142	0	0
Grand total			1,341	1,324	16	1

\* The gene frequencies for 100 random Tolai were:  $Fy^a$  0.9360  $Fy^b$  0.0650  
and for 36 random Sulka were: 0.944 0.056

revealed the presence of an unsuspected frequency of the  $Fy^b$  gene. In a total of 136 random samples tested out of 457 blood samples received, we found 120 out of 136 (88.2 per cent)  $Fy(a+b-)$ , fifteen (11.0 per cent)  $Fy(a+b+)$  and one (0.7 per cent)  $Fy(a-b+)$ . Thus, two linguistic groups in New Britain have been proved to possess the blood group gene  $Fy^b$  as well as  $Fy^a$  and this also applies to a small sampling from the Solomon Islands.

The inheritance of both  $Fy^a$  and  $Fy^b$  genes in a number of Tolai and Sulka families was checked with no evidence of any atypical inheritance, or the presence of a third Duffy gene being indicated. The results of these family investigations form part of a series of blood group genetic investigations, the results of which will be presented elsewhere.

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## BIOLOGY

### *Macrostomum tuba* (Graff), 1882 (Turbellaria, Rhabdocoela), recorded in British Freshwater Aquaria

THE macrostomid rhabdocoel *Macrostomum tuba* has its main distribution in central and southern Europe and Japan, and appears to be absent from northern Eurasia<sup>1,2</sup>. It has been found, however, in a few warmed aquaria and warmed pools in botanical gardens in the latter region for example, Stockholm, Berlin, Munich, Warsaw and Helsinki, and also in New York.

The species has been present in considerable numbers in a few warmed aquaria (21° C) at the I. M. Marsh College of Physical Education, Liverpool, since January 1966. There is every reason to believe that it will persist here, because Luther<sup>3</sup> comments that the species has lived in warmed aquaria in Helsinki for many years. The source of the rhabdocoels was traced to a shop called "The Liverpool Aquaria" which supplies the College with biological material, and an examination of their warmed aquaria revealed one specimen; the paucity of animals is not surprising, because the tanks are cleaned at frequent,

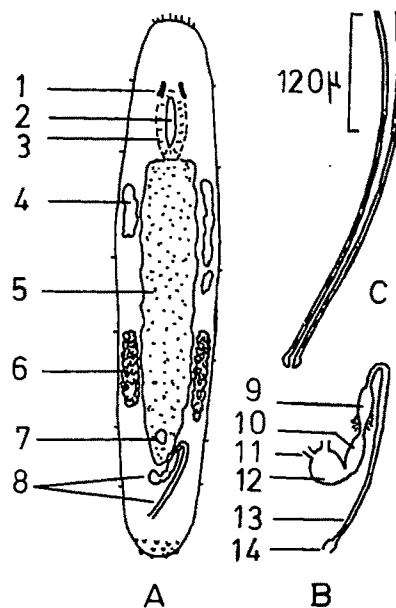


Fig. 1. *A*, *Macrostomum tuba*, a mature example of 2 mm length ( $\times 54$ ). *B*, Male copulatory apparatus of same ( $\times 120$ ). *C*, Penis stylet (varied from 280 to 420  $\mu$  in sixteen specimens examined). 1, Eye; 2, mouth; 3, pharynx; 4, testes; 5, intestine; 6, ovary; 7, female atrium; 8, male copulatory apparatus; 9, vesicula granulorum (prostatic vesicle); 10, vesicula seminalis; 11, vas deferens; 12, false seminal vesicle (spermiducal vesicle); 13, penis stylet; and 14, bulb-shaped distal tip.



regular intervals. It is probable that the living rhabdocoels, or a resistant phase, were unintentionally imported into Britain with animal or plant consignments from one of the regions, namely North and South America, Singapore, India, Holland and East Germany, which supply the importers. Thus, the animal may be present in other warmed aquaria in Merseyside and indeed could be widespread in aquaria in Britain. In view of the recent introduction of the American freshwater triclad *Dugesia tigrina* (Girard) to Britain, probably through the activities of aquarists<sup>3,4</sup>, and its subsequent spread into natural habitats, the authors advocate that similar introductions should be put on record. It is pertinent to mention that an extensive sampling programme for rhabdocoels in diverse freshwater habitats in Merseyside has not yielded any *M. tuba* as yet.

The anatomy of *M. tuba* has been described adequately<sup>5-7</sup> but Fig. 1, drawn from the animals found in Liverpool, has been included to aid the easy identification of mature animals. These specimens apparently conform with the widely spread form of the species which has a long stylet with a relatively small vesicula granulorum; the short styleted form with larger vesicula granulorum appears to be limited to Germany<sup>8</sup>.

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### Effect of Amino-acids on the Transport of Bovine Immune Lactoglobulin across New-born Pig Intestine

THE everted small intestine of the newborn pig, formed into sacs and incubated in a preparation of dialysed bovine colostrum, transports bovine immune lactoglobulin to the serosal surface; most of the protein transport occurs across sacs made from the middle third of the intestine<sup>1</sup>. This part of the small intestine is not outstanding in its ability to transport either water, sodium or glucose, but in other mammals this is the area for maximal transport of several amino-acids<sup>2</sup>. Amino-acids can either induce or suppress endocytosis in amoebae<sup>3</sup> and it was these two observations which suggested that amino-acids could influence protein transport in the pig intestine.

Akedo and Christensen<sup>4</sup> have shown that certain amino-acids cross mucosal cell membranes mainly by facilitated diffusion while others make use of an active transport mechanism. In the present investigation, L-leucine and L-alanine were chosen to represent amino-acids crossing by these two processes respectively. L-Methionine was also used; it is thought to cross cell membranes by both mechanisms<sup>5</sup> and is known to inhibit the intestinal transport of other amino-acids<sup>6</sup>.

Piglets, removed from the sow immediately after birth, were used within 2 h of birth. The induction of anaesthesia, dissection of intestine and preparation of everted sacs have been described in detail elsewhere<sup>1</sup>. The whole of the small intestine was divided into ten segments of equal length and segments 6 and 7 were removed for the following experiments. Everted sacs, formed from these two segments, six sacs in all, were partly filled with 0.3 ml. of bicarbonate saline<sup>7</sup> and incubated in bovine colostrum,

dialysed previously against bicarbonate saline. An amino-acid was sometimes added to the bovine colostrum after dialysis in a concentration of 10 mmol/l. Incubation was for 2 h at 37°C in a medium gassed with 95 per cent oxygen + 5 per cent carbon dioxide. A smear of silicone antifoam emulsion around the top of each tube controlled frothing produced by gassing. The dialysed bovine colostrum contained 138 mmol/l. of sodium; 6.7 mmol/l. of potassium; 25 mmol/l. of glucose; total protein 10.4 per cent (w/v); (immune lactoglobulin 6.3 per cent w/v). The pH of this solution when equilibrated with the mixture of oxygen and carbon dioxide was 7.4. Transmural potentials were recorded during incubation and fluid transfer calculated at the end of incubation<sup>1</sup>. At the end of incubation the serosal fluids were analysed for glucose by the method of Hansen<sup>8</sup> and for bovine immune lactoglobulin by the quantitative immunodiffusion test of Geil<sup>9</sup>. Serosal transfers were calculated as µg of bovine immune lactoglobulin, or ml. of fluid, or mg of glucose appearing/g of intestine/h of incubation. Serosal transfers were determined across sacs incubated in colostrum containing the amino-acid which was being tested and alternate sacs served as controls. The significance of differences was assessed using the Wilcoxon test<sup>10</sup>.

The mean control serosal transfers, determined on forty-eight sacs, were: bovine immune lactoglobulin, 61.6 µg/g/h; fluid, 0.5 ml./g/h; glucose, 1.9 mg/g/h. The mean transmural potential was 4.4 mV. Changes from these control values, measured in the presence of different amino-acids, are shown in Fig. 1. L-Alanine did not significantly change any of the parameters measured. L-Leucine inhibited protein transport, but it was impossible to say whether this was a direct effect or only secondary to the inhibition of water and glucose transport which occurred when this amino-acid was present. L-Methionine inhibited protein transport without changing the transmural potential or the serosal transfer of fluid or glucose. None of these effects were seen when the D-isomers were used (see Fig. 2).

The specificity of L-methionine as an inhibitor of protein transport implies a direct action on some part of the protein transferring mechanism; the absence of any effect using the D-isomer of methionine eliminates the possibility that the inhibitory action is dependent on the electrical charge carried by the molecule at pH 7.4. We have already suggested that different protein molecules show different specificities for binding sites at the luminal border of the mucosal cell and that selectivity in absorp-

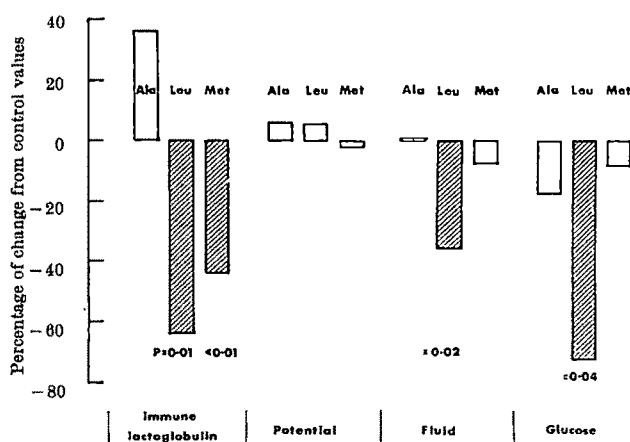


Fig. 1. Effect of L-amino-acids on the transmural potential and serosal transfers of bovine immune lactoglobulin, fluid and glucose, measured across the everted small intestine of the newborn pig. Everted sacs were incubated in dialysed bovine colostrum for 2 h at 37°C. The initial concentration of amino-acid in the mucosal fluid was 10 mmol/l. Comparisons were made with alternate sacs incubated in colostrum containing no added amino-acid. Each histogram gives the mean change from comparisons. Open histograms, no significant change; cross-hatched histograms, change significant at the level of probability *P* shown in the figure.

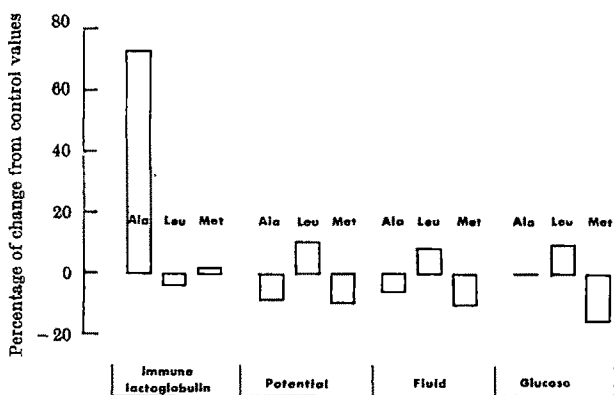


Fig. 2. Comparison of control transmural potentials and serosal transfers of bovine immune lactoglobulin, fluid and glucose, across the everted pig small intestine, with those found in the presence of D-amino-acids. Conditions of incubation were the same as in Fig. 1. Histograms give the mean change from six comparisons. None of the changes were statistically significant.

tion could occur before endocytosis takes place<sup>1</sup>. This would be analogous to the process of endocytosis of protein in amoebae<sup>2</sup>. If methionine and leucine, but not alanine, were to compete with bovine immune lactoglobulin for a common receptor at the mucosal membrane, this would explain the inhibitory action of these amino-acids and suggest that the postulated receptor is one normally responsible for the facilitated diffusion of amino-acids into the mucosa. The possibility that protein could be transported across intestines at a rate which depends on an affinity for a mechanism of facilitated diffusion was first suggested by Danielli in 1954 (ref. 11).

This idea can now be tested in greater detail using other amino-acids with known affinities for a facilitated diffusion mechanism, in the hope of finding some which inhibit protein transfer without changing fluid movements.

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### *Gwynia capsula* (Jeffreys), an Articulate Brachiopod with Brood Protection

THE articulate brachiopod *Gwynia capsula* (Fig. 1), which is only about 1 mm in diameter, was described by Jeffreys in 1859 and recorded during the last half of the nineteenth century from several marine localities in Great Britain, as well as from the continental side of the Channel. Because of the very small size of the animal, it was suggested that it might be the juvenile stage of some larger known species<sup>1</sup>. In spite of older records<sup>1,2</sup> the species is not listed in the *Plymouth Marine Fauna*<sup>3</sup> and it is quoted as a doubtful species in modern text-books<sup>4,5</sup>.

According to the description<sup>6,7</sup>, *Gwynia capsula* has some morphological details which are interesting from the point of view of brachiopod phylogeny. One of these features is the lophophore, which represents the simplest (trocholophous) type. A revision of the morphology of *Gwynia capsula* is now in progress in this laboratory.

I found *Gwynia capsula* living in sub-tidal sand at Church Island, near the Marine Science Laboratories, Menai Bridge, in 1956. It was found there again in 1964 and 1965. The particular sediment of the locality is a mineral sand containing a high portion of fine broken shell. The population seems scarce and the individuals were found one by one sheltered in tiny fragments of serpulid tubes. Others previously found *Gwynia capsula* fixed to the undersides of stones in the sub-tidal region. The findings at Menai Bridge indicate that the species can also be included in the rare category of sedentary animals of the interstitial fauna of marine sand<sup>8</sup>. Live specimens have been kept in the laboratory for

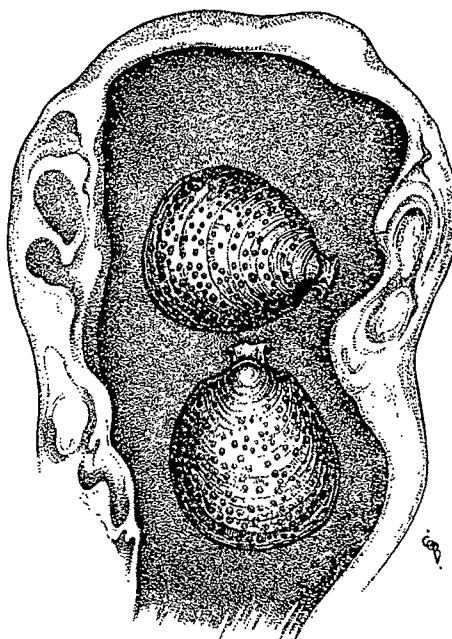


Fig. 1. *Gwynia capsula* (Jeffreys), two specimens living inside a fragment of a serpulid shell.

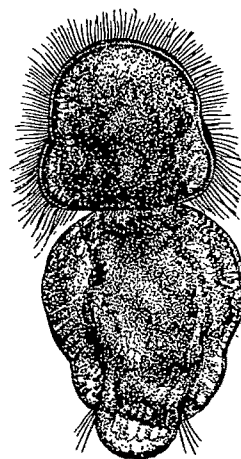


Fig. 2. *Gwynia capsula* (Jeffreys), larva from brood pouch of female.

considerable periods. Animals caught at Church Island in October 1964 were still alive at Kristineberg in August 1965, although no food had been added. It is believed, therefore, that *Gwynia capsula* feeds on micro-organisms.

If *Gwynia capsula* is interesting from a morphological point of view, it is also certain that it has a peculiar type of reproductive biology. Like many organisms of very small body size<sup>8</sup>, *Gwynia capsula* produces only a limited number of gametes. In the female gonad the eggs develop in the body cavity of the mother animal in a kind of brood pouch, at least up to the stage where three segments have been formed (Fig. 2). Only two embryos develop at one time. Biologically, brood protection has been considered to be an adaptation which favours the maintenance of populations of species producing low numbers of gametes<sup>9</sup>.

It is concluded, from the foregoing observations of reproduction at Bangor, that *Gwynia capsula* (Jeffreys) is a distinct species, which reaches the adult stage at a body size of only about 1 mm. It is the smallest articulate brachiopod known so far.

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### Blood Concentrations of Caesium-137 in People in the Far East and the Pacific Region

CAESIUM-137, a fission product with a long half-life, is liable to be transmitted through the food chain to man when the environment is contaminated by radioactive fall-out. From the behaviour of caesium-137 concentrations in humans observed in several countries since 1959, it has been suggested that the concentrations in people vary with the current rates of fall-out and the cumulative concentrations in the environment as well as the food habits. Special emphasis should be laid on the last factor, because in some groups of Scandinavian Laplanders and Alaskan Eskimos average body burden in 1961 reached concentrations forty to sixty times greater than the average for northern temperate regions. The enhanced concentrations are believed to depend on particular food chain mechanisms comprising high consumption of highly contaminated meat of caribou or reindeer<sup>1</sup>.

Measured values of caesium-137 in humans have been reported from about a dozen countries representing altogether a population of 500 million, but this is only a small fraction of the world population of 3,200 million and data for people living in Asia and the southern hemisphere are very scant. Measurements of the total body burden of caesium-137 have been made conveniently with whole body counters. A world survey of whole body monitors revealed that 67 per cent of the apparatuses were used to measure caesium-137 in the general public, but in fact the main use of these apparatuses is for occupational groups and patients<sup>2</sup>. Although mobile counters are available and used in the survey of Laplanders and Eskimos, the world distribution of the apparatuses is too uneven to give worldwide information about the concentrations of caesium-137 in the populace.

A method of blood analysis was proposed by Yamagata<sup>3</sup> as a promising substitute for whole body counting in countries where rather expensive counters are not available or limited in use for the general public. This method

makes use of the citrated whole blood which can be made available through blood bank or blood transfusion systems. A minimum quantity of about 100 ml. of blood is required currently for radiochemical determination of caesium-137 (ref. 4) and the amount of added anticoagulant has to be known for correction purposes. Measurement of activity is made by a low-background counting system in order to measure  $\beta$ -activity as low as a few d.p.m.

For the estimation of total body burden, the factor relating the concentration of caesium-137 in blood to the total body burden must be known. To obtain such a factor, the blood analysis and whole body counting were conducted monthly for a number of laboratory personnel between June and December 1964, when a stationary state was likely to exist in the body burden, indicating a quasi-equilibrium condition between the diet and body<sup>5</sup>. The factor relating the concentration of caesium-137 in blood to the total body burden as expressed in the formula

$$f = \frac{\text{Total body burden of caesium-137 (pc.)}}{\text{Body weight (kg)} \times \text{caesium-137 in blood (pc./kg)}}$$

was found to average 5.99, with a range from 5.59 to 6.53. The variation in the value of  $f$  among individuals was less than either that of the body burden or the blood concentration, indicating a rather consistent value of the factor.

Table 1. BLOOD CONCENTRATIONS OF CAESIUM-137 IN PEOPLE IN THE FAR EAST AND THE PACIFIC REGION

Source	Sample No.	Date of collection	Age	Caesium-137 in blood pc./kg	Mean standard deviation
Thai Red Cross Society, Bangkok, Thailand	A	November 18, 1965		21.7	
	B	" "		34.8	
	C	" "		20.3	
	D	" "		44.3	
	E	" "		34.6	
	F	" "		41.0	31.9
	H	" "		32.8	± 9.5
	I	" "		46.5	
	J	" "		22.7	
	K	" "		31.0	
Philippine National Red Cross, Manila, Philippines	L	" "		21.6	
	188,092	April 1, 1966	20	24.6	
	188,752	" "	18	38.2	
	188,757	" "	18	38.6	
	188,822	" "	19	40.5	
	189,511	" "	19	27.5	36.5
	191,381	" "	18	32.9	± 9.1
	190,788	April 2, 1966	34	40.7	
	195,894	" "	29	56.5	
	195,896	" "	22	37.7	
Red Cross Society of China, Taipei, Taiwan	190,806	April 3, 1966	25	27.8	
	7,195	December 11, 1966	36	28.4	
	7,321	December 15, 1965	37	15.2	
	7,335	December 16, 1965	29	24.5	
	7,346	December 17, 1965	44	20.6	
	7,351	" "	22	20.5	20.6
	1,576	April 1, 1966	32	13.6	± 4.5
	1,581	" "	41	23.9	
	1,583	" "	25	18.3	
	1,601	" "	42	18.1	
Indian Red Cross Society, New Delhi, India	1,607	April 4, 1966	38	22.4	
	948	December 31, 1965		23.8	
	949	" "		15.0	
	950	" "		17.0	
	951	" "		29.6	
	952	" "		14.9	25.1
	953	" "		38.4	± 8.5
	954	" "		38.5	
	955	September 27, 1965		22.8	
	956	December 31, 1965		26.9	
Red Cross Blood Transfusion Service, Queensland Division, Brisbane, Australia	957	" "		24.9	
	1	April 13, 1966	42	18.8	
	2	" "	40	28.0	
	3	" "	40	22.1	
	4	April 7, 1966	49	23.7	
	5	April 13, 1966	28	17.4	21.8
	6	" "	35	26.7	± 4.0
	7	" "	44	17.4	
	8	" "	37	24.4	
	9	April 14, 1966	24	18.4	
Burma Red Cross, Rangoon, Burma	10	April 13, 1966	48	20.0	
	1	April 27, 1966	29	18.7	
	2	" "	33	13.9	
	3	" "	45	20.7	
	4	" "	32	23.5	
	5	" "	28	19.8	18.7
	6	" "	19	19.9	± 3.3
	7	" "	19	14.1	
	8	" "	21	22.1	
	9	" "	26	19.4	
	10	" "	23	14.9	

Day to day fluctuation of dietary intake of caesium-137 is very likely to occur and this can cause day to day fluctuation of blood concentrations because of a rapid transition of ingested caesium. An *in vivo* experiment on the metabolism of caesium in human blood was conducted in order to estimate the degree of fluctuation mentioned here<sup>6</sup>. The result suggested that possible errors would amount to up to 30 per cent in an estimate based on individual determination, but the use of a pooled blood sample representing a number of individual or an average for a number of individual determinations could afford a means of assessing the concentration in the population with satisfactory accuracy.

The validity of assessing the population concentrations of caesium-137 has become possible on the basis of blood analysis, and so a research project was initiated in November 1965 and a first series of collections of blood from the Far East and the Pacific region was made. It should be noted that the relation factor mentioned here is only valid for male, so that blood samples were collected from males only. Red Cross societies in these regions were requested to participate in the project and ten samples each were air-mailed to this Institute. The results of analyses are summarized in Table 1.

To estimate the body concentrations of caesium-137, the relation factor of 6 was multiplied by the blood concentration to get the body burden/unit body weight (pc/kg), which was then divided by an assumed concentration of potassium/unit body weight (2 g/kg) to obtain the concentration of caesium-137/g of potassium in the body (pc/gK). The total body burden is expressed in this way because most data are available in these units and for convenience in comparison.

In Table 2 are shown the estimates thus obtained for people in the Far East and the Pacific region. The values are comparable in magnitude with those obtained by whole body counting for other parts of the world except Norway (Table 3). Naturally, our knowledge of the latitudinal distribution of radioactive fall-out can never explain the variable concentrations found in different parts of the world even if local climatological circumstances such as rainfall are taken into consideration. One of the indications of such discrepancy can be found between the body concentrations in Japan and the Philippines or Thailand and Burma. Food habits are believed to be a cause of

such discrepancies and high consumption of sea food as a source of animal proteins has been considered to reduce the body concentrations of caesium-137, but further inquiry into the composition of the diets in respective countries is needed.

The method of blood analysis seems to be a useful means by which the worldwide information on the concentrations of caesium-137 in the general public can be obtained to determine the risks of radioactive materials discharged into the environment on a worldwide scale.

I thank the blood banks and blood transfusion services which took part in this project. This is part of a co-ordinated programme of research under a research agreement with the International Atomic Energy Agency.

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## Host Specificity of *Sitona* Beetles

MEMBERS of the genus *Sitona* (Coleoptera: Curculionidae) are well known pests of leguminous plants. Their larvae are subterranean feeders and have been shown to feed on the root nodules, roots or both<sup>1-4</sup>. Observations I have made recently on *Sitona regensteinensis* Hbst. suggest that the feeding preference of its larva is not only restricted to the root nodule cells of the host plant (*Sarothamnus scoparius* L. Wimm), but to the cells containing bacteria.

Preliminary observations made by growing broom seedlings between glass plates and introducing larvae onto the soil surface showed that the larvae found their way down and fed only on root nodules. It was noticed that feeding was confined to the inner contents of the nodules and the outer regions were left as "coverings" or "skins". Examination of numerous root nodules from field samples showed that a larva pierces the nodule by eating its way through and feeds on the contents of the nodule after entering it. Once the contents of a nodule are fully consumed, the empty "skin" is abandoned and the larva shifts to a fresh nodule.

As the nature of the damage done by the larvae to the root nodules is not known, an investigation of this was made by taking sections of damaged and undamaged nodules and staining them. The nodules were doubly embedded in 'Celloidin' and paraffin; sections 10 $\mu$  thick were cut, and then stained with carbolthionin followed by orange G. This staining method was used by Stoughton<sup>5</sup> to demonstrate bacteria in plant tissue; the bacteria are stained deep blue, cellulose walls yellow or green and lignified tissue light blue. A normal undamaged nodule (Fig. 1) shows an epidermis, a narrow cortical region of parenchymatous cells, an endodermis, a pericycle and then a ring of vascular tissue; the region of the pith is occupied by cells filled with bacteria which are deeply stained. The bacteria-containing tissue which occupies the greater part of the nodule is clearly distinguishable in Fig. 1.

The examination of sections of the damaged (and abandoned) nodules (Fig. 2) revealed that the *Sitona* larvae have a specialized feeding habit in that they consume only the cells containing bacteria; the epidermis, cortex, endodermis and the vascular bundles are not eaten. Parts of a normal root do not seem to be included in the diet of a *S. regensteinensis* larva.

Table 2. TOTAL BODY BURDEN OF CAESIUM-137 AS ASSESSED BY BLOOD ANALYSIS

City	Country	Approximate latitude	Body burden of caesium-137 pc/g of potassium $\pm$ standard deviation	Date
Bangkok	Thailand	14° N	96 $\pm$ 29	November, 1965
Manila	Philippines	15° N	110 $\pm$ 27	April, 1966
Taipei	Republic of China	25° N	62 $\pm$ 14	December, 1965–April, 1966
New Delhi	India	29° N	75 $\pm$ 25	December, 1965
Brisbane	Australia	27° S	66 $\pm$ 12	April, 1966
Rangoon	Burma	17° N	56 $\pm$ 10	April, 1966
Sapporo	Japan	43° N	76 $\pm$ 6	April–May, 1966
Hiroshima	Japan	34° N	62 $\pm$ 10	March–May, 1966

Table 3. RECENT DATA FOR CAESIUM-137 IN MAN MEASURED BY WHOLE BODY COUNTERS

Region, country or area of residence	Approximate latitude	Body burden caesium-137 pc/g of potassium	Date	Ref.
Oslo, Norway	60° N	270	November, 1965	*
West coast, Norway		1,610	November, 1965	*
Berkshire, U.K.	50–55° N	104	January–March, 1966	7
Belgium	50° N	105	" "	7
Northern France	49° N	103	" "	7
Los Alamos, U.S.A.	36° N	79–67	November, 1965–April, 1966	†
Los Angeles, U.S.A.	34° N	63	January–March, 1966	7
Adelaide, Australia	35° S	62–45	October, 1965–March, 1966	‡

\* Personal communication from K. Koren.

† Personal communication from C. R. Richmond.

‡ Personal communication from B. Worthley.

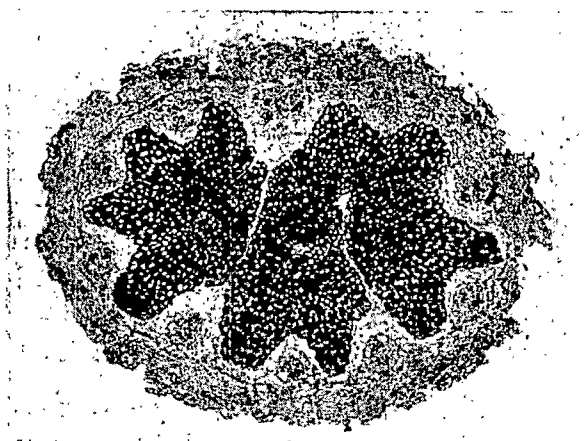


Fig. 1. Transverse section of root nodule of broom (*Sarthamnus scoparius* L. Wimm).

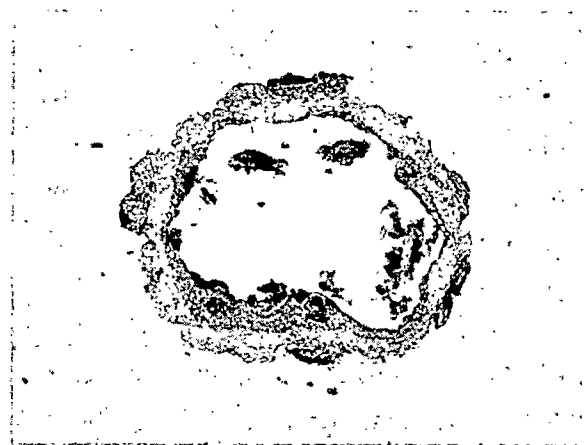


Fig. 2. Transverse section of damaged and abandoned root nodule of broom.

The preference of *Sitona* larvae for root nodules is not surprising from a nutritional point of view, because nodules contain bacteria and their function is nitrogen fixation. Nevertheless, it is interesting to note that nitrogen fixing nodules of leguminous plants contain a substance similar to haemoglobin and known as leghaemoglobin. The role and mode of this haemoglobin are unknown, but it is formed only in cells containing bacteria\*. In conclusion, these observations suggest an interesting relationship between the host plant and the pest, and the question arises as to whether the host specificity of *Sitona* beetles to legumes is associated with leghaemoglobin.

This work was carried out at Imperial College Field Station, Silwood Park, Ascot. I thank Professor O. W. Richards for providing facilities.

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## Elimination of Foreign Particles by the Snail, *Helix aspersa*

Brown and Brown<sup>1</sup> have described techniques for investigating the elimination of foreign, non-metabolizable particles from the bodies of invertebrate animals and have applied these techniques to the sandy-beach snail, *Bullia*. In this prosobranch the particles are phagocytosed by macrophagic haemocytes which migrate to the exterior mainly through the wall of the heart into the pericardial cavity and then through the renopericardial canal into the kidney, leaving the body through the nephropore. Some migration also takes place through the mantle and into the kidney from the surrounding sinuses. Tripp<sup>2</sup>, using different techniques, found that in the fresh water pulmonate, *Australorbis*, laden amoebocytes migrate mainly through the mantle epithelia and adjacent surfaces. This work has now been extended to the terrestrial snail, *Helix aspersa*.

Large aestivating individuals of *Helix* were collected, caused to emerge by being placed over water in a cold-room, and fed on lettuce. The following day, 0.35 ml. of 'Thorotrast' (a colloidal suspension of thorium dioxide) was injected into the pedal sinus of each of twelve individuals, while a further three were subjected to injections of the same volume of gastropod Ringer and retained as controls. The animals were kept on damp lettuce throughout the experiment, at a temperature of 15° C ( $\pm 1.0$ ). A test animal was killed each day for 12 days and preserved in alcohol; radiographs of each animal were taken later by means of a Siemens diagnostic X-ray machine with an exposure of 2.0 sec at 60 kV and 16 m.amp; the shutter-to-film distance was 120 cm.

In radiographs of snails killed within 48 h of injection, no pattern of internal organs is visible and the entire body casts a single, diffuse shadow. By the third day, however, certain of the organs and tissues cast hard shadows, in contrast with the rest of the body, and these shadows reach maximum intensity by the fourth or fifth day. The shadows then fade and by the tenth day are barely distinguishable from the shadow cast by the body as a whole. Even after 12 days, however, the body of the snail is more opaque to X-rays than is a control animal killed at the same time, indicating that some thorium remains in the tissues after that period.

In test animals, the organs which cast the most intense shadows are the reproductive organs, particularly the penis and the lower part of the female duct, including the vagina, and the mantle. In some animals, part of the mid-gut can also be seen in the radiographs. Sections through various regions of the reproductive organs, viewed by the method of Baxter<sup>3</sup>, reveal thorium-laden amoebocytes in the walls of these organs throughout their length, but particularly in the female side of the common duct. The intense shadows cast by the vagina and penis are seen to be caused not so much by laden haemocytes migrating through the walls of these structures as by an accumulation of them in the lumina of the ducts. Sections also reveal thorium-laden cells in the mantle epithelia and in the wall of the mid-gut, though they are relatively rare in the lumen of the alimentary canal. The heart does not appear to be involved at all as a migratory pathway and the involvement of the kidney is either very slight or lacking altogether.

In all three species of gastropod examined the mantle epithelia are involved in the elimination of foreign particles, but here the similarity ends. The chief route taken by migrating amoebocytes in *Bullia*, starting through the wall of the heart, is not evident in either *Australorbis* or *Helix*; in the former snail the mantle epithelium is of great importance as a migration route, but in *Helix* other pathways are exploited and the burden on the mantle epithelium appears to be no greater than in *Bullia*. It may be argued from this evidence that the importance of the mantle epithelium as a migratory pathway is related



to the closeness of the snail's association with water; however, work in progress on a variety of intertidal prosobranchs indicates that the relationship is by no means as simple as such a statement may suggest. Nevertheless, the chief migratory pathways in a terrestrial snail like *Helix* could be expected to differ from those found in aquatic snails, an expectation which is fully supported by the foregoing evidence.

Of particular interest is the use of the reproductive organs as a migratory route in *Helix*. Not only are these organs not involved in either *Bullia* or *Australorbis*, but their use for the elimination of foreign particles appears to be unknown also among the lamellibranchs<sup>1</sup>. The accumulation of laden haemocytes in the lower regions of the reproductive organs is clearly temporary, for the shadows cast by the penis and vagina have both disappeared by the tenth or eleventh day after injection. The fact that there was no accumulation of haemocytes in the gut may be accounted for by the conditions in which the experiment was conducted, the animals being allowed to eat as much as they wished after a period of aestivation; presumably the rapid translocation of food through the gut carried away haemocytes which had migrated through its walls.

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### Insect Population Control by the Use of Sex Pheromones to inhibit Orientation between the Sexes

SEVERAL people have speculated on the thesis that if a sufficiently high concentration of an insect sex pheromone could be maintained in the atmosphere, the sexes could not find each other for mating purposes<sup>1-3</sup> (for a review, see Jacobson<sup>4</sup>). Their conclusion was that this could lead to control or possibly eradication of the species. In the only large scale experiment to test this principle, gyplure, an analogue of the gypsy moth sex pheromone, was distributed over an island infested with gypsy moths<sup>5</sup>. Mating of males with females was not prevented; the failure of this experiment was attributed to the presence of a "masking" substance in the synthetic pheromone<sup>6</sup>. We have for the first time obtained experimental confirmation that pre-mating communication between the sexes can be disrupted by permeating the atmosphere with an insect pheromone. This general phenomenon was demonstrated using *Trichoplusia ni* (Hübner), the cabbage looper, as the test organism.

The experiment was carried out in a 27 m<sup>2</sup> plot which was cross-hatched with 100 stakes set at 3 m intervals. Each stake was about 1 m above the ground. A stainless steel ringed planchet, 25 mm in diameter, was attached to the top of each stake. A cylindrical trap, 11 cm in diameter and 64 cm long, of a design suggested by Howland (personal communication) was placed at an elevation of 1 m in the centre of the plot. The trap was divided into three compartments of equal length. The centre compartment, separated from the end compartments by a copper screen, contained ten virgin *T. ni* females. The open end compartments were lined with a sticky material. The experiment was carried out during a period of six nights. At the start of the experiment 20 µl., about 17 mg, of synthetic *T. ni* pheromone<sup>7</sup> was added to each of the 100 planchets. Each planchet was covered with wire gauze to prevent stimulated males from falling into the phero-

none. At the start of the fourth night, an additional 10 µl. of pheromone was added to each planchet. An identical plot 600 m away with a female baited trap, but without synthetic pheromone, was used as a check on male *T. ni* activity. The locations of the pheromone-treated and the check plots were randomized every other night. The female traps in the check plots caught a total of 102 *T. ni* males whereas the female traps in the pheromone-treated plots caught no *T. ni* males.

The absence of *T. ni* males in the female traps in the treated plots is interpreted as indicating that the synthetic pheromone concentration in the air was sufficiently high to prevent *T. ni* males from orienting to the additional increment of pheromone released by the living females. The females probably were prepared for mating and released pheromone during the time interval between 10 p.m. and 5 a.m.<sup>8</sup>. The temperature range during that time for the experiment was 21° C to 9° C. At these temperatures the pheromone release rate ranges from 110–40 µg h<sup>-1</sup>/planchet with the lowest release rate for one night being 57–40 µg h<sup>-1</sup>/planchet (unpublished work of Gaston, Shorey and Saario). Experiments are in progress to establish the minimum pheromone concentration required to prevent male orientation to females.

The successful disruption of male orientation to females may be caused by sensory and (or) central nervous system adaptation to the pheromone. In addition, the relatively large concentration of pheromone near the individual planchets could have caused the males to orient there rather than to the females.

The result of this experiment indicates that economic control of an insect over large areas may be possible by behavioural control using sex pheromones. Large scale release of sex pheromones presents numerous potential problems: (a) cost of pheromone; (b) cost of distribution; (c) how to keep a sufficiently large concentration of pheromone in a large area, and (d) mammalian hazards. Since a large part of the cost of the behavioural control method may be in the distribution system, the pheromones of two or more different insect species can be distributed as economically as one.

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### New Phenolic Plant Growth-regulating Compounds

It has been reported that 2,6-dichlorophenol and the corresponding dibromo- and diiodo- compounds show appreciable activity in promoting cell elongation when examined in the pea segment and pea curvature tests, and that they induce severe epinastic responses when applied through the soil to tomato plants<sup>1</sup>. Further work has now revealed other 2,6-substituted phenols which are active in these tests. Thus, in the pea tests, 2-chloro-, 2-bromo- and 2-iodo-6-nitrophenols possess activities approaching that of 2,4-dichlorophenoxyacetic acid (2,4-D); the corresponding 6-cyanophenols are also active (Table 1). 2-

Methyl-6-nitrophenol is inactive in the pea tests over the concentration range investigated, but replacing the methyl by trifluoromethyl, a strongly electron-attracting group, confers appreciable activity (Table 1). 2,6-Dinitrophenol is very phytotoxic and was reported inactive in our earlier tests<sup>1</sup>. At very small concentrations, however, it does show activity in the two pea tests, although it is completely inactive in the wheat cylinder test. (For details of all bioassay methods used see Fawcett *et al.*<sup>2</sup>.)

From all our results, for a phenol to show appreciable growth-regulating activity in the pea tests, both positions in the ring *ortho* to the hydroxyl group must be occupied by electron-attracting groups, although it would appear that certain steric requirements must also be fulfilled, because when one or both of the *ortho* substituents in the ring is fluorine the compound is inactive (Table 1). While in some cases a *meta* position in addition to the two *ortho* positions may be substituted by halogen or nitro without complete loss of activity, results already reported and all those of our more recent work indicate that the *para* position must not be substituted for the compound to be active.

As already reported<sup>1</sup>, 2,6-dichloro-, dibromo- and diiodo-phenols, in addition to exhibiting activity in the pea and tomato tests, show slight activity in the wheat cylinder test. When one of the halogens is replaced by —NO<sub>2</sub> or —CN, however, the compounds are appreciably active in the pea tests, but are completely inactive in the wheat test. Epinastic responses are observed with the above-mentioned nitro compounds when applied in 1 per cent solution in lanolin to the stem of tomato plants and also when applied in aqueous solution to the soil. With the corresponding cyano compounds the epinastic responses in tomato plants are transitory and disappear within 48 h. Furthermore, no effects are observed when these cyano compounds are applied as aqueous solutions of their sodium salts to the soil of potted tomato plants at the rate of 2.5 mg/pot (6 in. diameter). Such observations indicate that the molecules are rendered inactive both in tomato plants and in the soil.

Metabolism experiments with 2-bromo-6-nitro- and 2,6-diiodo-phenols in wheat coleoptile and pea stem tissue have indicated that in the former tissue, hydroxylation of the *para* position occurs. Thus, for example, we have shown by chromatographic techniques that 2-bromo-6-nitrophenol is rapidly converted in wheat coleoptile tissue to 2-bromo-6-nitro-1,4-hydroquinone. This compound has been synthesized and as expected,

having a *para* position substituted, it has no growth-regulating activity. 2,6-Diiodophenol is similarly converted in this tissue to 2,6-diiodo-1,4-hydroquinone which again is completely inactive. We have found that such inactivation does not occur readily in pea tissue, thus accounting, at least in part, for the activity of these phenols in the pea tests.

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## SOIL SCIENCE

### Diffusion of Potassium from Mica-like Clay Minerals

DIFFUSION coefficients for the release of potassium from phlogophite and biotite in the range 1 to 50 × 10<sup>-10</sup> cm<sup>2</sup>/sec have been reported by Rausell-Colom, Sweatman, Wells and Norrish<sup>1</sup>. Similar measurements have not yet been obtained for muscovite or its counterpart in soils, the fine grained materials generally referred to as illite.

We have conducted a number of experiments on the rate of release of potassium from the fraction of Fithian illite (*API* 35) less than 2 micron, and an illite from Tumut, New South Wales, supplied by Dr. K. Norrish. The potassium contents of these micas are respectively 4.7 and 7.4 per cent.

Samples (0.75 g) were shaken in 200 ml. of 0.3 and 0.03 normal sodium chloride solution at 20°, 40° and 60° C. To keep the potassium concentration in the external solution to a minimum, 180 ml. of sodium chloride was replenished daily, and the solution was analysed for potassium using an 'SP 900' flame photometer. In Fig. 1, the amount of potassium released per day from the Fithian illite to the solution is given in relation to time; the potassium released at 20° C in 0.3 normal sodium chloride falls to about 5 µequiv./g/day within 4–6 days to give a solution concentration of approximately 2 × 10<sup>-5</sup> molar. Similar curves were obtained for the Tumut illite, but because the mean

Table 1. GROWTH-REGULATING ACTIVITY OF CERTAIN 2,6- AND 2,3,6-SUBSTITUTED PHENOLS IN THE PEA SEGMENT AND PEA CURVATURE TESTS

Compound	Pea segment test			Pea curvature test		
	10 <sup>-3</sup>	10 <sup>-4</sup>	Molar concentration	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
2,6-Difluorophenol	100	100	102	0	0	0
2,6-Dichlorophenol	101	102	113†	0	1	6
2,6-Dibromophenol	102	101	115†	0	2	6
2,6-Diiodophenol	101	118†	131†	0	6	6
2-Fluoro-6-iodophenol	100	100	99	0	0	0
2-Fluoro-6-nitrophenol	100	100	100	0	0	0
2-Chloro-6-nitrophenol	102	133†	124†	4	6	5T
2-Bromo-6-nitrophenol	108*	133†	119†	5	6	5T
2-Iodo-6-nitrophenol	100	128†	121†	4	6	3T
2-Methyl-6-nitrophenol	100	99	103	0	0	0
2-Trifluoromethyl-6-nitrophenol	101	115†	126†	2	6	6
2-Fluoro-6-cyanophenol	100	100	100	0	0	0
2-Chloro-6-cyanophenol	101	101	113†	0	1	6
2-Bromo-6-cyanophenol	100	101	123†	0	4	6
2-Iodo-6-cyanophenol	100	105*	121†	0	5	6
2,3,6-Tribromophenol	100	101	101	0	1	5T
2,6-Dichloro-3-nitrophenol	99	99	102	0	0	1
2,5-Dichloro-6-nitrophenol	100	101	110†	0	2	2T
2,5-Dichloro-6-cyanophenol	100	100	105*	0	0	1
2,4-D	119†	135†	131†	4	6	6

Results in the pea segment test are expressed as the percentage of the final length of the control segments.

Results in the pea curvature test are assessed on an arbitrary scale of 0 (inactive) to 6 (highly active). T signifies that toxic effects were observed. Figures significantly different from the control are indicated thus:

\* At 5 per cent level.

† At 1 per cent level.

‡ At 0.1 per cent level.

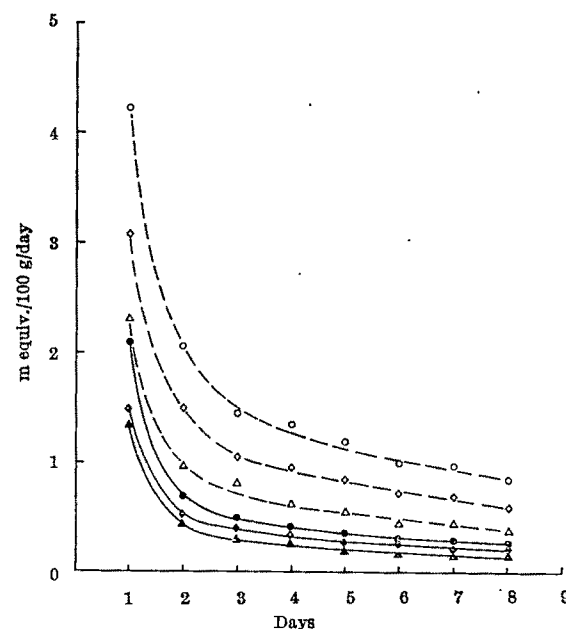


Fig. 1. Rate of potassium release from Fithian illite. ---, 0.3 normal sodium chloride; —◇—, 0.03 normal sodium chloride; —△—, 20° C; —◇—, 40° C; —○—, 60° C.

particle size was larger than the Fithian illite the actual amount of potassium released to the solution was correspondingly less.

Most measurements were carried out at the pH of the sodium chloride solution (pH 5.7). No observable changes in the rate of potassium release occurred in the pH range 4-8.

The experimental observations indicated that release of potassium was controlled by diffusion; thus to calculate diffusion coefficients, a model was adopted based on radial diffusion in a cylinder in which the concentration of potassium on the cylindrical surface is constant, and initially the concentration of potassium throughout the cylinder is uniform. It is assumed that the diffusion of potassium through the upper and lower faces of the cylinder (corresponding to external cleavage faces) is negligible. Following Crank<sup>3</sup>, if  $a$  is the radius of the cylinder,  $M_t$  is the quantity of diffusing substance which has left the cylinder in time  $t$  and  $M_\infty$  is the corresponding quantity after infinite time, then,

$$\frac{M_t}{M_\infty} = \frac{4}{\pi^{\frac{1}{2}}} \left( \frac{Dt}{a^2} \right)^{\frac{1}{2}} - \frac{Dt}{a^2} - \frac{1}{3\pi^{\frac{1}{2}}} \left( \frac{Dt}{a^2} \right)^{\frac{3}{2}} \dots \quad (1)$$

For the relatively short times in our experiments the third and subsequent terms may be ignored, and therefore

$$\frac{M_t}{M_\infty} = \frac{4}{\pi^{\frac{1}{2}}} \left( \frac{Dt}{a^2} \right)^{\frac{1}{2}} - \frac{Dt}{a^2}$$

or

$$\frac{1}{t} \left( \frac{M_t}{M_\infty} \right) = \frac{4}{\pi^{\frac{1}{2}}} \left( \frac{D}{a^2} \right)^{\frac{1}{2}} \frac{1}{t^{\frac{1}{2}}} - \frac{D}{a^2} \quad (2)$$

and thus a plot of

$$\left( \frac{M_t}{M_\infty} \right) / t \text{ versus } \frac{1}{t^{\frac{1}{2}}}$$

should give a straight line with a slope

$$\frac{4}{\pi^{\frac{1}{2}}} \left( \frac{D}{a^2} \right)^{\frac{1}{2}} \text{ and intercept } -\frac{D}{a^2}.$$

Consequently if  $a$  is known  $D$  may be calculated from both the slope and intercept (Fig. 2).

In practice the values obtained from the intercept may vary considerably for two reasons: the actual magnitude of the intercept is very small, and this gives rise to uncertainty from scale errors and because the diffusion equation is not strictly obeyed during the initial period of potassium release (up to an hour). The diffusion equation

is not strictly obeyed because of mass action exchange between sodium and potassium at sites on the external surface of the illite particles. The magnitude of this effect

may be estimated from the straight line plots of  $\frac{M_t}{M_\infty}$  versus  $t^{\frac{1}{2}}$ ; if the diffusion equation is obeyed from time zero, the line should pass through the origin. The actual intercept on the ordinate is a measure of the amount of potassium initially released from surface sites on the external surface of the crystal or frayed crystal edges. The final values for the diffusion coefficients have therefore been calculated from the slopes (Fig. 2) after allowance for this effect.

The values for mean particle radius ( $a$ ) were calculated from nitrogen surface area measurements on the assumption that there was a 10:1 ratio between particle diameter and thickness<sup>2</sup>. This assumption was confirmed by observation of shadowed particles in the electron microscope. For the < 2 $\mu$  fraction of Fithian and Tumut illite the mean particle radii were 500 and 1,800 Å respectively.

Fig. 2 shows the potassium release data in Fig. 1 plotted according to equation (2). Each curve results from a single experiment.

The mean diffusion coefficients at 60° C calculated from a number of experiments are  $7.7 \pm 2.8 \times 10^{-20}$  and  $1.1 \pm 0.5 \times 10^{-20}$  cm<sup>2</sup>/sec for 0.3 and 0.03 normal sodium chloride respectively. (Errors quoted are at the 95 per cent confidence level.)

Under similar conditions the diffusion coefficients for the Tumut illite were the same within experimental error for the 0.3 normal solution and were a factor of two higher for the 0.03 normal solution.

These values of the diffusion coefficients for potassium release from illites are therefore comparable with those for the diffusion of hydrogen in nickel and iron hydroxides<sup>4</sup> and are about ten orders of magnitude lower than for potassium release from biotite.

This difference may be explained from the observation of Bassett<sup>5</sup> that the dipole moments of the hydroxyl ions in trioctahedral micas are perpendicular to the cleavage, whereas those in muscovite, a dioctahedral mica, are obliquely oriented, and as a result the proton is further from the ion and places the negative or oxygen end of the dipole closer to the potassium with a resulting increase in binding energy.

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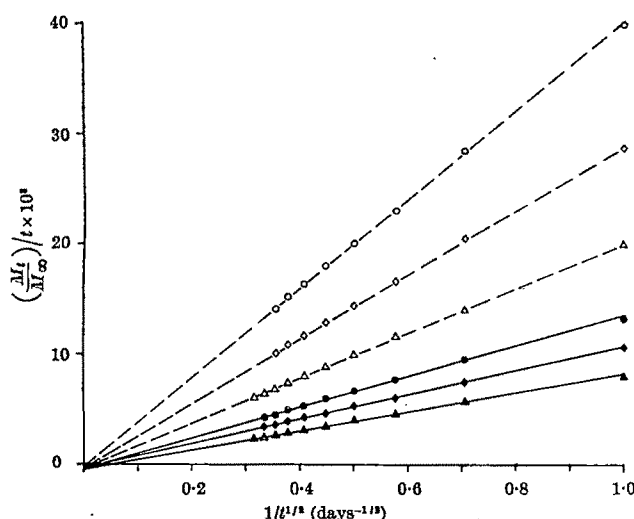


Fig. 2. Potassium release from Fithian illite. ---, 0.3 normal sodium chloride; —, 0.03 normal sodium chloride; ○●, 60° C; ◇◆, 40° C; ▲▲, 20° C.

## Effects of Pulverized Fuel Ash on the Moisture Characteristics of Soils

THE available water capacity (AWC) of a soil has been shown to be positively correlated with the percentage of fine sand (0.2-0.02 mm) and organic carbon present, and negatively correlated with the percentage of coarse sand (2.0-0.2 mm)<sup>1</sup>. Pulverized fuel ash, produced from the combustion of finely ground coal, is a dust containing between 50 and 70 per cent of "fine sand" size particles<sup>2</sup>. It occurred to us that pulverized fuel ash could be applied to increase the AWC of coarse textured soils. To test this hypothesis a field experiment was carried out on a coarse sandy loam of the Newport Series. Rates of ash, equiva-

lent to 0, 50, 100 and 200 tons/acre, and containing 65 per cent of particles of size 0.2–0.02 mm, were incorporated into the top 12 in. of soil in small plots by putting soil and ash through a concrete mixer. There were five replications of each treatment. A satisfactory crop of red beet was grown on all plots, but there was a reduction of 9 per cent in the yield of roots with the highest rate of ash applied, which was probably caused by the presence of toxic quantities of boron. After harvest the various moisture characteristics of the soil were determined<sup>3</sup>: the upper limit of available water by the gravimetric method on samples taken 48 h after rewetting and the lower limit by the 15 atm. percentage method.

The largest addition of ash significantly increased the AWC and there were small but non-significant increases with the lower rates of application (Table 1). This effect resulted from the progressive increase in the moisture retained at the upper limit of available water with increasing quantities of ash; there was little effect on the 15 atm. percentage.

To assess the effect of ash on other soils as quickly and simply as possible, experiments were carried out in the laboratory. First, to determine whether an effect comparable with that obtained in the field could be obtained under laboratory conditions, a quantity of the soil from the site of the field experiment was air-dried. Ash was mixed with the soil at rates equivalent to 0, 50, 100, 150, 200 and 300 tons/acre in the top 12 in. of soil. After thorough wetting, triplicate samples of each mixture were subjected to a suction of 0.05 atm. to determine the upper limit of available water<sup>3</sup>; the bulk density was obtained from the same samples and the 15 atm. percentage determined from a further three samples.

The suction of 0.05 atm. gave lower values for the upper limit of available water than the gravimetric method under the conditions of this experiment and for this reason the measured AWC of the untreated soil under field conditions was 1.29 in. (Table 1) but only 1.11 in. in the laboratory study (Table 2). Differences of a similar order of magnitude were obtained with comparable ash treatments. Nevertheless, the percentage increase in AWC resulting from the addition of ash in the laboratory was greater than those obtained in the field and possibly resulted from the better mixing of ash and soil that could be achieved in the laboratory. Despite differences in magnitude, the results from the two types of experiment were similar and therefore the effect of ash on four other coarse textured soils was assessed using the same laboratory techniques; the results are given in Table 2.

Without exception the AWC of all soils increased progressively with increasing quantities of ash, the relationship between AWC and the rate of ash applied being

Table 1. EFFECT OF PULVERIZED FUEL ASH ON THE MOISTURE CHARACTERISTICS OF THE TOP 12 IN. OF A COARSE SANDY LOAM

Rate of ash (tons/acre)	0	50	100	200	Standard error (12 d.f.)
Upper limit (% water, w/w)	14.8	15.0	15.3	17.0	±0.40
Lower limit (% water, w/w)	6.9	7.2	7.1	6.9	±0.25
Bulk density (g/c.c.)	1.40	1.40	1.41	1.41	—
AWC (in./ft.)	1.29	1.31	1.39	1.71	±0.096
% increase	—	1	7	32	—

Table 2. AVAILABLE WATER CAPACITY (IN./FT.) (Percentage increase over control in parentheses)

Soil texture <sup>4</sup> and series	0	50	100	150	200	300
Coarse sand	0.70	0.78	0.93	1.08	1.32	1.62
Bridgnorth	(11)	(11)	(33)	(54)	(89)	(131)
Loamy sand	0.79	1.02	1.07	1.34	1.39	1.51
Bridgnorth	(29)	(29)	(34)	(70)	(76)	(91)
Sand	0.98	1.19	1.56	1.60	1.68	1.97
unknown	(21)	(21)	(59)	(63)	(71)	(101)
Loamy coarse sand	1.06	1.25	1.40	1.49	1.55	1.72
Newport	(18)	(18)	(32)	(41)	(46)	(62)
Coarse sandy loam	1.11	1.17	1.24	1.42	1.67	1.77
Newport	(5)	(5)	(12)	(28)	(50)	(59)

Standard error of differences between treatments within a soil = ±0.122 (20 d.f.)

approximately linear for three soils and possibly curvilinear for two soils. The largest increase in AWC occurred when ash was added to the coarsest textured soils (those having only small percentages of fine sand present in their natural state) and smaller, though still significant, increases occurred on the slightly heavier soils.

Further work is proceeding on other soils under field conditions to study the relationships between rate of ash application, increase in AWC and the particle-size composition of different soils and to determine whether better growth of crops and higher yields are obtained from soils with AWCs increased in this way.

Although much work has been carried out on the phytotoxicity associated with the high boron contents of some ashes and on the nutrient status of pulverized fuel ash in relation to crop growth, particularly where ash has been used as a filling material in land reclamation<sup>2</sup>, its use in comparatively small quantities to alter the moisture retaining properties of soil does not appear to have been considered. Provided any problem of phytotoxicity, particularly from excess boron, can be overcome and if the beneficial effects of pulverized fuel ash on AWC are confirmed and result in increased crop yields, then pulverized fuel ash could have an important use as a soil improver. For example, its application on the cultivated sand lands in areas of low rainfall in Britain<sup>5</sup> may reduce the need to irrigate crop plants. Further, with the aforementioned proviso, it may possibly be used to improve the productivity of the marginal sand lands, that is, soils characterized by poor water retention and high acidity which make up more than 800,000 acres of England and Wales<sup>6</sup>; the addition of highly alkaline pulverized fuel ash<sup>2</sup> may simultaneously increase the water retention of these soils and reduce the high acidity.

We thank Dr. E. W. F. Gillham of the Central Electricity Generating Board for kindly providing the pulverized fuel ash and for the data on the particle-size composition of the ash.

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## APPLIED SCIENCE

### Tensile Strength of Granular Materials

Smalley and Smalley<sup>1</sup> have commented on a theoretical expression for the tensile strength of an ideal granular material (in the pendular stage of moisture distribution), due to Rumpf<sup>2</sup>, and have proposed an alternative approach. Their final equation 10a, namely,

$$\sigma \approx 4.6 \frac{Bt}{D^3}$$

(where  $B$  is the interparticle force per contact,  $D$  is the diameter of the particles, and  $t$  is the thickness of the fracture zone) includes in the constant term the value of the co-ordination number,  $k$ . The authors quote a value  $k = 8.5$ , taken from data published by Bernal and Mason<sup>3</sup>; my re-examination of these data (given in Table 1, ref. 3) indicates that the value should be  $k = 8.0$ , which makes a proportionate change in the constant of equation 10a. While equation 10a is dimensionally consistent, the authors' intuitive reasoning in their final paragraph<sup>1</sup> would seem to require that  $\sigma$  be inversely proportional to  $D^2$  and not  $D^3$ , as the number of bonds in a section of constant size is inversely proportional to particle cross-section, and not volume; in this respect, Rumpf's

equation  $\sigma \approx 2B/D^2$  is more plausible, though of rather academic interest. Rumpf<sup>2</sup> measured the tensile strengths of limestone pellets, and found that as moisture content decreased, so also did the strength, contrary to his theoretical expectations for the pendular state. I have determined the tensile strengths of various size fractions of a fine coal, using a split cylinder method. Fig. 1 illustrates some typical results, which show a trend similar to that found by Rumpf for limestone.

Quite recently, Tanaka *et al.*<sup>4</sup> have published a theory, together with some results of measurements made on a fly ash; the measured strengths tend to zero as moisture contents become very small and the authors remark that this is consistent with the theory they propound. I submit that the qualitative agreement cited by these authors is fortuitous, because their theory is crucially dependent on the fallacious premise that at low water contents capillary pressure can be ignored when calculating the cohesive force between two spheres having a lens of water at their point of contact. Fisher<sup>5</sup> was the first to deduce theoretically that both the tension in the air-water interface and the pressure deficiency must be considered, and that as the water lens diminishes the cohesive force between two spheres increases to the limiting value  $B = \pi DT$  ( $T$  = surface tension), just before dryness (when molecular dimensions are reached and elementary theory becomes inapplicable). Cross and Picknett<sup>6</sup> have convincingly confirmed this experimentally. Despite the seeming contradiction, it can be concluded that the tensile strength characteristics reported here, and in refs. 2 and 4, are typical of real granular materials.

Consider first a pendular distribution of moisture achieved by removing liquid from a rigid assembly of particles, initially in the saturated condition. It is to be expected that once the pendular stage is reached, decreasing the moisture content results in the gaps between neighbours not quite in contact progressively (according to their size) ceasing to be bridged by liquid; while the strengths of the remaining bridges presumably approach the theoretical maximum value ( $\pi DT$  for spheres), the number of "dry joints" increases and could result in a net

decrease in the tensile strength of the assembly. If the spatial distribution of near neighbours is known<sup>7</sup>, then the "strength versus liquid content" characteristic for the case of spherical particles should be amenable to computation.

It seems necessary, however, to differentiate between the foregoing "static" method of forming the moist agglomerate, and the more usual "dynamic" processes, in which granules and moisture are mixed together and then consolidated (as in the tests yielding the data given in Fig. 1). Consider now the hypothetical case of such a consolidation process resulting in an assembly of particles identical with that obtained for the static method: then at identical moisture contents in the pendular state, the occurrence of "dry joints" is more likely in the "dynamic" than in the "static" process, because particles, or rather the liquid films surrounding them, must now make direct contact before a liquid bridge can be established. The formation of a bridge decreases the film thicknesses on the two particles, and to some extent reduces the number of subsequent bridges formed. Thus the dynamic assembly should have lower tensile strength than the static assembly, and this should decrease as moisture content diminishes. If the particles are not perfectly wetted, even direct contact is not a guarantee of bridge formation, and when bridges do form liquid may be completely withdrawn from other sites on the surfaces of the particles, so that subsequent encounters during the compaction process are even less likely to result in bridges.

The final number of bridges, in a given cross-section, for the dynamic case of perfectly wetted spheres may be soluble using an incremental mathematical technique and given the end point spatial distribution and the amount of liquid present; for imperfectly wetted spheres some assumptions would have to be made concerning the liquid distribution at the start of compaction. It is unlikely that the case of imperfectly wetted, irregularly shaped particles could be better than very approximately treated.

I thank Dr. Cutress for helpful comments.

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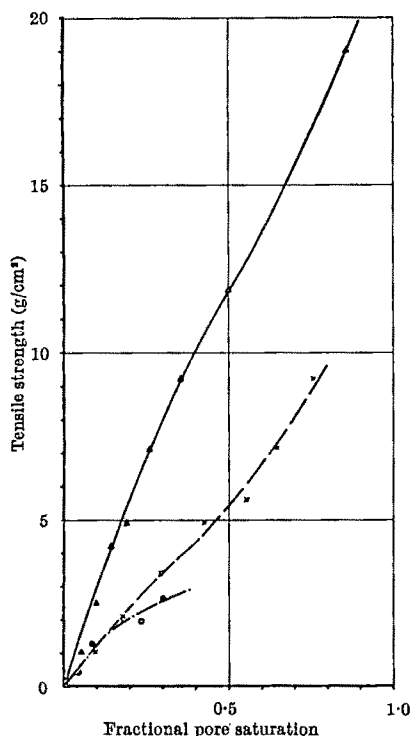


Fig. 1. Tensile strength of Aberpergwm coal fractions (▲) 0-64μ, (×) 124-211μ, (●) 422-788μ. Nominal mesh sizes. Porosity 0.50 approximately.

## GENERAL

### Normal or Log-normal: Appropriate Distributions

I WISH to point out that for the statistical analysis of certain types of data the assumption that the data are drawn from a normal population is usually wrong, and that the alternative assumption of a log-normal distribution is better. This alternative is widely used by statisticians, economists and physicists, but for some reason is often ignored by scientists of some other disciplines.

The normal distribution is unlikely to hold for the class of data in which the possible range of values is closed at the lower end, for example by zero or the concentration required to maintain life, but is effectively open at the higher end, and the empirical mean lies near the lower limit. One expects in such cases an asymmetric distribution with a "tail" of high values. Concentrations of many materials in body fluids are in this class. Particular examples are glutamate-oxaloacetate transaminase<sup>1</sup> and



free fatty acids<sup>2</sup> in rat plasma, and dieldrin in human blood and fat<sup>3</sup>. The normal distribution is plainly not applicable. Not only is the observed distribution asymmetric, but in two cases<sup>1,2</sup> the standard deviations are such that in large samples more than 10 per cent of the values would be expected to be negative, which is, of course, impossible.

These examples have been tested for log-normality. For two<sup>1,2</sup> the log-normal distribution holds. For the third<sup>3</sup> it is much better, but some tailing remains. Several other less conclusive cases have come to my attention (for example, isopropoxyphenol equivalents in human urine<sup>4</sup>), and in none is the log-normal distribution worse than the normal.

I suggest that when an *a priori* decision about distribution has to be made the log-normal distribution should always be preferred to the normal distribution for data of this general type. (The choice is trivial when the standard deviation is less than 15 per cent of the mean value, because the results of tests are very nearly the same.)

For calculations the logarithms of the data are treated in exactly the same way as the original data when a normal distribution is assumed. The mean obtained, the antilog (mean log), is the geometric mean. Standard deviations may either be expressed as ranges, for example, geometric mean = 2, standard deviation range = 1-4; or all the results may be given in logarithms, in this example: mean log ( $\pm S.D.$ ) =  $0.30 \pm 0.30$ . A *t*-test gives the significance of the difference between logarithms, and thus the significance of the ratio of the values corresponding to the logarithms.

It should be emphasized that for data the range of which is open at one end it is better when in doubt to use logarithms; but when the range is closely restricted at both ends, as, for example, in the case of percentages, the normal distribution is likely to be better, and any other distribution including the log-normal should only be used when there is statistically significant evidence from the data in its favour.

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Received November 23, 1966; revised January 4, 1967.

<sup>1</sup> McLean, A. E. M., and McLean, E. K., *Biochem. J.*, **100**, 564 (1966).

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## Random Generation of Optimal Codes

THE problem dealt with in this communication is that of generating an optimal code for the transmission of a long sequence of binary digits over a noisy channel. The channel is supposed to produce errors at random,  $p$  ( $< \frac{1}{2}$ ) being the probability that a particular digit is wrong, and  $q$  ( $= 1 - p$ ) being the probability that it is right. The capacity of such a channel is

$$(p \log p + q \log q) - \left(\frac{1}{2} \log \frac{1}{2} + \frac{1}{2} \log \frac{1}{2}\right) = 1 - X \text{ bits per digit,}$$

where  $X = -(p \log p + q \log q)$  lies between 0 and 1, and all logarithms are referred to the base 2. Consequently, if it is desired to transmit  $B$  bits of information reliably, a total of  $N$  digits must be sent, where  $N > B/(1 - X)$ . One way of doing this is to begin by sending  $B$  message digits, and to follow them with  $C$  checking digits, where  $C = N(X + \delta)$  and  $\delta$  is a positive finite number. Then  $B/N = 1 - C/N = 1 - X - \delta < 1 - X$  as required. The checking digits are made to depend on the message digits so as to enable the recipient, in spite of the transmission errors, to reconstruct the complete set of  $N$  digits with virtual certainty. The question is: can this be done with a number of checking digits which is

close to the theoretical minimum? In other words, is it possible to specify an actual coding procedure such that when  $N$  is sufficiently large  $\delta$  can be made arbitrarily small? It is possible, and I now describe such a procedure.

Set  $C$  equal to  $N(X + \delta)$ , and proceed to treat  $\delta$  as a small positive quantity. Now set up a matrix  $M$  with  $B$  rows, corresponding to the  $B$  message digits, and  $C$  columns, corresponding to the  $C$  checking digits. Each element of the matrix is assigned the value 0 or 1 according to the toss of a coin, so that each row may be read as a random binary number in the range 0 to  $2^C - 1$ . The sender uses the matrix  $M$  for assigning values to the  $C$  checking digits. If  $d_b$  is the  $b$ th message digit, then the  $c$ th checking digit is assigned the value 0 if  $\sum_b d_b M_{bc}$  is even and the value 1 if it is odd. The complete sequence of  $N$  digits is then transmitted.

When the message arrives the recipient tests the  $C$  checking digits against the others to see if they satisfy the appropriate parity relations. If the message is long (as we are assuming), about half these relations will be violated. A possible interpretation of the observed disparities is that all the errors of transmission have occurred in the checking digits; but this interpretation is highly implausible, because the total number of errors in the message will almost always be close to  $pN$ , which is substantially less than  $\frac{1}{2}C$ , by virtue of the fact that when  $p < \frac{1}{2}$ ,  $p < -\frac{1}{2}(p \log p + q \log q)$ . The recipient's best policy is to search among the  $N$  digits for a set of about  $pN$ , such that if they were all wrong, and the others all right, the resulting disparities would be precisely those observed. The search will not, however, be worth undertaking at all unless the recipient can be assured that if he finds an interpretation of the observed disparities which implicates about  $pN$  digits, this interpretation is indeed correct. Such an assurance emerges from the following argument.

Suppose that the recipient's search has been rewarded by the discovery of a certain set of digits, numbering about  $pN$ , such that if these digits, and no others, were altered, the disparities would all disappear. The prior probability of these particular digits being wrong, and all the others right, is  $p^{pN}q^{qN}$ . The prior probability of the sequence having been corrupted in any other manner is therefore  $1 - p^{pN}q^{qN}$ . But because  $M$  is a random matrix the probability of any other corruption resulting in the observed disparities is  $2^{-C}$ . Hence the odds on the recipient's provisional interpretation being right are

$$p^{pN}q^{qN} : 2^{-C} (1 - p^{pN}q^{qN}),$$

which for large  $N$  becomes  $2^C p^{pN}q^{qN} : 1$ . But by construction

$$C = N(\delta + X) = N(\delta - p \log p - q \log q).$$

Therefore as  $N$  tends to infinity the odds in favour of the provisional interpretation, namely,  $2^{N\delta} : 1$ , become infinite. Hence there is no need for the recipient to continue the search.

It is always possible, of course, that the number of erroneous digits in the sequence is substantially greater than  $pN$ , or that there are two or more alternative interpretations of an observed set of disparities, both interpretations implicating about  $pN$  digits. These possibilities, however, become more and more improbable as  $N$  increases, so that the random matrix code here described is indeed optimal in the strict sense. As an engineering proposition it suffers from the grave disadvantage that the recipient is given no guidance as to how to conduct his search for the offending digits; but with sufficient patience he could in principle locate them by the exhaustive enumeration of all possible sets containing about  $pN$  members.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, March 20

INSTITUTE OF ELECTRICAL ENGINEERS (joint meeting with the Royal Society and the I.E.R.E. Computer Group, at Savoy Place, London, W.C.2), at 2.30 p.m.—Colloquium on "Automated Cartography: Scientific Needs and Engineering Possibilities".

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Prof. P. G. Moore: "Operational Research in Business".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5 p.m.—Dr. P. S. Hewlett: "Synergism and Potentiation in Insecticides".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Dr. Sydney Smith: "Charles Darwin's 'Species' Work 1837-1842".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Rendez-vous du Diable" (colour film).

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. F. Bennett: "Monomer Approach to Flame Retardancy".

## Monday, March 20—Tuesday, March 21

GRIMSBY COLLEGE OF TECHNOLOGY (at Nuns Corner, Grimsby)—Symposium on "Modern Developments in Food Preservation".

## Monday, March 20—Wednesday, March 22

BIOCHEMICAL SOCIETY (at Trinity College, Dublin)—469th Meeting. Included in the programme will be an Ordinary Meeting and a Discussion Meeting on "Metabolic Role of Vitamin A".

## Tuesday, March 21

UNIVERSITY OF SURREY (at the University of Surrey Annexe, 14-16 Falcon Road, Battersea, London, S.W.11), at 5.30 p.m.—Prof. E. Gutmann (Czechoslovak Academy of Science): "Problems of the Specificity of the Neuromuscular System".

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion Meeting on "Rapid Fault Diagnosis of the Future", opened by Commander L. E. Land, R.N., and Mr. A. J. Cope.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the HEAVY ORGANIC CHEMICALS GROUP, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. M. M. Baizer (U.S.A.): "Electrolytic Reductive Coupling of Acrylonitrile".

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (in the Lecture Theatre "C", Chemistry Department, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Dr. G. Nickless: "Principles and Practices of Mössbauer Spectroscopy"; Dr. T. C. Gibb: "Mössbauer Spectra of Elements other than Iron and Tin".

## Tuesday, March 21—Thursday, March 23

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Weir Hall, 10 Upper Belgrave Street, London, S.W.1)—Spring Meeting.

## Wednesday, March 22

CHEMICAL SOCIETY (at the University of Keele, Keele, Staffordshire), at 10.30 a.m.—Symposium on "Heterocyclic Chemistry".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Dr. I. J. Good: "A Bayesian Significance Test for Multinomial Distributions".

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 6.30 p.m.—Dr. R. Spence and Mr. P. J. Baxandall: "The Theory of Oscillators".

INSTITUTE OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. A. M. Angelini: "The Development of Electricity Supply in Italy".

UNIVERSITY OF LONDON (at the Institute for Child Health, Gullford Street, London, W.C.1), at 5.30 p.m.—Mr. R. G. Burwell: "The Scientific Basis of Bone Homotransplantation".

INSTITUTE OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-Acoustics GROUP (at 8-9 Bedford Square, London, W.C.1), at 6 p.m.—Mr. K. O. Bäder: "Dynamic Properties of Audio Frequency Compressors".

INSTITUTE OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (joint meeting with the GRADUATE AND STUDENT SECTIONS OF THE INSTITUTIONS OF CIVIL AND OF MECHANICAL ENGINEERS, at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 6.15 p.m.—Mr. Ove Arup, C.B.E.: "Design".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—FOOD ENGINEERING PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting and Chairman's Address.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

BIOLOGIST, SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER grade in the SYSTEMS SYNTHESIS SECTION of the DEPARTMENT of ECOLOGY, to work on theoretical aspects of plant and crop growth—The Secretary, Grassland Research Institute, Hurley, Maidenhead, Berkshire (March 24).

SENIOR LECTURER (preferably medically qualified and an interest in human physiology) in the DEPARTMENT of PHYSIOLOGY—The Secretary, The University, Aberdeen (March 25).

BIOCHEMIST (with at least D.Sc. or Ph.D. in biochemistry) at the National Nutrition Research Institute, South African Council for Scientific and Industrial Research, Pretoria, for duties which will involve studies of the biochemical and metabolic effects of various toxins (mainly mycotoxins) on the tissues of experimental animals and of man—The South African Science Office, Chichester House, 278 High Holborn, London, W.C.1 (March 28).

LECTURER (preferably with experience in X-ray or optical diffraction, thermionic emission, luminescence or acoustics) in the DEPARTMENT of PHYSICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (March 29).

LECTURER or ASSISTANT LECTURER in STATISTICS—The Secretary, The Queen's University, Belfast, Northern Ireland (March 31).

LECTURER (with special research interests in genetics) in the DEPARTMENT of BOTANY—The Deputy Secretary, The University, Southampton (March 31).

RESEARCH ASSISTANT (honours graduate in agriculture, agricultural science or geography) in the DEPARTMENT of AGRICULTURE for work in connexion with the effects of shelter on physical environment, animal performance and behaviour—The Professor of Agriculture, University College of North Wales, Bangor, North Wales (March 31).

SOIL SURVEY OFFICER (male, with a first- or upper second-class honours degree in science) for the SOIL SURVEY OF SCOTLAND—The Secretary, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (March 31).

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LECTURER (honours graduate with research or industrial experience) in BREWING SCIENCE in the DEPARTMENT of BREWING and BIOCHEMISTRY—The Secretary, Heriot-Watt University, Edinburgh (April 1).

UNIVERSITY LECTURER in HISTORY OF SCIENCE or PHILOSOPHY OF SCIENCE—Dr. M. A. Hoskin, Whipple Museum, University of Cambridge, Free School Lane, Cambridge (April 1).

HYDROBIOLOGIST (with a good degree in biology (preferably zoology) and research experience in hydrobiology) at the University of Malawi to conduct research on planktonic and benthic organisms in Lake Chilwa—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 3).

CHAIR OF PATHOLOGY at the Western Infirmary—The Secretary to the University Court, The University, Glasgow (April 5).

UNIVERSITY LECTURER and a UNIVERSITY ASSISTANT LECTURER in PURE MATHEMATICS—The Appointments Committee, Faculty of Mathematics, University of Cambridge, Silver Street, Cambridge (April 5).

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LECTURER in GENETICS (any field) at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 1).

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Growing Points in Physics. (A booklet for use with two related series first broadcast in Study Session, Third Network, January-March 1967.) Edited by Rosemary Jellis. Pp. 27. (London: British Broadcasting Corporation, 1966.) 3s. [1612]

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Roads in England: Report by the Minister of Transport for the year ended 31st March, 1966. Pp. v+110+16 plates. (London: H.M. Stationery Office, 1966.) 10s. 6d. net. [1612]

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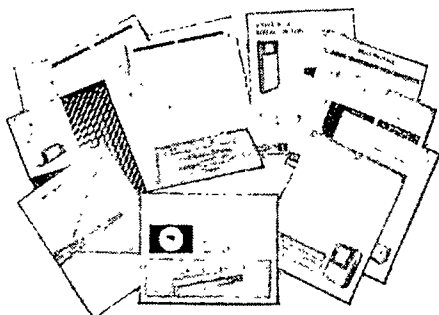
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##### QUALIFICATIONS:

S.O. 1st or 2nd class hon. degree or Dip. Tech. or equivalent or higher qualifications in appropriate subject. Age under 29.

S.S.O. As above plus at least 3 years' post-graduate research. Age normally 26-31.

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(1242)



## MORE PLACES TO FILL

NOBODY should be surprised that British universities have once more failed to fill all their vacant places in science and applied science (see page 1165). It has been clear for the best part of five years that the rapid expansion of departments of science and technology in the late fifties would be difficult to sustain indefinitely. Certainly nothing has happened in the last year or so to halt the slow drift away from science, or even to suggest what the root causes may be. The interim report of the Dainton sub-committee of the Council for Scientific Policy, now nearly a year old, served chiefly as another reminder that the problem remains unsolved. In the circumstances it is important to recognize that things could well be worse, but that the vacancies are not a problem in their own right but, rather, consequences of deep-seated tensions in the British educational system. Obviously there can be no quick solution.

The supposed drift away from the sciences is, in the first place, strictly relative. Admissions of potential scientists to British universities increased by 40 per cent in the five years from 1962. In the same period, admissions to courses in technology increased by 28 per cent. In strictly numerical terms, the universities should therefore be turning out steadily increasing numbers of graduates in science and technology in the years ahead. But the output of graduates in other faculties is likely to increase still faster. In the past five years the overall rate of admissions to universities has increased by 54 per cent, and has thus more than kept pace with the projections of the Robbins Commission. At the same time, however, admissions to courses in the social sciences have increased by nearly 120 per cent. This remarkable growth of interest by undergraduates in the social sciences is numerically just enough to explain why admissions in science and technology declined from 46 per cent of the total in 1962 to 40.6 per cent in 1966. It is also consistent with the recent popularity among senior students at schools of subjects such as economics. Another sign of the same tendency is the growing entry to the school-leaving examinations at advanced level of students with patterns of study which bridge whatever gap there may be between science and the humanities. The last report of the Universities Central Council on Admissions suggests that the numbers of sixth-formers following courses like these increased by 89 per cent between 1962 and 1966—far more quickly than the school population as a whole. Although there is nothing which can be called a stampede away from science and technology, the signs of disaffection are so consistent that there is obviously a real phenomenon to be dealt with.

What, then, should be done? The first thing to be said is that it would be inconsistent for a nation which spends a good deal of its time worrying about the loss of scientists and engineers by emigration, and about the tendency of skilled people to stay in academic life rather than to find their way into industry, unthinkingly to lament what seems to be a failure of school-leavers to become scientists. And it is entirely possible that the reasons why trained scientists tend to emigrate or to stay in universities, whatever they may be, may be linked in some way with a tendency for school-leavers to fight shy of science courses. The three phenomena would certainly be expected to follow naturally from a state of affairs in which there was a surplus of scientists, defined in the strictly economic sense that the demand is less than the supply. It is true, of course, that there are good reasons for believing that British industry would profit enormously from a much fuller use of scientific people, but that is only another way of saying that the demand for scientists is now lower than it should be. In other words, there is good reason to expect that a great many problems would be simplified if there were a general improvement of the conditions under which scientists and technologists are expected to work, particularly in industry. Nobody should be surprised if young people leaving school are hard-headed enough to know on which side their bread is buttered.

In the competition for school-leavers, numbers are not everything. Quality also matters, and there are signs that science departments at British universities are less able to recruit bright undergraduates. Certainly the UCCA report shows that science departments were having to accept as entrants students whose attainment, on paper at least, is hardly spectacular. In the humanities, on the other hand, students are being turned away with qualifications which indicate considerable ability. This feature of the balance between university entrants in various disciplines is in part, of course, a consequence of the numerical shortage of suitable entrants, but it is also clear that the science departments would do better if the curriculum were more flexible—and more interesting—at school and at university. The way in which sixth-formers are required to specialize in one set of subjects or another is absurd and damaging as well. To the extent that university departments insist that studies at school should be a narrow preparation for what happens at university, they are slowly cutting their own throats. The tendency for students at British schools to follow courses with one foot in science and one in the humanities is not an accident but a rebellion. In the long run, the universities would benefit if there were a more

liberal education in the sixth form. But university curricula are also much in need of being made more liberal. Courses which combine studies such as engineering and economics should be much more common than they are, for example. And the style of teaching in many departments could well be made more stimulating (and more educative) by borrowing from the schools some of the innovations which have recently transformed the teaching of science. In other words, although the most important reasons for the drift from science are probably beyond the reach of the universities, they could do a lot to help themselves.

## LABORATORY PEOPLE

THE White House panel on the conflict between personal privacy and the needs of scientific research has started some interesting hares (see page 1165). Although most of its recommendations are at once moderate and sensible, the issues of principle involved are bound to be contentious where they are taken seriously. Historically, the panel has its roots in exercises such as those in which groups of people were locked in air-raid shelters for days on end, and in which their conversation was recorded, allegedly unknowingly. The chances are that tactless investigations like those, whose claim to the title "experiment" is dubious to say the best of it, will not lightly be undertaken in future, but the panel is right to insist that the question remains of what rules should govern the relationship between biologists and the public. In one sense, of course, the question is as old as the hills, for there have always been doctors and doctors have always been doing things for the first time. But the arrival on the scene of great numbers of experimental biologists, with no written code of ethics nor great body of precedent as a guide, is a real complication.

The most obvious difficulty is that there can be no simple code of ethics. In the treatment of laboratory people, as of laboratory animals, the only general rule is that experiments should be designed so as to minimize pain, intrusion and other offences against the animal—human or otherwise. When unpleasantness seems unavoidable, an honest attempt must be made to balance this against such benefit as there may be. Often it will seem best not to carry out the experiment. But things will not always turn out as easily as this. Where people are concerned, the fact that they can be asked for their consent is a great help, but the committee is right to ask that they should be given a full understanding of what is involved. Whether the committee is right to ask grant-giving bodies to censor proposals is another matter. Institutions—and journals—are much more able to act objectively.

## ENERGY FROM WHERE?

THE British government is about to make a muddle of its fuel policy. Ever since natural gas was discovered

in the North Sea more than a year ago, the Ministry of Power has found it hard to suppress its excitement at the consequences. Naturally enough, the ministry is aware of how natural gas has transformed the economy of the Netherlands in the last two decades, and of how liberal supplies of it are an essential part of the economy of the United States. Indeed, it has probably not forgotten that the discovery of coal in Britain, and the development of techniques for mining coal, was the foundation for the great upsurge in British productivity nearly two centuries ago. It is understandable, then, that the steady pace of the discovery of gas wells in the North Sea has occasionally gone to the ministry's head. The trouble, unfortunately, is that the discoveries of gas coincided with the point at which nuclear power stations have become decisively cheaper than conventional power stations. If it were not for the gas, the ministry would no doubt be cock-a-hoop about nuclear power instead. But as recently as two years ago the British government also committed itself to the coal industry by letting Lord Robens feel that a reduction of coal production to about 170 million tons a year would be about the limit of the sacrifice expected of him in the interests of cheaper energy production. In the circumstances, it is no wonder that the Ministry of Power is torn between different and alternative sources of energy. Things have changed a lot since the fifties, when one report after another foretold great scarcity. What is the government to do?

In the first place, on gas, it would be a great benefit if the minister could restrain his enthusiasm and also the Gas Council. The production of the North Sea gas field may well turn out to be as great as the optimists forecast, but it does not follow that it is most economical to exploit it quickly. Indeed, the rapid conversion of existing plant from coal gas to natural gas upon which the Gas Council has embarked may in the long run turn out to be exceedingly wasteful of resources. To begin with, at least, it would probably have been more economic to use natural gas for providing big industrial consumers with gas supplies. Even power stations might have come high on the list of potentially good customers, even though the Gas Council has set its face against such a development. As things are, and whatever the outcome of the negotiations on the price for North Sea gas, the gas will be used chiefly in supplying customers for whom distribution costs are in any case much higher than the cost of gas, so that the economic benefit will be marginal—and imperceptible.

On nuclear power, the government's policy is most of all deficient in its passivity—a strange contrast with the involvement of the fifties, when the utility undertakings were overridden by the government and were required to build nuclear capacity that they did not want. Now the government appears to be letting events take their course, which means in practice that the comparatively high initial cost of nuclear power stations is tending to reduce the pace at which nuclear power is exploited. The most urgent need may now be to persuade the minister to transfer a part of his enthusiasm for gas to nuclear power.

## NEWS AND VIEWS

### Swings and Roundabouts

THE swing away from science gets another push in the fourth report of the Universities' Central Council on Admissions (UCCA, 29 Tavistock Square, London, W.C.1, 6s.). The end of the bulge in post-war births seems to have been a sharper shock in science and technology than in other disciplines. Last year there were more places at British universities for undergraduate scientists than there were applicants qualified to take them up; it has become significantly harder to get a place in the humanities than in the sciences. Pure scientists, who make up 19.2 per cent of all applicants, gain 26.2 per cent of the places, while social scientists make up 29.7 per cent of the applicants and get only 22.5 per cent of the places. The council demonstrates that the situation exists and goes to some lengths to translate it into figures. Last year there were, it says, 500 too few applicants in technology, and 1,100 too few in science, figures open to all sorts of criticisms. The council produces no evidence, for example, that the projections made by the universities of the number of places available are consistent from department to department. This is important because scientific departments gain much more from expansion than others do and as a result their optimistic predictions are less likely to be met.

For all that, the trend is convincing and, as the report notes, disturbing. Since 1962, admissions in pure science have grown by 41 per cent, in technology by 28 per cent, in arts subjects by 55 per cent, and in social studies (economics, sociology, psychology, law, geography) by no less than 118 per cent. Chary of falling off the fence, the council is content to record the figures and leave others to argue about them. It would be fascinating to learn, for example, where the shortfall is concentrated—well known and respected departments claim to have no difficulty in filling their places—and it may be that students are helping to create centres of excellence willy nilly by voting with their feet. The council bases its judgments on the premise that university entrance should be exactly as easy for arts undergraduates as for scientists. It might well be argued, though, that the hurdles to entry should be lower for scientists, as an encouragement. Perhaps, indeed, the shortfall should be welcomed for the effect it will have on the generation now in the schools.

### No Unwarrantable Intrusion

A WARNING that research into human behaviour may sometimes imply an unwarrantable interference with the privacy of people, and a code of values for the protection of individual privacy, are spelled out in a report of a panel under Dr. Kenneth E. Clark, chairman of the College of Arts and Sciences at the University of Rochester, and now published by the Office of Science and Technology in Washington (*Privacy and Behavioral Research*, U.S. Government Printing Office, 15c.).

The report says that the panel began work because of the threat to individual privacy implied by wire-tapping, electronic eavesdropping and similar innovations. On a number of occasions recently, the possibility that scientific enquiry might similarly intrude on personal matters has been raised in the United States Congress. While rejecting any notion that the 35,000 scientists who at present spend the \$300 million a year which the federal government devotes to behavioural sciences may be unaware of these problems, the panel does admit to having discovered a "limited number" of investigations in which privacy has been invaded.

The panel concludes that participation of experimental subjects in investigations must be voluntary and based where possible on "informed consent". Experiments should be designed in such a way that there is no permanent physical or psychological harm to participants. Similarly, published reports of research must protect the privacy of individuals, and government agencies supporting research should "satisfy themselves that the institution which employs the investigator has effectively accepted its responsibility to require that he meet ethical standards". Legislation, says the committee, is not necessary.

To the extent that these recommendations imply the supervision of the character of a person's work by his institution, they will in themselves be of some importance. In practice, the committee's interpretation of what is meant by privacy will raise other issues. As an example of intrusion, it cites an attempt at a sociometric measure in which children are asked questions designed to reveal their relationship to other children in the class. The committee says that this invades privacy because "it forces children to think about certain qualities of behaviour shown by one another and to reach firm conclusions about what is best or worst". The panel points out that in an experiment in which experimental subjects are given a sense of failure, the experimenter has a duty to see that they leave the laboratory with their natural spirits restored.

### New Fellows

THE Royal Society has broken new ground by the election of a number of technologists to the fellowship. The list of elections of March 16 includes eight people whose present work is more in industry than in academic life. The inclusion of technologists in the fellowship, not an entirely novel idea, has been given especial importance in the last few years, and Professor P. M. S. Blackett, the president, drew attention to the virtues of electing industrial people to the fellowship in his presidential address on November 30, 1966. Many of those now elected as technologists have distinguished records in pure research as well. The list of elections on March 16 is the following:

E. J. W. Barrington, Professor of Zoology, University of Nottingham; K. L. Blaxter, Director of the Rowett Research Institute, Aberdeen; E. S. Booth, Member for Engineering of the Central Electricity Generating Board; C. W. Bunn, Dewar Research Fellow of the Royal Institution; C. S. Cockerell, Consultant, Hovercraft Development Limited, and Chairman, Ripplecraft Limited, Southampton; J. V. Dacie, Professor of Haematology, Postgraduate Medical School, London; N. A. de Bruyne, Managing Director,

Techne Limited, Duxford; F. J. Dent, Director of the Midland Research Station of the Gas Council, Solihull; C. A. Fleming, Chief Palaeontologist, Geological Survey of New Zealand; H. Ford, Professor of Applied Mechanics, Imperial College of Science and Technology; S. D. Garrett, Reader in Mycology, University of Cambridge; M. A. Grace, Nuclear Physics Laboratory, University of Oxford; E. J. Hanson, Medical Research Council, Biophysics Research Unit, King's College, London; A. A. C. Issigonis, Technical Director of the British Motor Corporation, Birmingham; F. E. Jones, Managing Director, Mullard Ltd.; R. U. Lemieux, Professor of Organic Chemistry, University of Alberta; I. Maddock, Controller, Ministry of Technology; N. A. Mitchison, Head of the Division of Experimental Biology, National Institute for Medical Research; J. A. C. Nicol, Plymouth Laboratory, Marine Biological Association; D. C. Phillips, Professor of Molecular Biophysics, University of Oxford; C. H. B. Priestley, Chief, Division of Meteorological Physics, Commonwealth Scientific and Industrial Research Organization; C. R. Rao, Professor of Theoretical Statistics, Calcutta; R. Riley, Plant Breeding Institute, Cambridge; M. J. Seaton, Professor of Physics, University College, London; N. Sheppard, Professor of Chemistry, University of East Anglia; D. H. Smyth, Professor of Physiology, University of Sheffield; F. Sondheimer, University of Cambridge; H. P. F. Swinnerton-Dyer, Trinity College, Cambridge; D. F. Waterhouse, Chief, Division of Entomology, Commonwealth Scientific and Industrial Research Organization; A. Williams, Professor of Geology, Belfast; R. T. Williams, Professor of Biochemistry, St. Mary's Hospital Medical School, London; J. M. Ziman, Professor of Theoretical Physics, Bristol.

## Automatic Medicine

THE varied display of equipment at the International Medical Engineering and Automation Exhibition in London last week was a measure of the extent to which the engineers are now moving in on the medical field. Medical research, even in Britain, seems to have left string and sealing wax firmly behind, and the hospitals are also taking advantage of the new methods and techniques. One popular exhibit at Medea 67 was the use of fibre light guides in such instruments as the gastroscope developed by the Atomic Weapons Research Establishment of the U.K. Atomic Energy Authority. The same principles are also being used in an instrument which can be passed into a blood-vessel to show the colour of the walls and contents, and which can thus be used to localize blood clots. Cardiac pacemakers, shown by a number of manufacturers, are typical of the miniaturized equipment now coming into service. Some of the pacemakers on show could perform such tricks as altering their pace automatically when the patient is exercising and even switching on only when the heart stops. Dialysis machines were much in evidence, and several manufacturers had something useful to show.

## Keeping Fruit

SOME of the farmers who attended the open day of the East Malling Research Station on Wednesday, March

15, may have needed to be reassured about the value of continuing with the research on fruit storage going on at the station. If this was so, and Dr. H. B. S. Montgomery, assistant to the director, seemed to think so when he spoke to the large group of fruit growers at the station, it is not because the research carried out so far has been a failure. The research team in the pomology unit has been able to define the conditions of storage best suited to the different types of apples and to the different requirements of the grower. It has also made some progress in finding the causes of the gradual decline in the quality of apples stored for some time. All this research has been valuable in direct cash terms to the growers, who have supported the study by sending samples of their fruit to the laboratory for study. Because of the improvement of fruit storage resulting from the work of the laboratory, farmers no longer have to rush to market with their produce the moment it has been harvested. By a quirk of economics the results benefit not only the farmers who invest in the complex machinery required for the new methods of storage, but also those who do not have any special equipment. This may have produced some feeling that research into storage methods is not really worthwhile. The research staff at the station considers, however, that it has captured the enthusiasm of farmers up and down the country and that, if it asks, it will continue to get help from these farmers.

The station is also expanding the more general aspects of its research. A new electron microscope has just been installed to be used for studies of plant viruses and the fine structure of plant cells. It will have to turn to more fundamental studies of plant parasites because the experimental farm has swallowed up all the available land in the area around the laboratory.

## Maps by Machine

MAPS as a means of displaying information have been caught up in what is known as the information explosion. Partly this is because the amount of information available to map makers has increased at enormous speed since the introduction of automatic data collection and logging; the next step logically seems to be automatic map drawing by computer. A one day discussion meeting on the subject of automated cartography was held on March 20 by the joint computer groups of the Institution of Electrical Engineers, the Institution of Electronic and Radio Engineers, and the British National Committee for Geography of the Royal Society.

Mr. D. P. Bickmore of the Clarendon Press at Oxford defined the need. "The first—and urgent—need is to reconstitute an existing map from digital information, automatically, and so that it conforms to traditional standards of appearance and accuracy. This will show how much hand work is still necessary, and what modifications in style, information or even accuracy should be considered". Forms of output other than lithographic printing on paper must be considered, he said; high speed plotters or cathode ray tubes may give a quick preliminary look at a map. Professor D. Linton of the University of Birmingham pointed out that only a tiny proportion of the mass of demographic and climatic information collected by governments ever sees print in map form. Information is simply



tabulated by area, and the possibility of spotting regularities or correlations—say the incidence of pellagra, family expenditure on food, and the provision of medical services in the eastern United States—is very remote indeed. Such interesting relations as have been found are the result of years of searching, and merely increase the sense of frustration that there is no better way of doing it. Other speakers, from the General Register Office, the Ordnance Survey, and the Meteorological Office, discussed their own approaches to the problem. As Mr. Bickmore sadly admitted, nothing so far qualifying as a real map has been produced automatically in Britain or in the United States. He was hopeful, though, that something would be done before the end of this year.

## Reactors at Sea

THE economics of marine propulsion by nuclear reactor have been derided so often—most recently by Sir William Penney, Chairman of the Atomic Energy Authority—that believers need either considerable courage or blind optimism. Despite the figures recently issued for the U.S.S. *Savannah*, the United States nuclear ship, which requires an operating subsidy of close on a million dollars a year, and will cease to operate when the subsidy runs out, Mr. J. A. Teasdale, lecturer in Naval Architecture and Shipbuilding at the University of Newcastle upon Tyne, believes that a competitive nuclear powered nuclear ship could be built. In a paper read before the Royal Institution of Naval Architects on March 22, he described his design.

The ship would be in two parts, one containing the motive power and the other the cargo holds. The chief advantage would be to make full use of the expensive machinery in the motive part of the ship by doing away with long periods of idleness during loading and unloading. On delivery of a cargo, the pusher section would separate itself and depart almost immediately with another cargo unit which had been loaded in readiness. For the same power source, Mr. Teasdale claims that this concept would have both lower capital and running costs than an equivalent number of conventional ships. The design could of course be used with normal power sources, but since nuclear power plant would not require large oil storage tanks some of the difficulties in matching draught and trims would be removed.

The joint between the two sections should be either flexible or completely rigid. The flexible design would have to accommodate large forces in several degrees of freedom, and it is probable that the rigid design would be preferable. For the actual link, Mr. Teasdale visualizes a ratchet arrangement with a V-shaped bow for the pusher unit and a corresponding recess in the cargo unit. After positioning the pusher unit correctly, water ballast would be taken on to engage the ratchet. To get the best advantage from the design, the voyage time should be equal to half of the loading or discharging time of the cargo. A suitable route, Mr. Teasdale suggests, would be Liverpool–New York, at a service speed of 21 knots. One pusher unit and three cargo units, he says, would be equivalent in handling capacity to 2.43 conventional ships. After what he admits to be an elementary cost comparison, he concludes that a nuclear composite ship could be competitive with existing vessels. Using the design with a diesel main engine

would, he thinks, lead to “considerable savings in cost”.

## High Powered Co-operation

THE Cavendish Laboratory at Cambridge, the Ministry of Technology and Associated Electrical Industries, Ltd., are all agreed that there are substantial scientific and economic gains to be had from the development of high voltage electron microscopes. Dr. V. E. Cosslett at the Cavendish has designed and built a microscope operating at 750,000 volts, and AEI has decided to build the microscope, scaled up to 1 million volts and with minor design modifications, and to sell it commercially for a price in the region of £180,000. Where the ministry comes in nobody is quite sure, but it is on the scene and beside itself with pleasure at this example of collaboration between industry and the university.

In order to build the microscope at Cambridge, some structural alterations were necessary at the Cavendish; a students' laboratory was annexed for the electron accelerator, a large hole made in the floor, and the actual microscope sited in the cellar below, previously a battery room. The microscope is beautifully engineered, about twice as large as conventional ones, and has been in use for a year. Results have been very encouraging, and it seems that some of the doubts expressed about high voltage microscopes were unjustified. Apart from the opportunity to use much thicker specimens in transmission, there has been a considerable improvement in clarity, and fine detail invisible at 250 kV can be seen at 750 kV. For biological specimens damage is less than at 100 kV, perhaps surprisingly, and for metallurgical work the interpretation of the electron micrographs is not unduly complicated by the increase in thickness; the use of stereo pictures can help. The microscope has the advantage of operating over the whole range from 100 kV to 750 kV, and AEI intends to preserve this feature when it puts the design into production.

So far, the microscope has been used, among other things, for the examination of wool fibres, porcupine quills and membranes for artificial kidney machines, but the obvious application is in metallurgy. Foils

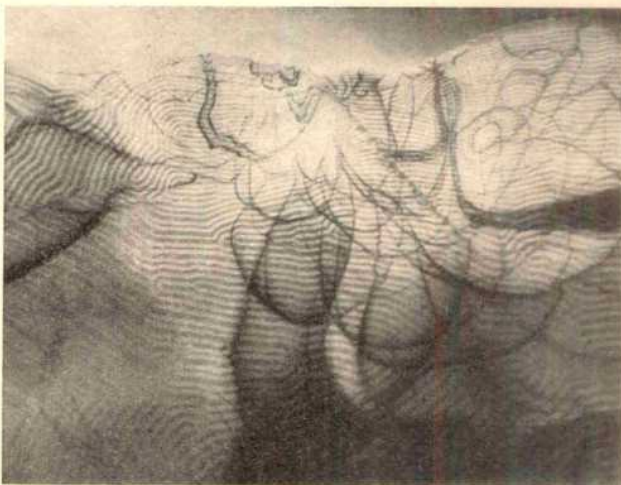


Fig. 1. Domains in a wedge-shaped cobalt foil, photographed by Lorentz microscopy in the Cavendish Laboratory microscope. Voltage 700 kV, magnification about 5,000. (P. J. Grundy and J. P. Jakubovics, Cavendish Laboratory.)



up to  $1.4\mu$  thick can be examined, and the instrument already has one triumph behind it—the identification of an alloy inclusion which would have fallen out if conventional thinning techniques had been needed. AEI is confident that a number of 1 MV microscopes can be sold, and has orders from the National Physical Laboratory, the Atomic Energy Authority at Harwell, and the Science Research Council. Deliveries will start in two years time, which gives some Japanese companies a substantial lead—they are already marketing 500 kV and 1 MV instruments. In France and the United States the talk is now in terms of 3 MV and 5 MV instruments.

## Select Committee

THE Select Committee on Science and Technology continued its search for something to be shocked about when it discussed the British nuclear reactor programme with a delegation from the Central Electricity Generating Board on March 16. In earlier discussions, Sir William Penney had suggested that substantial savings could be made by using standardized designs, but Mr. F. H. S. Brown, chairman of the CEBG, did not agree. He thinks that greater savings can be made by technological development than are possible by replication of design. While standardization could doubtless give a price reduction of 7–10 per cent, development had brought down the cost of reactors from £180 a kW for the "Magnox" reactor to £100 a kW for the Advanced Gas Cooled reactor. Standardization in the United States, Mr. Brown believes, has only been possible because water moderated reactors are at the end of their development. To give up development of the AGR for the sake of marginal cost reductions would be foolish.

Mr. Brown went on to give the committee the following comparison of the costs of two AGR power stations and one new coal fired power station.

	Capital costs (per kW)	Running costs (d/kWh)
Dungeness B	£104.8	0.511
Hinkley Point B	£95.5	0.476
Drax (coal fired)	£55.2	0.577

During its proceedings last week the committee did finally sight a hare to chase. Apparently the capital expenditure of the CEBG for the financial year that begins at the end of March has not yet been approved by the Ministry of Power. Some members of the committee were clearly taken aback although the CEBG patiently explained that it would make very little difference as long as capital projects already under way would not have to be brought to a shuddering halt. This had not happened, although the start of the Hinkley Point B station had had to be delayed by 12 months, and the CEBG would therefore have to wait another year before reaping the economic advantages of the new station. As for natural gas, the men from the electricity industry would not be drawn—they would, of course, be well placed to use the gas for electricity generation, but all depended on the price fixed for the gas. Was the Ministry of Power influenced by greedy thoughts of what natural gas could do for British power generation? Did this make it reluctant to approve new expenditure on nuclear plant? On their best behaviour, the CEBG delegation refused to guess.

## Parliament in Britain

REPLYING to a request for a statement on the future of the National Reference Library of Science and Invention, the Minister of State at the Department of Education and Science, Miss Jennie Lee, said in the House of Commons on March 13 that the provision of a new building was a matter for the Minister of Public Building and Works. A working party representing the British Museum, the Treasury, the Ministry of Public Building and Works and the Department of Education and Science was reviewing the space required. The 1966 report of the Trustees of the British Museum revealed encouraging progress in staffing, acquisitions, physical reconstruction and improvement, and the next few years should see considerable further developments.

In a written answer in the House of Commons on March 14, the Secretary of State for Commonwealth Affairs, Mr. H. Bowden, stated that the Government had under consideration the recommendation of the mission under the chairmanship of Sir Charles Morris (now Lord) that a comprehensive university institution to serve the needs of the English speaking territories in the South Pacific area should be established at Laucala Bay, Fiji. Mr. Bowden said that the British Government would be prepared to offer, subject to approval of Parliament, £1.25 million sterling over some 5 years towards the initial cost of the university, including related institutes, of which up to £500,000 could, if necessary, be devoted to recurrent costs. The provision of assistance is subject to a firm decision by the Fiji Government, after such consultation as it may find necessary with other governments in the area, to proceed with the establishment of the university. The New Zealand Government has already promised help.

On March 15 the Postmaster General, Mr. E. Short, told the House of Commons that in the past year the Post Office had earned 7.8 per cent overall on capital compared with a target of 8 per cent and the predicted return for 1967–68 was 7.4 per cent. Over the whole five year period it was anticipated the return on the postal side would be short of the target, but not on the telecommunications side. Much postal work offered little or no scope for increased mechanization, about 40 per cent of postal costs being incurred in collecting and delivering mail, and the profit of £4 million this year was made on an income and expenditure of about £340 million. Subscriber trunk dialling (STD) was now available on about three-quarters of the telephones in Britain and by the end of 1967 fewer than 3 per cent of subscribers would be connected to manual exchanges. There would be none after 1970–71.

MR. SHORT said that the number of staff trained in the latest management techniques of work study and operational research was being increased; about £4 million worth of computers were already installed and working; others to the value of £3 million were on order, and by 1971, 20 large computers would be in operation. It was expected that in about 2 years time use of computers in telephone accounting would save over 2,500 clerical staff, and in posts and telecommunications was expected to save over 12,000 staff in the next few years.

## University News:

## London

THE following appointments to chairs have been made: Dr. J. L. Hancock, a member of the A.R.C. Animal Research Organization, to the chair of anatomy tenable at the Royal Veterinary College; Sir Francis Knowles, professor of comparative endocrinology in the University of Birmingham, to the chair of anatomy tenable at King's College; Dr. E. Leader, research fellow at Clare College, Cambridge, to the chair of theoretical physics tenable at Westfield College; Dr. I. W. Roxburg, reader in astronomy in the University of Sussex, to the chair of applied mathematics tenable at Queen Mary College.

Sir Hans Krebs, Whitley professor of biochemistry in the University of Oxford, has been appointed as visiting professor of biochemistry in the Royal Free Hospital School of Medicine, a position he will continue to hold after his retirement from the Oxford chair. This is the first appointment in a new scheme the school has adopted for the appointment as visiting professors of men and women, from other academic or research institutions or from government departments, industry or commerce, who are distinguished in the fields of medicine and science allied to medicine.

## Announcements

THE International Agency for Research on Cancer is awarding travel fellowships during 1967-68. The fellowships are intended to provide opportunities for visits to other laboratories for consultation, collaborative planning of research projects, and the acquisition and standardization of new research techniques, and applicants must be actively engaged in cancer research and qualified in natural or medical sciences. The Agency is also awarding research training fellowships in 1968 and applications are invited from junior scientists wishing to be trained in any aspect of cancer research, including epidemiology and biostatistics. Applicants should preferably be already engaged in research in medical or allied sciences and intend to pursue a career in cancer research. Further information concerning both fellowships can be obtained from the Chief of the Education and Fellowships Programme, International Agency for Research on Cancer, 16 avenue Maréchal-Foch, 69-Lyon (6e).

PROFESSOR J. GRAHAME CLARK, Disney professor of archaeology in the University of Cambridge, has been awarded the Hodgkins gold medal by the Smithsonian Institution. This medal, which is accompanied by a \$3,000 honorarium, is awarded periodically for important contributions to knowledge of the physical environment bearing on the welfare of man.

THE 1966 Prince Philip Medal for outstanding promise or achievement in the promotion, theory or practice of science or technology has been awarded to J. B. Warman, departmental chief engineer, Telecommunications Division, Associated Electrical Industries Ltd.

THE James Watt International Medal has been awarded by the Institution of Mechanical Engineers to Professor I. I. Artobolevskii, a member of the Academy of Sciences of the U.S.S.R., in recognition of his contributions to mechanical engineering, particularly in the field of mechanisms.

THE 1967 Coblentz Society Award has been presented to Professor P. J. Krueger, professor of chemistry and chairman of the Department of Chemistry in the University of Calgary, in recognition of his contribution to the field of infra-red spectroscopy.

A SHORT course on "Mechanical Design" is to be held in the University of Bradford during May 1-5. Further information can be obtained from the Registrar, University of Bradford, Bradford 7.

CIBA Laboratories, Ltd., Horsham, have donated, initially for a period of seven years, a silver medal and prize of £50 to be awarded annually by the Biochemical Society for outstanding research in any branch of biochemistry. Nominations are now invited for the third award, which will be made in 1967, and further information can be obtained from the Executive Secretary, The Biochemical Society, 20 Park Crescent, London, W.1.

THE National Technical Committee of the Society of Instrument Technology is to set up a Study Group on automation in design for manufacturing processes. Further information can be obtained from the Society of Instrument Technology, 20 Peel Street, London, W.8.

A SECOND conference on "Static Electrification", arranged by the Institute of Physics and the Physical Society, will be held at the Institution of Electrical Engineers during May 8-10. Further information can be obtained from the Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

AN international symposium on "Molecular Associations in Biology" will be held at the Institut de Biologie Physico-Chimique, Paris, during May 8-11, in celebration of the fortieth anniversary of the foundation of the institute. The main object of the symposium will be to discuss the principal forces involved in such associations, the means for their detection and the principal biological systems and problems in which they are in operation. Further information can be obtained from Professor B. Pullman, Institut de Biologie Physico-Chimique, 13 rue Pierre Curie, Paris 5e.

A SYMPOSIUM on "Composite Polymeric Materials" is to be held in the University of Bradford during May 12-13. Further information can be obtained from the Registrar, The University of Bradford, Bradford.

A SYMPOSIUM on "Diagnosis and Treatment of Deposited Radionuclides", sponsored by the Battelle Memorial Institute-Pacific Northwest Laboratory, Hanford Occupational Health Foundation, Inc., and the U.S. Atomic Energy Commission, is to be held in Richland during May 15-17. Further information can be obtained from Dr. J. F. Park, Symposium on Diagnosis and Treatment of Deposited Radionuclides, P.O. Box 999, Richland, Washington 99352.

A SECOND national symposium on radioecology entitled "Nuclear Energy in Man's Environment: Past, Present and Future Problems", sponsored by the Atomic Energy Commission, the Ecological Society of America, and the University of Michigan, will be held in the University of Michigan at Ann Arbor during May 15-17. Further information can be obtained from Dr. F. C. Evans, Department of Zoology, University of Michigan, Ann Arbor, Michigan.

AN international colloquium on "Solid Inorganic Phosphates" will be held in Toulouse during May 16-20. Further information can be obtained from the Secrétariat Général du Colloque International sur les Phosphates Minéraux Solides, 38 rue des Trente-Six Ponts, 31-Toulouse.

THE fifth international conference on "Non-Destructive Testing", sponsored by the Canadian Council for Non-Destructive Technology, will be held in Montreal during May 21-26. Further information can be obtained from the Secretary, The British National Committee for Non-Destructive Testing, Redfields Home Farmhouse, Church Crookham, Aldershot, Hants.

THE fourth international seminar on "Gel Permeation Chromatography" will be held in Florida during May 22-24. Further information can be obtained from the chairman

of the Fourth International Seminar, c/o Waters Associates, Inc., 61 Fountain Street, Framingham, Massachusetts 01701.

A CONFERENCE on "Frequency Generation and Control for Radio Systems" will be held at the Institution of Electrical Engineers during May 22-24. The aim of the conference is to review the developments in techniques of frequency generation, synthesis, control and distribution as applied to radio communications, navigation and radar systems. Further information can be obtained from the Conference Section, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

AN international congress on "Sulphur Oxides" will be held in the University of Toulouse during May 22-26. Further information can be obtained from the Secrétariat du Congrès, Association des Ingénieurs du Génie Chimique, Institut du Génie Chimique, Chemin de la Loge-Emplot, 31-Toulouse.

**CORRIGENDUM.** In the article entitled "Activity of Ribosomal Phosphodiesterase in a Protozoan", by L. H. Lazarus and O. H. Scherbaum (*Nature*, 213, 887; 1967), the following should be added to the end of the last sentence of para. 5: ", either as magnesium and/or calcium ions.". Lines 10 and 11 of para. 6 should read: "... This ribosome-bound enzyme with its high pH for optimum activity is ...". In line 3 of the legend to Table 1 "magnesium chlorate" should read "magnesium chloride". The fifth line of the legend to Table 2 should be "... tryptophan plus potassium chloride relative to potassium chloride alone.". In the first column in Table 2 the word "Plus" should be deleted from entries 5-8. The last two entries in this column should then be moved under entry 8.

**ERRATUM.** In the article entitled "Preliminary Observation on the Lipids of Bovine Retinal Outer Segment Disks" by S. Fleischer and D. G. McConnell (*Nature*, 212,

1366; 1966), the second line of the third paragraph should read "resolved into six fractions. . . .".

**ERRATUM.** In the article "Reduction of Methaemoglobin in Haemoglobin Samples using Gel Filtration for Continuous Removal of Reaction Products" by H. B. F. Dixon and R. McIntosh (*Nature*, 213, 399; 1967), second paragraph, line 5, "12 mg sodium dithionite" was incorrectly given as "12 ml. . . .".

## CORRESPONDENCE

### Asbestos in the Lungs

SIR,—The first paragraph of this review (*Nature*, 213, 855; 1967) states that mesotheliomas of the pleura and peritoneum only occur in association with exposure to crocidolite asbestos dust. This statement is not in accordance with our present knowledge. These tumours have occurred in people in whom no evidence of exposure to asbestos dust was established. Furthermore, crocidolite is not necessarily the only type of asbestos implicated. Investigations in the United States<sup>1,2</sup> have indicated that other types of asbestos may be involved. The international investigations which are described later in your article are planned to measure the absolute and relative risk of tumour development with all types of fibres.

Yours, etc.,

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J. C. WAGNER

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<sup>1</sup> Selikoff, Irving J., Churg, Jacob, and Hammond, E. Cuyler, *New Eng. J. Med.*, 272, 560 (1965).

<sup>2</sup> O'Donnell, Ward M., Mann, Richard H., and Grosh, John L., *Cancer*, 19, 1143 (1966).

## THE NIGHT SKY IN APRIL

All times are in Universal Time

MOON		CONJUNCTIONS WITH THE MOON	
New Moon	9d 22h	Venus	13d 06h, 0.8° S.
Full Moon	24d 12h	Mars	23d 17h, 0.4° N.
		Jupiter	17d 16h, 5° S.
		Saturn	8d 15h, 0.8° N.

### PLANETS

Name	Times of rising (R) and setting (S) during the month				Mag.	$D_g$ (10 <sup>6</sup> miles)	Zodiacal position
	R/S	Beginning	Middle	End			
Mercury	—	Unfavourable for observation			—	102	Pisces
Venus	S	21h 45m	22h 30m	23h 20m	-3.5	113	Taurus
Mars	R/S	20h 00m (R)	18h 40m (R)	4h 10m (S)	-1.3	56	Virgo
Jupiter	S	3h 20m	2h 25m	1h 30m	-1.7	481	Gemini
Saturn	—	Unfavourable for observation			—	970	Cetus

$D_g$  is the distance of planet from the Earth on the 15th of the month.

### OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
47 Gem	D	17d 00h 13.3m	+5.6
$\omega$ Cnc	D	17d 19h 45.3m	+5.9

(D disappearance; R reappearance)

### METEORS

Name	Active period	Date of maximum	Radiant	Remarks
Lyrids	19d-23d	22d	271° R.A. +33° Dec.	Unfavourable

### OTHER PHENOMENA

8d 15h, Saturn occulted by the Moon, visible in S. America.  
13d 06h, Venus occulted by the Moon, visible in N.E. Europe and Asia.  
23d 17h, Mars occulted by the Moon, visible in Central and East Africa, S. Australia.  
24d 12h, Total eclipse of the Moon, Moon enters umbra 10h 25m, visible in the Americas, Asia, Australasia.

Total eclipse begins	11h 28m
Mid eclipse	12h 07m
Total eclipse ends	12h 46m
Moon leaves umbra	13h 49m

# Morphological Conversion of Cells *in vitro* by *N*-Nitrosomethylurea

by

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A system is described for investigating the primary events involved in the induction of tumours by certain chemical carcinogens. It is based on the finding that such compounds alter the mode of growth of cells from a Chinese hamster lung tissue cell line in culture, and that the effect accompanies an ability to cause tumours when such cells are later injected into foreign host animals.

WHEN intact animals are treated with tumour-inducing agents, tumours usually appear only after many weeks and often in places remote from the original site of carcinogen application. Because tumours may arise from one, or at most a few, altered cells, experiments of this kind provide little knowledge of the nature of the initial, irrevocable, interaction between a carcinogen and cells directly affected by it. Thus, most current hypotheses concerning the primal events of cancer induction draw their support from information regarding the behaviour of carcinogens under metabolism by cells and tissues in short-term biochemical experiments<sup>1</sup> and their interaction with cell constituents *in vitro*<sup>2</sup>. Recently<sup>3,4</sup>, after successful experiments with oncogenic viruses<sup>5,6</sup>, cells have been treated in tissue culture with chemical carcinogens in order to search for cell colonies which have been "transformed" morphologically, the constituent cells of which may have the ability to cause tumours when later transplanted into animals. Although success was achieved, carcinogenic hydrocarbons of proved efficacy in animal experiments, compounds of notoriously low solubility in aqueous media, were used, and left in contact with the treated cells for several cell generations. In one experiment<sup>3</sup>, the way in which the carcinogen was applied made it difficult to be certain how much of it actually reached the cells, and in the second study<sup>4</sup> failure to detect clear-cut morphological changes *in vitro* made it obligatory to test for cell conversion by transplanting the cultured cells into animals.

This article describes a system whereby cells from a single serially cultured clone of mammalian cells, treated only briefly with a carcinogen *in vitro*, acquired (a) a capacity for altered growth when cultured later, as well as (b) an ability to grow as tumours in a heterologous host. The compounds used are all soluble in water, and the rate of cellular conversion is very high. These facts suggest that the system may be valuable for further investigations of the primary events involved in the induction of tumours.

The cells used came from a pseudodiploid, male Chinese hamster lung tissue cell line, serially cultivable *in vitro* as monolayers on glass or plastic. They came from the same source as the agar-passage line used by Borenfreund *et al.*<sup>4</sup>, but had not been so adapted; they were obtained from the Sloan Kettering Institute, New York, in 1965. A large stock of cells kept frozen in 10 per cent dimethylsulphoxide at  $-70^{\circ}\text{C}$  was used throughout. Cells for experiments were obtained by sub-culture from this frozen stock, but were not used at a passage level more than fifty cell generations later than that of the frozen material. Experimental batches of cells were grown up in stoppered Roux flasks and babies' bottles in a medium consisting of Eagle's basal medium (Hanks salts), 10 per cent tryptose

phosphate broth and 10 per cent foetal calf serum<sup>7</sup>. Experiments, using the same medium, were conducted in 5 cm plastic Petri dishes, incubated at  $37^{\circ}\text{C}$  in a water-saturated atmosphere containing 5 per cent of carbon dioxide.

Three compounds were tested, all of them obtained from Dr. P. N. Magee: (i) *N*-nitrosomethylurea (NMU)—this is one of the most effective carcinogenic substances known, causing primary gastric cancer in rats after a single oral dose<sup>8</sup>; (ii) dimethylnitrosamine (DMN)—a powerful representative of a class of hepatotoxic substances, also capable of causing liver cancer in rats after

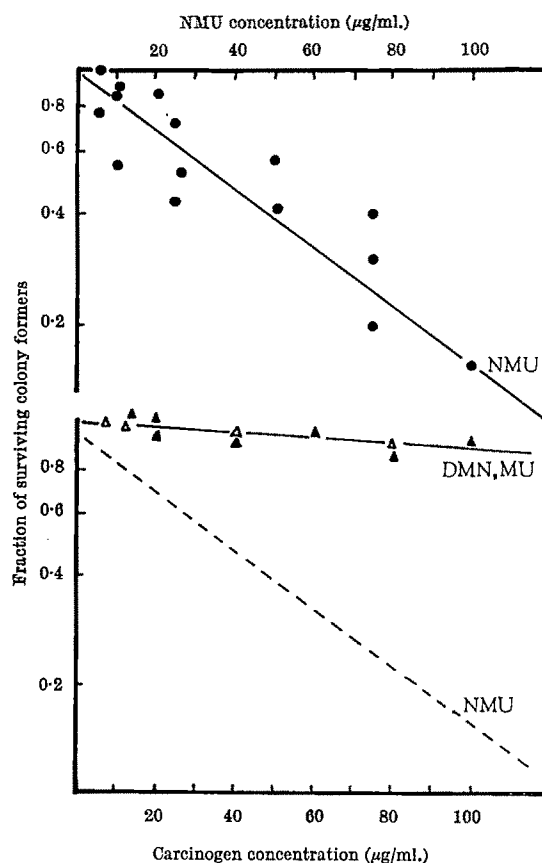


Fig. 1. Toxic effect of nitrosomethylurea (NMU), dimethylnitrosamine (DMN), or methylurea (MU), on the capacity of Chinese hamster cells to form colonies *in vitro*. The upper part contains the pooled results of three experiments with NMU. The lower part compares NMU with DMN and MU.

oral or intravenous administration<sup>9</sup>; (iii) 1-methylurea (MU)—chosen essentially as a non-toxic control.

All three compounds were used as a 1 mg/ml. solution in phosphate buffered saline (PBS), stored at +4° C in the dark. Trypsinized, washed cells were suspended in growth medium or PBS, the proper concentration of test substances added and the mixture incubated with gentle shaking at 37° C; after 2 h, or shorter times when the time necessary for carcinogen action was being investigated, the mixture was diluted and plated in one of two ways: (1) A volume estimated to contain 10<sup>5</sup> cells was plated in 5 ml. of liquid growth medium. After 14 days the number of colonies formed, and their morphology, was recorded. Under these conditions untreated cells had a plating efficiency of about 20 per cent. Alternatively, (2) 0.1 ml. of diluted incubation mixture containing 1–2 × 10<sup>4</sup> cells was plated in semi-solid agar<sup>10,11</sup>. The overlay contained 0.33 per cent washed agar, and under these conditions the plating efficiency of untreated cells was 5–10 per cent.

The ability of treated, as well as untreated, cells to grow as tumours was tested by injecting 0.1 ml. of culture medium containing 1 × 10<sup>6</sup>–1 × 10<sup>8</sup> cells into the cheek pouches of cortisone-treated (0.1 ml. cortisone acetate, 25 mg/ml., subcutaneously, twice weekly), 3–4 week old hamsters<sup>12</sup>. Cheek pouch nodules were removed after 10–20 days and prepared for histological examination as well as further investigation in tissue culture.

0.3–0.5 µg/ml. of either 7–12 dimethylbenzanthracene, 3-methylcholanthrene or 3.4 benzo(a)pyrene reduced the plating efficiency of Chinese hamster cells in agar by

up to 90 per cent<sup>4</sup>. The three compounds tested here were much less toxic. Fig. 1 shows that two of them, namely DMN and MU, were virtually non-toxic up to a concentration of 100 µg/ml. in liquid medium. NMU, at the same concentration, reduced the number of cells potentially capable of forming colonies by 85 per cent. A similar toxicity was found when cells were plated in agar. It should be remembered, however, that all three compounds were only allowed to act briefly before dilution of the mixture to a concentration below that at which any activity could be demonstrated. Any lack of toxicity compared with the hydrocarbons can then perhaps be ascribed to the short time during which they were allowed to act. With NMU a further factor may be the innate instability of this compound in biological media under the conditions used.

Cells treated with NMU at all concentrations greater than 10 µg/ml. grew into colonies which could easily be distinguished from their normal counterpart. The majority (more than 80 per cent) of the colonies given by the latter were flattened, up to five cells thick at the centre, but consisting peripherally of a broad monolayer the constituent cells of which exhibited a marked parallel orientation (Fig. 2a). The rest consisted either of small nests of epithelial cells or diffuse collections of small fibroblasts one cell thick, but nevertheless exhibiting an essentially parallel orientation. Most colonies from cells treated with NMU, however, had centres up to twenty cells thick; the periphery of such colonies tended to remain multilayered and the cells comprising them to grow over one another with a criss-cross orientation (Fig. 2b).

Epithelial and small fibroblastic colonies were present, but were also disordered as compared with their normal counterpart.

Up to seven passages, either of clones derived from single colonies, or mass populations obtained by trypsinization of plates containing up to a hundred colonies of cells treated with NMU, gave cultures consisting of colonies the morphology of which remained essentially as described. It is therefore clear that treatment with NMU causes cells to change their mode of growth *in vitro*, and that this change is hereditary, persisting through subsequent generations under conditions when significant quantities of the carcinogen can no longer be present. Plating, and sub-culture, of NMU-treated cells in agar did not give a similar result. Individual colonies were larger than those given by untreated cells but had essentially the same morphology.

Toxicity, and cell conversion, of a similar degree was found when NMU was allowed to interact with cells either in growth medium or in PBS; it would appear that simultaneous rapid cellular syntheses are not essential to the eventual expression of the NMU effect. Fig. 2b illustrates the morphology of colonies arising from cells treated with NMU.

In contrast to NMU, colonies of cells treated with DMN, at whatever concentration, exactly resembled those given by untreated cells, whether plated in liquid medium or in agar (Fig. 2c).

A surprising result followed the treatment of cells with MU. This substance, not so far listed as a carcinogen, gave rise in all concentrations exceeding 40 µg/ml. to altered colonies with a morphology like those found after treatment with NMU (Fig. 2d). When first found, it was thought that this effect was due to an impurity in the sample tested, but repetition

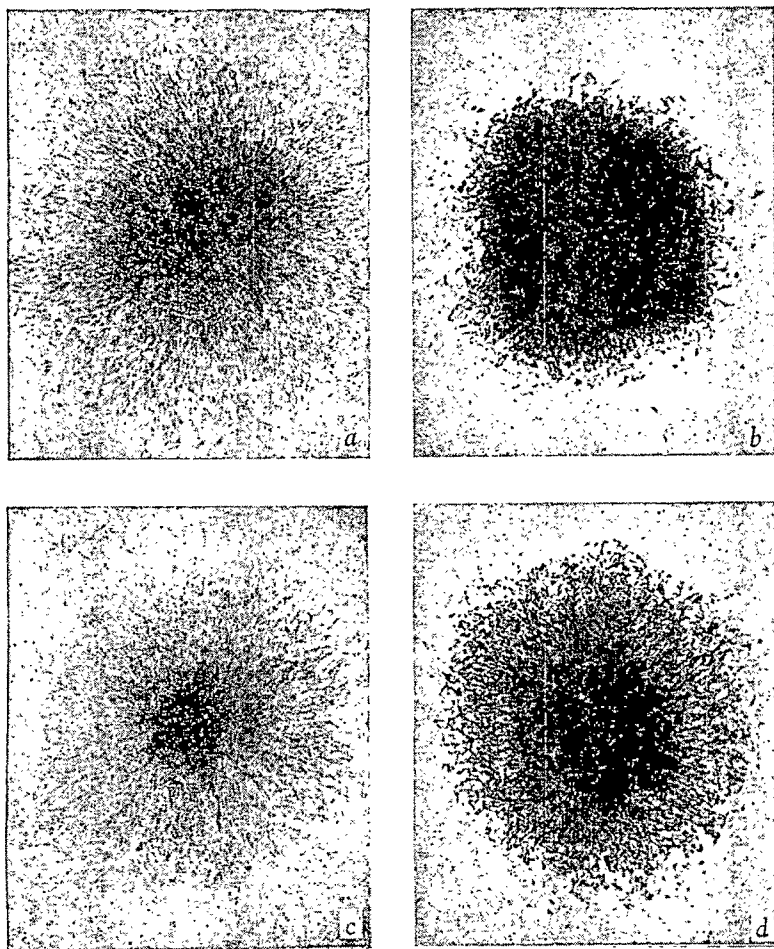


Fig. 2. Photographs, at the same magnification, of crystal violet-stained colonies of Chinese hamster cells, arising from: (a) untreated cells; (b) cells treated with 20 µg/ml. NMU; (c) cells treated with 80 µg/ml. DMN; (d) cells treated with 80 µg/ml. MU.



of the experiments with a re-crystallized sample of MU gave the same result over the same range of concentrations. Experiments are in progress involving further related compounds, in order to unravel the nature of this effect.

When cells treated *in vitro* were tested for their ability to grow *in vivo* by inoculation into the cheek pouch of Syrian hamsters, all cell populations derived from tissue culture colonies "converted" by NMU were able to grow in the alien host (Table 1). Untreated normal cells, or cells treated with DMN, failed to grow in the cheek pouch. It is as yet too early to know whether cells from different colonies vary in their ability to give rise to nodules in the cheek pouch or whether several alternating passages *in vitro* and *in vivo* are necessary to select a cell line with a high capacity to cause tumours when only a few cells are injected<sup>13</sup>.

Table 1. CHEEK POUCH NODULES IN SYRIAN HAMSTERS AFTER INOCULATION OF CHINESE HAMSTER CELLS

	Tumours after inoculation of $1 \times 10^6$ cells
Cells from untreated colonies	0/10
Cells from colonies after NMU treatment (10 $\mu\text{g}/\text{ml}$ )	5/10
Cells from colonies after NMU treatment (100 $\mu\text{g}/\text{ml}$ )	10/10
Cells from colonies after DMN treatment (80 $\mu\text{g}/\text{ml}$ )	0/10

An attempt was made, using NMU, to determine the minimum time of cell/carcinogen interaction necessary to ensure morphological conversion.  $1 \times 10^6$  cells/ml. preheated to  $37^\circ\text{C}$  were treated with 20  $\mu\text{g}/\text{ml}$ . NMU, the reaction stopped at intervals by five-hundred-fold dilution in medium chilled to  $4^\circ\text{C}$  and the samples plated in liquid medium. Fig. 3 shows that contact between cells and this concentration of NMU for only 1 h is sufficient to achieve conversion. This time is far shorter than the mean generation time of these cells ( $\geq 12$  h in our hands) and its significance in relation to intracellular events remains to be determined.

Cells treated *in vitro* with carcinogenic hydrocarbons, besides being capable of giving rise to tumours, are also able to grow in the presence of hydrocarbon concentrations which would otherwise be lethal<sup>3,4</sup>. This suggests that, with hydrocarbons, selection must play a part in the appearance of such cells, although, when rendered neoplastic by other means, such as virus infection, cells also resist high hydrocarbon concentrations<sup>14</sup>. It was therefore of interest to determine whether cells converted *in vitro* by NMU also became resistant to the toxic effects of this compound. Fig. 4 shows the result of an experiment in which the plating efficiencies of untreated cells, as well as cell populations grown up from colonies converted after treatment with 10  $\mu\text{g}/\text{ml}$ . and 100  $\mu\text{g}/\text{ml}$ . NMU, were compared after further treatment with various concentrations of NMU. The results suggest that NMU converted cells do not have an augmented resistance to this compound. The lack of acquired resistance, the marked effect of a brief treatment and the fact that a high proportion of converted colonies is found at rela-

tively non-toxic levels of NMU all suggest that the effect of NMU on the cells may well be a direct one, and not the selection of a previously existing cell type. It would thus be of particular interest to determine whether conversion of cells by NMU endows them with the ability to resist otherwise lethal concentrations of carcinogenic hydrocarbons.

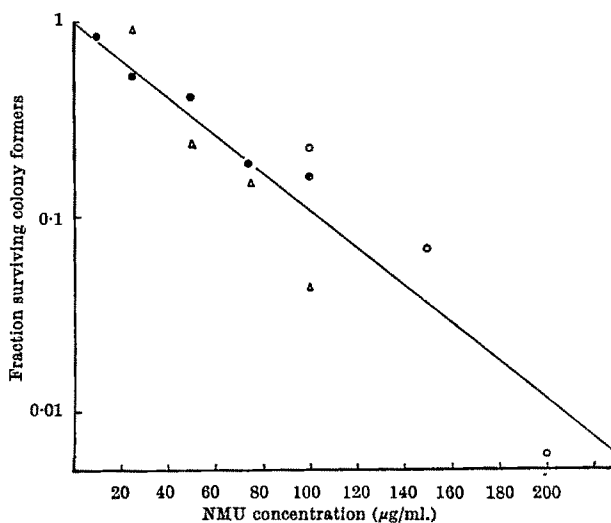


Fig. 4. Resistance of the colony-forming capacity of Chinese hamsters to NMU. ●, Untreated cells; △, cells from colonies "converted" by 10  $\mu\text{g}/\text{ml}$ . NMU; ○, cells from colonies "converted" by 100  $\mu\text{g}/\text{ml}$ . NMU.

One feature of great interest to have emerged from this work is the contrast between the *in vitro* behaviour of NMU and DMN. In addition to its properties as a direct acting carcinogen<sup>2</sup>, the former is known to be mutagenic in *Drosophila*<sup>15</sup>, *Saccharomyces*<sup>16</sup> and *Arabidopsis*<sup>17</sup>. DMN, by contrast, acts indirectly on the liver as a carcinogen<sup>2</sup>, and, while inducing mutations in *Drosophila*<sup>18</sup>, lacks mutagenicity for *Neurospora*<sup>18</sup> or bacteria<sup>19</sup>. These facts, and our observation that DMN fails to convert Chinese hamster cells in tissue culture, support the suggestion<sup>20</sup> that DMN only becomes an active carcinogen after having been acted on by an enzyme. While the mammalian liver and *Drosophila* contain this enzyme, *Neurospora*, *Escherichia coli*, and Chinese hamster fibroblasts presumably do not possess enough of it to convert DMN into an active form.

We thank Dr. P. N. Magee for the gift of chemical compounds, as well as much helpful discussion, and Mr. J. Green for technical assistance.

Received March 14, 1967.

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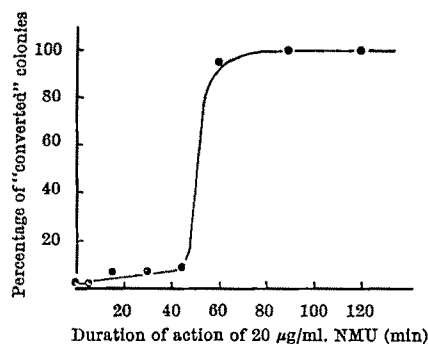


Fig. 3. Percentage of Chinese hamster cells giving rise to "converted" colonies after different times of treatment with NMU.

# Ecology of Aldabra Atoll

by

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The Royal Society made public on February 22 its concern that the island of Aldabra might be used as an air staging post and transmitting station. The island ecosystem of Aldabra is of great interest to biologists. The authors visited the island during 1966, and Dr. Stoddart will return with the Royal Society Expedition to Aldabra this summer.

SINCE the time of Darwin and Wallace it has been recognized that the biology of remote islands is of particular significance in evolutionary theory, and in the study of the dispersal, colonization, and speciation of plants and animals. Much work has been done on these problems both in low-latitude islands such as the Galapagos and Hawaiian groups, and more recently in the high volcanic islands of the Southern Ocean<sup>1</sup>. The small and relatively simple range of habitats and biota on islands suggests that insular ecosystems be used as models for more complex systems, forming natural laboratories for the study of ecological and evolutionary problems<sup>2</sup>.

As such problems have been formulated, however, the natural ecosystems of many islands, particularly in the Tropics, have been transformed, either by mining, by plantation agriculture, or more drastically in the past thirty years, by their use for airstrips, military bases and weapon-testing grounds. Recent proposals to locate a Ministry of Defence air staging post and B.B.C. transmitting station at Aldabra Atoll in the south-west Indian Ocean have caused considerable concern<sup>3</sup>, for Aldabra is one of the least disturbed of all low-latitude islands, and for historical and environmental reasons possesses an exceptionally rich and interesting fauna and flora<sup>4</sup>.

Aldabra is situated 260 miles north-west of Madagascar and 400 miles from the coast of Africa (Fig. 1). It is an elongate atoll, 21 miles long and up to 9 miles wide, with a land area of 60 square miles, and a total area, including the central lagoon, of 141 square miles (Fig. 2). Like a number of adjacent islands, it has been slightly elevated and the land rim now consists of eroded coral reef and reef-flat deposits, with maximum elevation of the solid rock of 15–20 ft. above sea-level, and some dunes rising to 50 ft. A general distinction may be made between two types of surface—champignon and platin<sup>5</sup>. Champignon consists of uplifted peripheral atoll reefs, intricately “dissected” by salt-water and brackish-water. The sharp, pinnacled surface is interrupted by numerous deep, steep-sided solution holes. Platin, on the other hand, is formed from raised detrital back-reef deposits modified by freshwater solution. The surface is flatter and lower than the champignon, and dimpled with solution pans. These contain freshwater pools which change size considerably with the transition from the wet (January–March) to the dry seasons: the annual rainfall is unknown, but may reach 50 in. The main platin area, of 14 square miles, is at the east end of the atoll, on South Island, where it is fringed towards the sea by champignon, partly overlain by dunes, and towards the lagoon by mangroves. The lagoon itself is shallow, and large areas dry out at low water; active coral growth is restricted to the margins of Main and East Channels. On the seaward side of the atoll there is a narrow wave-cut platform, which lacks flourishing coral reefs and which is backed by undercut champignon cliffs up to 15 ft. high. Beaches are small and few in number, though sand dunes have built up along the seaward edge of the champignon on the south side of the atoll.

The distinction between platin and champignon is of direct ecological importance because it corresponds to a distinction between two main types of vegetation<sup>6</sup>, which in turn are strongly correlated with faunal distributions. Champignon is covered with a dense thicket of *Pemphis acidula* which is almost impenetrable. There is virtually no soil except in the bottom of some potholes and the bare rock surface supports sparse tufts of *Mariscus ligularis* and *Eragrostis riparia*. Platin is covered with a more open mixed scrub of *Euphorbia abbotii*, *Thespesia populnea* and other trees, and an irregular turf of *Fimbristylis spathacea*. *Pandanus* forms conspicuous clumps around the larger freshwater pools. Hemsley<sup>7</sup> has compiled a flora of Aldabra, totalling some 173 species, of which 10 per cent are thought to be endemic.

Oceanic isolation combined with relative proximity to Africa and particularly Madagascar, together with the wide range of habitats presented by an uplifted compared with a sea-level atoll, have resulted in the development of an unusually rich fauna at Aldabra. The inhospitability of the environment, the scarcity of potable surface water, the lack of soil for growing coconuts, and above all the absence of workable guano deposits<sup>8</sup>, have meant that Aldabra has preserved its isolation while neighbouring islands such as Assumption have been drastically exploited by man.

Indigenous mammals are represented only by two insectivorous bats, and by an endemic fruit-bat, *Pteropus alda-branus*<sup>9</sup>. It is the giant land tortoise *Testudo gigantea*, however, which is of greatest interest and importance in the land fauna<sup>10</sup>. With those of the Galapagos Islands (*T. elephantopus*), the land tortoises of Aldabra form the only surviving natural populations of the giant form. In the early eighteenth century, *T. gigantea* was found on islands from Madagascar to the Seychelles, the Mascarenes, and even to the Chagos Archipelago (Fig. 1). Changes in habitat, competition from introduced animals and above all predation by man led to the disappearance of wild herds on all islands except Aldabra by 1840. Tortoises are now concentrated on the platin at the east end of South Island, where, during the dry season, they wallow in the drying freshwater pools during the early hours of the day and retreat to the shade of *Pandanus* when the sun is high. During the wet season, when large areas of platin are flooded by freshwater, they are probably more active and then breed. Despite a number of nineteenth century references to small or declining numbers, it is certain that the tortoise population now exceeds ten thousand. Tortoises feed on the *Fimbristylis* turf and the lower leaves of shrubs. Any attempt to move them to less favourable habitats on champignon would undoubtedly lead to considerable mortality. In addition to the tortoises, land reptiles are also represented by two geckos and a skink<sup>11</sup>.

The insects of Aldabra are fairly well known as a result of intensive collecting by Abbott<sup>12</sup>, Voeltzkow<sup>13</sup>, Fryer<sup>14</sup> and, more recently, by Legrand<sup>15</sup>. More than 350 species are recorded, with dominantly East African and Madagascan affinities; this compares with less than a hundred

species on most of the sea-level Indian Ocean atolls<sup>16</sup>. So little is known of the insect faunas of neighbouring islands that the degree of endemism is uncertain, but Legrand's recent monograph of the Lepidoptera<sup>15</sup> records thirty-five endemic species and twelve endemic sub-species in a total of 127 species. There is also a single earthworm, a common scorpion, several spiders and an inadequately known land mollusc fauna which includes one endemic species *Rhachistia aldabrae*<sup>17</sup>. The robber crab *Birgus latro* is common. A rich freshwater fauna has been found in the platin pools, including crustaceans (fairy shrimps *Streptocephalus* sp., conchostracans *Eulimnadia* sp. and ostracods *Heterocypris* sp.) and molluscs (notably a species of *Bulinus*). One freshwater "well" also contains a semi-freshwater fish of the gobiid genus *Tamanka* not previously recorded.

The birds of Aldabra<sup>18</sup> are interesting for two reasons: first, the presence of a number of endemic species and sub-species of land birds<sup>19</sup>, which are closely related to the land birds of Madagascar and the Comoros; and second, the existence of large breeding colonies of sea birds. Among the land birds the drongo *Dicrurus aldabranus* is most distinct.

Possible endemic sub-species include a fody *Foudia aldabrana*; a sunbird *Nectarinia souimanga*; a white-eye *Zosterops maderaspatana*; a turtledove *Streptopelia picturata*, and blue pigeon *Alectroenas gonzini*; a nightjar *Caprimulgus madagascariensis*; the coucal *Centropus toulou*; the kestrel *Falco newtoni*; the sacred ibis *Thres-*

*kiornis aethiopica*; and a rail *Dryolimnas cuvieri*. The flightless rail is now the last of the flightless birds of the Indian Ocean islands, which once included the dodo and the solitaire. On Aldabra it is now found on Middle Island since cats were introduced, the spread of which to Middle Island would clearly soon lead to the bird becoming extinct. Other larger birds, such as the sacred ibis and a flamingo *Phoenicopterus ruber*, are now only found at the east end of the atoll far removed from the small human settlement at West Island. Sixty years ago the flamingo probably numbered 500–1,000 individuals; now there are probably no more than fifty<sup>20</sup>.

Of the sea birds, frigates *Fregata minor* and *F. ariel* and pink-footed boobies *Sula sula* nest in great numbers on the mangroves of Middle Island. The frigates greatly outnumber the boobies and spend the day soaring to great heights over the eastern end of the atoll and diving to drink on the wing at freshwater pools on South Island. The Aldabra frigate colonies serve as the major breeding ground for the frigate population of the western Indian Ocean and a considerable non-breeding population certainly exists in this area (Bourne, W. R. P., personal communication). Other sea birds<sup>21</sup> include tropicbirds *Phaethon lepturus lepturus* and *P. rubricauda rubricauda*, terns *Sterna fuscata* and *S. sumatrana mathewsi*, fairy terns *Gygis alba monte* and noddies *Anous stolidus pileatus*. The most common shore birds include little egrets (*Egretta garzetta*), which show dimorphism as in Madagascar, and

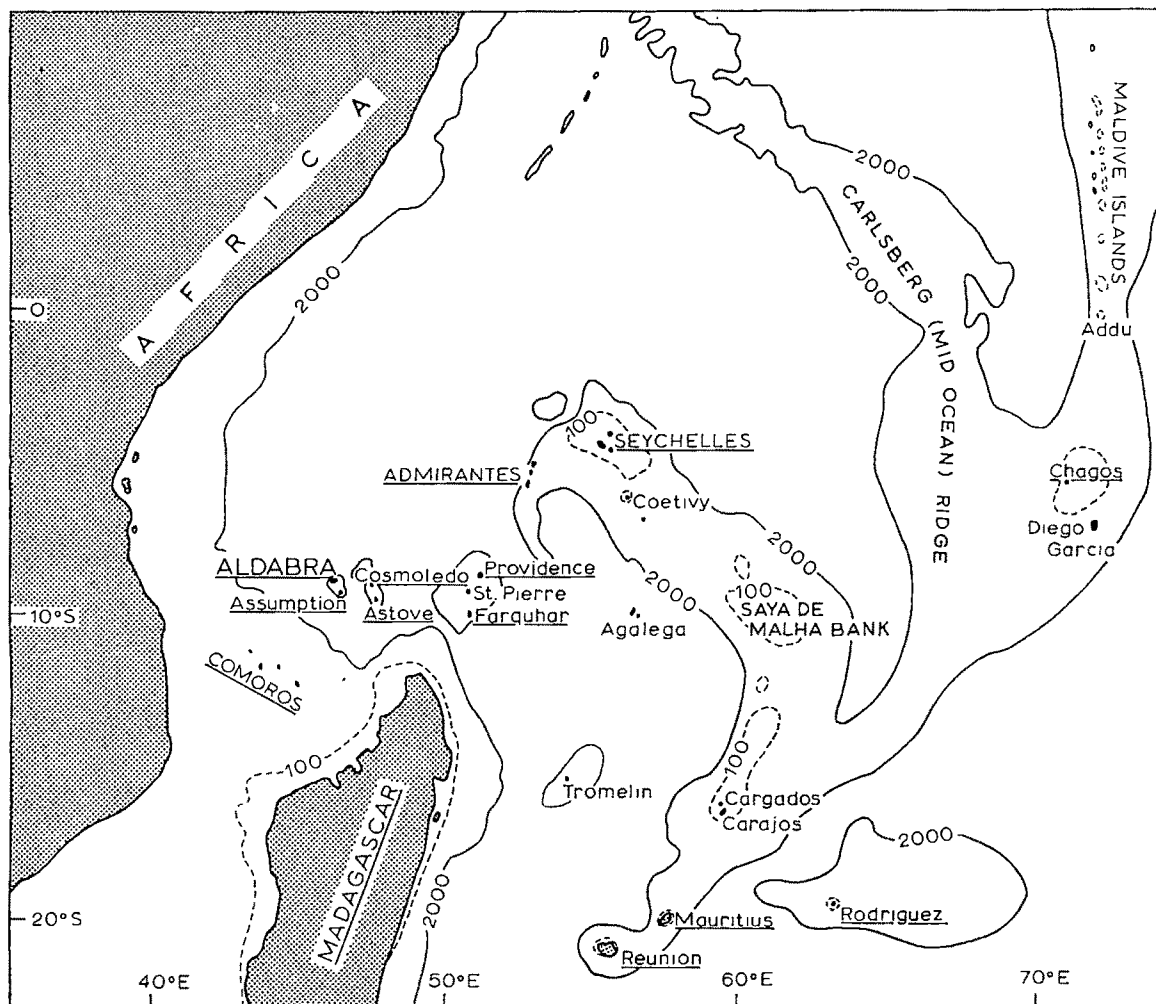


Fig. 1. Location of Aldabra in the western Indian Ocean. The islands underlined are those where the giant land tortoise existed in the eighteenth century, according to Rothschild (ref. 10).

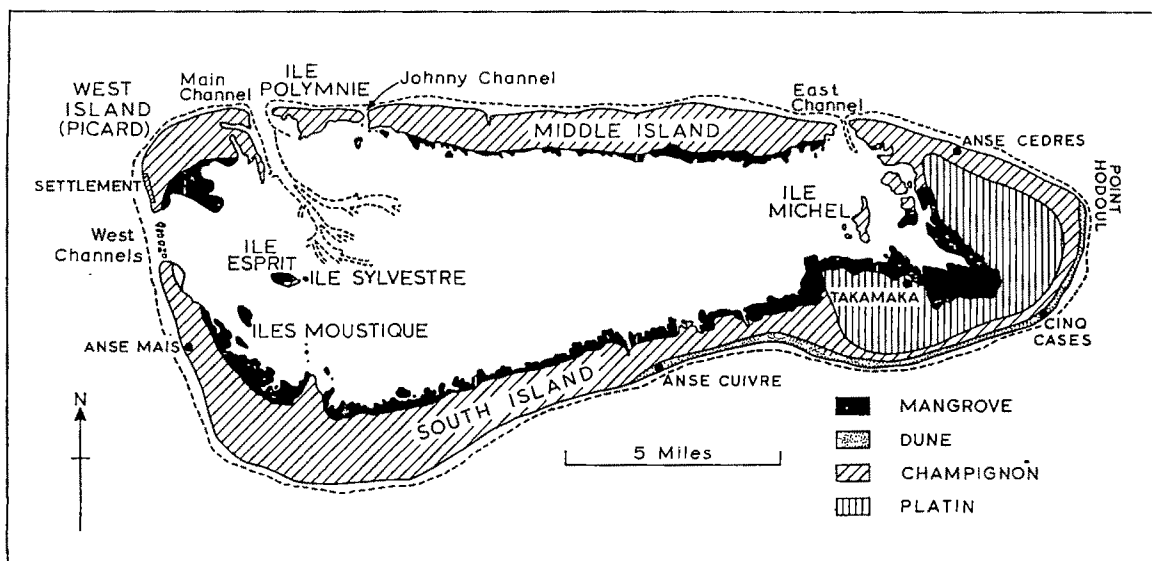


Fig. 2. Main terrestrial habitats of Aldabra Atoll.

the grey and little green herons *Ardea cinerea* and *Butorides striatus*. Large numbers of wading birds feed on drying sand flats in the lagoon at low tide.

Knowledge of the marine biota of Aldabra is particularly inadequate, though the brachyuran decapod crustacea have recently been reviewed by Guinot<sup>22</sup>. It is known that Aldabra, Cosmoledo and Assumption form the largest breeding ground for green turtle *Chelonia mydas* in the Indian Ocean<sup>23</sup>. Commercial exploitation has led to a catastrophic decline in breeding numbers despite protective legislation. The hawksbill *Eretmochelys imbricata* is also exploited and is declining in numbers. The green turtle population is thought to migrate from the Mozambique Channel, and breeds at Aldabra from February until September; however, no field study of this reptile has ever been carried out. The coral reef fauna appears to be poor and the prospects for commercial fisheries are also disappointing<sup>24</sup>. Studies are needed of marine algae, marine mollusca and other groups.

An attempt was made by Norwegians to colonize Aldabra in 1879, but the present settlement began with the first commercial lease of the atoll in 1888. A small group of Seychellois labourers, now numbering up to 100, takes turtle, fish and timber from the atoll. Within a few years of the first lease being granted, coconuts and other cultivated plants were introduced at the West Island settlement and other small beaches, together with weeds and domestic animals. As a result, there are now feral goats, dogs, cats, rats and mice at Aldabra. Large herds of feral goats have been described in the past, but few were seen in 1966; their role in modifying the island ecosystem remains unclear. Most of the introduced mammals are confined to South and West Islands, and have not yet reached the sea birds and the flightless rail of Middle Island.

Concern over the exploitation of Aldabra and the "imminent extermination of the Gigantic Land-Tortoises of the Mascarenes" was first expressed in a letter sent to the Governor of Mauritius in 1874 by a group of scientists which included Charles Darwin, Richard Owen, and Joseph Hooker. Even at that time it could be stated that "Aldabra is now the only locality where the last remains of this animal form are known to exist in a state of nature", and it was argued that:

"The rescue and protection of these animals is, however, recommended . . . less on account of their utility . . . than on account of the great scientific interest attached to them. With the exception of a similar tortoise in the Galapagos Islands (now also fast disappearing), that of the Masca-

renes is the only surviving link reminding us of those still more gigantic forms which once inhabited the continent of India in a past geological age. . . . It flourished with the Dodo and Solitaire; and whilst it is a matter of lasting regret that not even a few individuals of these curious birds should have had a chance of surviving the lawless and disturbed conditions of past centuries, it is confidently hoped that the present Government and people . . . will find a means of saving the last examples of a contemporary of the Dodo and Solitaire"<sup>25</sup>.

Protective legislation has been enacted from time to time by the Government of the Seychelles, mainly to protect the turtle industry and certain birds, and under the terms of the present commercial lease, granted in 1955, the whole of South Island, where it is planned to build an airfield, is designated as a nature reserve. Legislation in effect in 1965 under the laws of the Seychelles continues to apply under the new administrative arrangements whereby Aldabra forms part of the British Indian Ocean Territory<sup>26</sup>.

No major scientific studies have been carried out at Aldabra since J. C. F. Fryer's work in 1908-9. The relatively undisturbed character of the Aldabra ecosystem, the overriding importance of its population of the giant tortoise, its great sea bird breeding colonies and distinctive land birds, and its green turtle breeding grounds all require detailed study. Based on a reconnaissance expedition in 1966, conservation plans are being made in the event that the Ministry of Defence decides to proceed with the construction of an airfield, harbour and other facilities, and the B.B.C. with a transmitting station. At the same time, the Royal Society, after consultation with other scientific bodies, is organizing an expedition to make urgent scientific investigations before any disturbance takes place. This expedition, involving fourteen scientists, will last from August 1967 to March 1968, spanning both dry and wet seasons, and will cover both marine and terrestrial ecology. If development proceeds, the expedition will provide basic data for conservation planning, while if the present plans are abandoned, it will lay the groundwork for long-term scientific studies of an undisturbed island ecosystem unique in the Indian Ocean and perhaps in the world.

The 1966 scientific reconnaissance was made possible by the courtesy of the British Broadcasting Corporation and the Ministry of Defence, and by the support of the Royal Society. We thank the former Minister of Defence for the Royal Air Force, Lord Shackleton, and the Hydrographer, Rear-Admiral G. S. Ritchie, for their active interest; and Mr. A. Bosworth, British Broadcasting

Corporation, expedition leader; Mr. C. E. Loveridge, Ministry of Public Building and Works; Wing Commander P. A. S. Thompson, Ministry of Defence; Sir George Taylor, Director of the Royal Botanic Gardens, Kew; Dr. W. R. P. Bourne; and Dr. F. R. Fosberg, for their aid. The late Professor C. F. A. Pantin gave much initial encouragement.

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## From Practice to Theory and Back

by

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Should engineers be taught at university in the same way as scientists? In what follows, the author expands on a talk to the Midlands Branch of the Institution of Chemical Engineers at Loughborough University on February 22.

ENGINEERING is an activity which begins with practical problems and ends with practical solutions. It calls for an understanding of science and the ability to apply it, but most engineers are not primarily scientists. The object of this article is to suggest that the university education of engineers is too closely modelled on that of scientists and that, as a result, most graduates leave university with an insufficient range of knowledge to enable them to practise successfully as engineers. Industry is obliged to teach them not only skills but also principles and techniques which could have been learnt earlier.

It is important to distinguish between skill, which must be developed on the job, and knowledge, which is more easily acquired away from it. (Skill in this sense is not necessarily manual, it may be technical or managerial.) Skill is the product of experience; knowledge can also come from experience but is best inculcated by systematic teaching. The practice of engineering calls for both knowledge and skill, and therefore graduates are not recognized as fully qualified engineers until they have had some years of responsible experience. In this respect the British system of education is ahead of most others. In the interests of economy, however, the knowledge and skill which an engineer needs should each be imparted to him by the most efficient means. For developing skill there is at present no satisfactory substitute for experience on the job. The inculcation of knowledge, however, is the specialized function of schools, colleges and universities, and one in which they have considerable expertise. One might therefore suppose that the universities and colleges ought to give the young engineer the knowledge he needs, and industry should provide the requisite experience, neither side encroaching on the function of the other.

This is not quite what is happening. Apart from, and in addition to, the required period of responsible experience, there is a trend in industry towards formal courses lasting for 1 or 2 years in which the graduate has the status of a trainee and is instructed in subjects such as organization and management, work study, economics and costing, factory legislation and so forth as well as in company practice. Courses of this kind proliferated after the Second World War when industrial companies found that by

setting up training schemes they could recruit engineering graduates direct from the universities and bypass national service. In chemical engineering, where the apprenticeship system had never been customary, there were no graduate training schemes known to the Institution of Chemical Engineers in 1946. By 1960, when national service ended, forty-seven approved schemes were in operation. In 1965 the number had risen to fifty. The continued existence of these courses, which must be quite costly to the firms concerned, suggests that they are meeting a genuine need. It must be a need peculiar to the United Kingdom because little if any formal training in industry is to be found elsewhere.

The continued need for graduate training schemes in Britain is in part a reflexion on the universities. In so far as the training imparts knowledge as well as skill it is doing a job which could have been done, and possibly done better, in a teaching establishment. The report of the Bosworth committee<sup>1</sup> recommends an industrial training course for electrical and mechanical graduates lasting for between 1 and 2 years; but it was strongly represented to the Bosworth committee, especially by young graduates, that several elements of the proposed training could with advantage replace less relevant material in the undergraduate course. My own experience in industry was that formal graduate training schemes tended to be unpopular both with the graduates themselves and with the middle and lower ranks of professional engineers who were expected to do the teaching. The former are tired of being *in statu pupillari* and eager to get their teeth into a responsible job; the latter already have a full-time job and may not like teaching anyway. Perhaps, therefore, the universities ought to look more closely at the work an engineer is required to do and consider whether they could not provide a more useful tool kit.

Engineering achievement is a product of four factors—knowledge, time, money and men. The term "product" is used in a quasi-arithmetical sense; if any one of these factors is zero then the total achievement is nil. If one factor were unduly low, then it would be necessary to increase some or all of the others in order to achieve a given result. Universities quite properly concern them-



selves with the factor of knowledge, but it tends to be limited to a knowledge of what is called engineering science. The principles which govern efficient management of time, money and men are supposed to be learnt by experience. This supposition may have been justified when industry was managed by rule-of-thumb, but today there are techniques and disciplines which largely replace rule-of-thumb and which can be systematically taught. For example, the more sophisticated applications of method study, critical path planning, linear and non-linear programming, cost-benefit analysis, project evaluation, and organization analysis. The logical content and level of abstraction of these disciplines of thought render them at least as "educational" as some of the subjects now studied. Furthermore, most engineers are unlikely ever to acquire a thorough grounding in the principles involved unless they get it at the university.

A competent engineer must be able to do three things: (1) translate a practical problem into a theoretical problem; (2) solve the theoretical problem; (3) convert the theoretical solution into a practical solution.

University engineering education concerns itself primarily with stage 2; yet it is a common experience among engineers that the greatest difficulties often arise in stages 1 and 3. There are areas of knowledge not usually touched on in engineering courses, such as those mentioned in the previous paragraph, which can help in both these stages.

In stage 1 the first task is to determine what the problem actually is. In any real situation the engineer is usually faced with a confused mass of information, some of which is conflicting and most of which is irrelevant. The correct selection of the relevant variables and parameters and the construction of a simplified conceptual model of the system may be more than halfway towards a solution of the problem. In this respect the numerical "examples" worked by university students are largely unreal because the relevant data have already been selected by the examiner. It may be possible for university teachers to adapt and to use some of the newer techniques of thought which have been developed in industry, such as formal critical examination<sup>2</sup> and PABLA (problem analysis by logical approach)<sup>3</sup> in order to inculcate the habit of asking the right question before rushing in to give the answer.

Stage 3 is the creative stage of the work of an engineer. Here the kind of detail which was discarded in stage 1 must be reintroduced in order to convert the theoretical solution into a workable piece of hardware or a practicable procedure. The type of thinking required is no longer the abstract analysis of pure science but a faculty that might be called "physical imagination". Analysis raises the thinker from the concrete to the abstract level of thought; imagination returns him from the abstract to the concrete, an equally difficult transition. The good engineering designer, having calculated his theoretical design parameters, must create in his mind's eye a detailed image of the final product and must conduct imaginary experiments with it in order to arrive at the best arrangement. Similarly a good managing engineer must visualize in advance the effects of alternative decisions. How to develop in students the faculty of physical imagination is a major problem. A start might be made by substituting freehand engineering drawing for the drudgery of the tee-square, as is already done in some American colleges, and requiring all solutions of problems to be supported by dimensioned sketches of equipment. Works visits and vacation employment in industry are helpful in stocking the student's mind with images of actual plant.

Physical imagination is the faculty which above all others distinguishes the born engineer from the born mathematician or pure scientist. It is the source of invention as opposed to discovery, and is so important that perhaps a special test should be given by engineering schools to pick out applicants who possess this faculty. It can be further developed during the course by the use of

graphical and geometrical concepts wherever possible as well as by open-ended problems which exercise the imagination. The technique of formal critical examination<sup>2</sup> which was mentioned under stage 1 can also be used to encourage creative imagination in design. As developed and used in the I.C.I. organization it involves the following basic sequence: (1) uncritical imagination throwing up a set of possibilities; (2) critical examination of these possibilities; (3) decision on the course to be followed. These are, of course, the stages of all creative thought, but in the method referred to they are systematized in such a manner that no possibility, however unusual, is likely to be overlooked. The first response of undergraduates to this technique is sometimes completely wooden; but in the more imaginative it soon releases a flood of ideas and enthusiasm. Furthermore, the habits of thought which it engenders are of permanent value in every kind of enterprise.

Little need be said here about management education proper, because British universities are at last awakening to the importance of this subject. For the most part, however, management studies appear to be developing in the context of economic and social science rather than of engineering. It is therefore worth emphasizing that to a practising engineer management principles are at least as important as mathematics. Engineering is a large-scale activity which requires the directed and co-ordinated efforts of many different groups and individuals. If the direction and co-ordination are inefficient, then, however good the scientific work, the total engineering job will be done badly. Many of the complaints about British engineering arise from management failures; for example, poor inspection or the late completion of projects. It is often argued that management principles should not be taught until experience has been gained in industry. The short answer to this is that only a small number ever attend post-experience courses. If some knowledge of management theory is to reach all engineers, then it must figure in the undergraduate syllabus.

Another common criticism of engineers is that they are bad at expressing themselves and communicating ideas. This is sometimes treated as a lack of skill in the use of English, and classes in grammar and composition are established to remedy it. The writings of students often reveal a more fundamental defect—the inability to think logically and present a reasoned case. It is a curious fact that in universities logic is not taught to those who have most need of it, especially scientists and engineers. All engineering students would profit from a course of instruction in symbolic and applied logic designed both to introduce them to the computer and to sharpen their ordinary thinking.

If, in order to produce a more balanced undergraduate course, university engineering departments were to interest themselves in the non-engineering science subjects mentioned here, some members of the academic staff would inevitably be drawn into research in these fields. This would be of benefit to industry as well as to the universities. The new planning and programming techniques have been developed to meet urgent practical needs, and their theoretical foundations have not always been very thoroughly explored.

Any attempt to broaden university engineering curricula is certain to meet with the objection that engineering courses are already overloaded and have no room for additional material. This argument betrays a complete misunderstanding of the problem. There is no received corpus of engineering knowledge that must be transmitted inviolate to the undergraduate. Engineering is a vast subject and only a very small part of it can be taught during the 3 or 4 years of an engineering course. The problem is to fill the short time available with whatever is most valuable. Dr. D. G. Christopherson<sup>4</sup> once observed that the overloading of this curricula stems not from demands by industry and the institutions but from the

way in which academics force their pet subjects into the syllabus.

It is easy to add material to a university curriculum but difficult to take it out on account of the internal pressures and interests alluded to above: thus, the chronic overloading. In my experience the only instrument that can overcome these pressures is factual information about the sciences and techniques which engineers are currently using; not the opinions of the professors, nor the opinions of employers (who sometimes do not know what their engineers are really doing), nor even the opinions of the engineering institutions, but objective first-hand data derived from a systematic survey. This has been done among chemical engineers and the two surveys conducted in 1960 and 1965 (refs. 5 and 6) provide quantitative data on the relative usage of different subjects. Professor S. P. Hutton has carried out a somewhat similar exercise among mechanical engineers<sup>7</sup>.

When all these arguments are admitted, however, it may be found that 3 years is too short a period in which to give young engineers the minimum equipment they will need if these additional aspects are to be covered. The present conjunction of overloaded syllabuses at universities with further instruction in industry might seem to support this view. In fact, much of what has been advocated would involve changes in emphasis rather than added matter; nevertheless, the possibility must be faced that 3 years may simply not be long enough. It is then a straight choice between teaching the additional material in industry or extending the university course. At present there is no factual evidence on the efficiency of teaching in industry as compared with that in the universities. What is needed is an independent survey of existing graduate training schemes and an assessment of the quality of the teaching and the amount of knowledge gained by the trainee. I feel that a teaching institution is likely to teach more efficiently than an industrial organization: consequently everything that is teachable at the university should be taught there.

If university engineering courses are to move closer to engineering as it is actually practised, certain changes will be necessary in the make-up of the academic staff. It ought to contain a leavening of engineers with industrial experience at all levels of seniority. This is because it is not possible to practise engineering at a university in the sense that one can practise science. As the industrial experience of a university teacher recedes into the past he inevitably becomes less of an engineer and more of a scientist. Consulting work may delay but cannot arrest this gradual loss of touch. How to attract mature engineers of high calibre into university teaching is a problem which should not be insoluble. One way not to attract them is to insist that applicants for senior academic posts in engineering shall always be able to produce long lists of publications. It may be more relevant to enquire what plants they have designed, built and operated, and what projects they have carried through successfully.

This, then, is a plea for universities to pay greater attention to stages 1 and 3 of the engineer's task and provide a more adequate conceptual framework for the creative imagination of the designer, the diagnostic skill of the trouble-shooter, and the organizing ability of the managing engineer. The aim should be to produce a graduate who will be fit for responsible employment in a period of months rather than years.

<sup>1</sup> *Education and Training Requirements for the Electrical and Mechanical Manufacturing Industries*, Chairman, S. G. Bosworth, (H.M.S.O., 1966).

<sup>2</sup> Birchall, H., and Binstead, D. S., *Chem. and Indst.*, January 16, 1960. Also personal communications from the Central Work Study Department of I.C.I.

<sup>3</sup> Latham, R., *A Guide to the "Problem Analysis by Logical Approach" System* (A.W.R.E. Report, Aldermaston, 1965, unclassified).

<sup>4</sup> Christopherson, D. G., *Rep. Proc. Home Universities Conf.*, 1956, p. 23.

<sup>5</sup> Edgeworth Johnstone, R., *Trans. Inst. Chem. Eng.*, 39, 263 (1961).

<sup>6</sup> Edgeworth Johnstone, R., and Lax, C. B., *Chem. Engineer*, CE7 (Jan./Feb., 1966).

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## BOOK REVIEWS

### ALL ABOUT ALCOHOL

#### Alcoholism

*Mechanism and Management.* By Max Hayman. (A Monograph in the Bannerstone Division of American Lectures in Living Chemistry.) Pp. xv + 315. (Springfield, Ill.: Charles C. Thomas, 1966.) \$10.50.

MODERN films tend to open with an arresting scene—a burglary or murder—with which the subsequent action deals in more conventional fashion. In much the same way this book begins with a staggering list of facts about alcoholism, which become the more impressive for their presentation in this way.

The state of California (population about 17,500,000) had 886,000 alcoholics in 1962: it runs a close second to New York with 8,170 alcoholics in every hundred thousand of population more than twenty years old. In hospital in the United States as a whole alcoholism ranks fourth in the list of public health problems. These are just a few of the facts recorded.

The book is addressed to medical men, whether general practitioners or specialists. In so far as several chapters deal with psychoanalytic theory and practice in relation to alcoholism, it is meant for American doctors, whose medical training and orientation are such that they would feel the need for some presentation in these terms. The author, who has unrivalled experience in all aspects of alcoholism, makes it clear, however, that very few alcoholics are suitable for, or receive, formal psychotherapy of this kind, and that eclectic methods involving a wide range of skills and professions suit the majority of the sufferers best.

Alcoholism is presented as a medico-social problem, affecting human life at very many points. Certain selected aspects, biochemical, psychological, legal, psychiatric, pharmacological and others, are chosen for succinct review in relatively short chapters, each of which is followed by an excellent list of the most relevant publications. This design precludes an evaluation in great depth in any area, but has the advantage of covering the whole field in readable fashion, while giving the more sophisticated reader plenty of leads for pursuing a particular interest.

D. L. DAVIES

### PULMONARY CIRCULATION

#### Pulmonary and Bronchial Vascular Systems

*Their Reactions under Controlled Conditions of Ventilation and Circulation.* By I. de Burgh Daly and Catherine Hebb. (Monographs of the Physiological Society, Vol. 16.) Pp. xv + 432. (London: Edward Arnold (Publishers), Ltd., 1966.) 90s. net.

DR. I. DE B. DALY's published contributions to the study of pulmonary circulation extend over forty years, and he and Dr. Hebb between them have used each one of the experimental approaches discussed in this book.

The book opens with an account of the functional anatomy of the lung, which lays the essential foundation for the evaluation of subsequent experimental results. Important quantitative data on lung architecture are assembled and species differences are illustrated by

hitherto unpublished photomicrographs by Dr. Hebb. Two important conclusions emerge from an examination of the literature on the interrelation between bronchial and pulmonary circulations: that changes in pulmonary vascular flow and resistance may be secondary to changes in the bronchial circulation, but, on the other hand, the functional interrelationships between the two circulations demonstrated in experimental conditions can be very different from those in the intact animal. All the work on the pattern of innervation of the lung was done until very recently with the use of silver or methylene blue staining—both methods with severe limitations. The results obtained with recent histochemical methods—Koelle's method for cholinesterases and Hillarp and Falck's for catecholamines and 5-hydroxytryptamine—are reviewed and recent results obtained by Dr. Hebb are included. The use of Hillarp and Falck's fluorescence method is likely to solve many unanswered questions. The discussion in the chapter on haemodynamics is relevant to problems beyond those of the pulmonary circulation and includes very valuable comments by L. E. Bayliss on Burton's theory of critical closing pressure. The chapter concludes with an examination of the complicating effects on pulmonary blood flow introduced by lung inflation and stroma stresses. The next chapter is concerned with the criteria for distinguishing primary pulmonary vasomotor responses from secondary or passive effects on the pulmonary circulation caused by changes in the bronchial circulation, in airway diameter or lung volume. This allows a critical evaluation to be made of the experimental results described in the remaining five chapters.

Chapters six and seven deal with the voluminous, conflicting and confusing literature on pharmacological experiments, using the acetylcholine family of drugs (including analogues, blockers, potentiators), the catecholamines and 5-hydroxytryptamine. To the authors' many reservations in the interpretation of the results with drug applications another could be added: recent evidence from fluorescence and electron microscopy has shown that the nerve fibres in most blood vessels are restricted to the medio-adventitial junction. The chemical transmitter released by the nerves, therefore, gains access primarily to the outer layer of the media. This cannot be mimicked by either intravascular injection of the drug or soaking a ring of blood vessel in solutions containing the drug.

The final three chapters deal with the effects of various ventilating gas mixtures, and of nerve stimulation on the pulmonary circulation and the problem of pulmonary vasomotor reflexes. Earlier workers failed to get results with nerve stimulation. Dr. Daly showed that survival of the nerve supply depended on the bronchial circulation. His pioneering work on lung perfusion technique is contained in an invaluable appendix. Using perfused lung preparations Dr. Daly in collaboration with M. de B. Daly has provided important evidence for pulmonary vascular reflexes from the carotid body and carotid sinus.

The great value of this book lies in the rigorous examination of a number of basic problems and the stepwise synthesis of a picture of an integrated physiological system.

MARIANNE FILLENZ

## PARASITE COLLOQUIUM

### Host-Parasite Relationships

By James E. McCauley. (Proceedings of the Twenty-sixth Annual Biology Colloquium, April 23-24, 1965.) Pp. 148. (Corvallis: Oregon State University Press, 1966.) \$4.50.

THE twenty-sixth annual Biology Colloquium, held in April 1965 at Oregon State University, was attended by six specialists in the subject of host-parasite relationships, whose contributions are published in full in this booklet

of 148 pages, edited by J. E. McCauley. J. F. Mueller, who has for years made a study of diphyllbothriid tapeworms, describes many aspects of *Spirometra mansonioides*, a parasite of carnivores most commonly found in domestic cats which harbour the adult worms and whose larval development takes place in copepods and reptiles. This paper is concerned mainly with the biology of larval stages in experimental animals and in *in vitro* culture; growth rate, immunity, weight gain and longevity of infected animals, and aberrant forms of the parasite are discussed in this beautifully illustrated article. H. W. Manter discusses the trematode parasites of fishes and their relationships with their hosts in regard to the information they convey both about their own geographical distribution and that of their hosts in ancient times; they also function as "indicators" of evolution in the same way as fossils provide information to palaeontologists. G. H. Ball investigated the relationship of the avian malaria parasite, *Plasmodium relictum*, to its mosquito host, by *in vitro* culture of the mosquito phase of the parasite, to determine its biochemical needs. His experiments have shown that the mosquito environment is not essential physiologically or biochemically for the development of the parasite, whose relationship to the mosquito's organs is an ecological one. J. R. Douglas and N. F. Baker are concerned with certain nematode parasites of the dog ("man's best friend"), particularly with *Toxocara canis*, which in the past decade has been shown to be a most unfriendly parasite, transmissible to human beings, sometimes with serious consequences. P. H. Silverman gives some immunological aspects of infection with helminth parasites and, by means of examples from the cestodes, nematodes and trematodes, he contrasts the mechanism of metazoan and microbial immunity. E. L. Schiller reviews the host-parasite relationship of members of the cestode genus *Echinococcus* from the ecological standpoint, with special reference to their occurrence in arctic wildlife and in man.

J. J. C. BUCKLEY

## ENVIRONMENT AND BEHAVIOUR

### Environmental Biology

Compiled and edited by Philip L. Altman and Dorothy S. Dittmer. (Biological Handbooks.) Pp. xxi + 694. (Bethesda, Maryland: Federation of American Societies for Experimental Biology, 1966.) \$15.

THIS handbook, prepared by the Federation of American Societies for Experimental Biology, attempts to bring together in a single publication the pertinent information relating to the effects of the environment on the behaviour of living organisms—plants and animals including man. The contents have been selected by an advisory committee comprised of R. L. Zwemer, A. R. Behnke, L. D. Carlson, H. J. Curtis, D. B. Dill, D. E. Goldman, R. A. McFarland, A. G. Norman, M. B. Russell and H. M. Webb, and prepared by some 450 biologists.

The information is classified in the first place in relation to one of ten major environmental factors. Under each heading the effects of various attributes of the particular environment on a particular biological function are stated where quantitative data are available. Most of the information is presented in tabular form with an introductory heading giving essential details about, for example, units of measurement, definition of terms, and followed by a citation of the literature from which the table has been prepared. Values are generally presented as the mean with either the standard deviation or the upper and lower limits of the range of individual values about the mean.

The first 120 pages of the handbook are devoted to temperature control and the effects of raised and lowered environmental temperatures on such varied biological quantities as body temperature, heart rate, cardiac output,

performance, growth of various mammals and rates of growth of bacteria, fungi and viruses. A section dealing with the effects of light and ionizing radiation occupies the next fifty pages and this is followed by a forty page section, the contents of which are devoted to the effects of sound, vibration and impact. The effects of acceleration and gravity are described in a twenty page section primarily in relation to man. The effects of the gaseous atmosphere on plants and animals are considered in three distinct sections totalling 180 pages, dealing with high and low concentrations of oxygen and carbon dioxide; total environmental pressure and atmospheric pollutants. The effects of water and solutes are considered in separate sections (with a total of 100 pages) primarily in relation to plants and aquatic animals. The tenth section of forty pages is devoted to biological rhythms. The book has two appendixes which give corresponding scientific and common names for animals and plants.

This handbook provides within a single cover a very extensive range of quantitative biological data. It is inevitable in a book of this type that some of the limitations of the value of particular groups of data are not apparent. In general, however, references to the literature are extensive and the book provides an easy method of access to the literature of fields outside a reader's normal sphere of knowledge. Thus, while the handbook could with advantage be held in the libraries of biological research and teaching institutions, the individual worker will probably find it of limited value in his own field.

J. ERNSTING

## LABORATORY BIOLOGY

### An Experimental Approach to Biology

By Robert G. Thomson and Peter Abramoff. Pp. xi + 253. (San Francisco and London: W. H. Freeman and Company, 1966.) 26s.

THIS laboratory guide contains protocols for thirty-five experiments in nine areas of biology, and it is clear that the authors are well aware of students' difficulties. An introductory section covers safety measures, cleaning glassware, preparing standard solutions, and the use of centrifuges and volumetric glassware, while appendixes deal with pH meters and colorimeters.

The book would prove useful to those planning sixth form and introductory university courses, although some caveats must be entered. The experiments on active transport through bags of frog's skin, and on the induction of ovulation by injecting frogs with pituitary extracts prepared from anaesthetized frogs, will require care if the spirit of the laws protecting animals is to be observed. Measuring the volume of gas produced by *Elodea* without further analysis gives misleading data on the production of oxygen—and to show that the gas turns pyrogallate brown merely demonstrates the presence of oxygen, not its concentration. A large water reservoir in potometers is an anachronism, and in any event transpiration is easy to estimate directly by weighing. Benedict's solution contains sodium carbonate, not bicarbonate.

These are errors which can be corrected in a second edition. The general standard of the book is high, and it can be recommended.

A. K. THOMAS

## STATISTICAL PAPERS

### The Selected Papers of E. S. Pearson

(Issued by the Biometrika Trustees to celebrate his 30 years as Editor.) Pp. vi + 327. (London: Cambridge University Press, 1966.) 40s. net.

THE journal *Biometrika* was founded by Karl Pearson, W. F. R. Weldon and Francis Galton in order that papers

on the applications of statistical method to biology and allied subjects might be published speedily and without hindrance from the editors of journals not specifically designed to cover such topics. The journal grew in circulation over the years but, such was the authority of Pearson in the statistical world, it remained very largely a house-journal until his death. When his son, E. S. Pearson, took over as editor in 1936 the statistical climate as well as the journal were changing. The proliferation of statistical centres all over the world was then beginning, which resulted in an outpouring of research papers which has grown steadily in volume until the present day.

*Biometrika*, the statistical world generally, and the English statistical world in particular owe much to E. S. Pearson's devoted and conscientious editorship during this climactic period. As ruler over a journal, still at the present day pre-eminent in England, he was able to influence to some extent the thinking of his contributors, and if we have not yet abandoned the search for common sense in data in favour of the hunting of the mathematical snark this is in part due to his careful refereeing and editing.

Much of E. S. Pearson's own work was published in *Biometrika* and it is fitting that the trustees should issue some of these papers to mark his retirement from the editorship. Out of the twenty-one papers all but three were first printed in the journal, half of them since 1945. A further volume, which will reflect his research activity in company with J. Neyman during the thirties, is promised in a short time. The papers republished in this present volume show Pearson's main interests over the years—non-normality, bases for the tests of hypotheses, analyses of accident data, approximations to probability distributions using moments.

It was a happy thought of the trustees to accompany this present volume with a volume containing the joint Neyman-Pearson papers and, in consort with the University of California Press, a volume of Neyman's own papers. Statisticians the world over will be grateful for their enterprise.

F. N. DAVID

## UNDERWATER SOUND

### Ocean Acoustics

Theory and Experiment in Underwater Sound. By Ivan Tolstoy and C. S. Clay. (Advanced Physics Monograph Series.) Pp. x + 203. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1966.) 124s. 6d.

THE rather few monographs in this field tend to lean strongly toward either applied mathematics or engineering. This short volume holds admirably to a middle course, balancing mathematical analysis against experimental knowledge, and the idealizations required for a coherent theory against the real inhomogeneities and fluctuations.

The theme is acoustic propagation in shallow and deep water; sources, receivers and (except in one chapter) signal processing are purposely given little attention. The main attack is by normal-mode theory, in terms of eigenfunctions and eigenvalues rather than of residues in the complex plane. Ray methods and the intermediate WKB approximation are, however, carefully related to normal modes, and are used frequently. Geophysical and oceanographic complexities of the medium are constantly emphasized as limitations on feasible theory and on comparisons with experiment. Many such comparisons are given, numerically or graphically, with both clarifying and cautionary effects. Citations from the literature, averaging thirty in a chapter, amplify mathematical details, extend the theory, and give experimental results.

The preface succinctly outlines aims and methods, and the first chapter is an excellent compact account of the chief acoustical properties of the ocean. Thereafter, mathematical description builds up step by step. Successive chapters treat basic theory; normal modes; shallow-water propagation; deep-water propagation; effects of irregularities; and measurements as problems in filter theory. A brief chapter of conclusions goes beyond a résumé by outlining the need for carefully planned combinations of theory, measurement and statistical analysis if much more progress is to be made.

The book can be highly recommended to neophytes, but also to experienced workers heretofore daunted by differing, widely scattered and often over-formal presentations. No one could make this subject easy, but the authors hold to essentials, develop them systematically, and discuss them in a physical context.

A. O. WILLIAMS, JUN.

## DYNAMIC STARS

### Dynamics of Stellar Systems

By K. F. Ogorodnikov. Translated from the Russian by J. B. Sykes. Translation edited by Arthur Beer. Pp. xii+359+28 plates. (Oxford, London and New York: Pergamon Press, Ltd., 1965.) 100s. net.

TEN years after it was written this book can hardly read as a fully balanced account of the subject. In those years stellar dynamics has been re-enlivened by contacts with plasma physics, stellar element abundance work, celestial mechanics and ergodic theory, so it is no surprise to find little mention of those. The book's main emphasis, however, that stellar systems have settled to some form of detailed statistical quasi-equilibrium, is still well placed. No detailed theory of the relaxation process exists although it still seems likely that it is the large scale density fluctuations or star clouds that cause "irregular" gravitational fields and thus cause relaxation of stellar orbits. Even from the swarming workers on plasma physics no full theory of the relaxation of a collisionless medium has emerged. Recently computer experimenters and theorists have tackled this problem which lies at the basis of galactic dynamics. So far Ogorodnikov's assumption that the rotating self-gravitating Maxwellian distribution will be the outcome seems not too far from the truth; however, it appears that equipartition of kinetic energy will be replaced by an equality of the velocity dispersions of stars of different masses.

The book has other strong points. First, the emphasis that Schwarzschild's ellipsoidal hypothesis, though locally a good rough approximation, is actually false, so that theory based on its exact truth everywhere leads to nonsense. Second, there is the importance given to work on the third integral, a subject that has developed considerably since the book was written and which has even given rise to developments in celestial mechanics.

There are also weak points, however. The author has chosen to ignore work on evolution resulting from individual stellar encounters based on Chandrasekhar's dynamical friction. The Fokker-Planck equation and the difficult task of deriving the Fokker-Planck diffusion coefficients are hardly mentioned and the student is thus given few clues as to how to set up the mathematics necessary for a full discussion of the evolution of star clusters.

It is argued that apparent smoothness of the form of a galaxy must imply the presence of a dissipative viscosity. In fact such forms could be attained quite otherwise as a result of mere orbital propagation of the distribution function. These are perhaps matters of author's licence; the following error is not. In equations 4.44 and 4.45 approximate expressions for changes in velocity resulting from an encounter are compared and contrasted. Unfor-

tunately in one  $\Delta V$  is the change of relative velocity while in the other  $\Delta V$  is the change of velocity of only one of the two interacting bodies. Had this been taken into account disagreement would have been avoided.

Physicists and astronomers who have not looked at graded exposures of galaxies should be warned against "theorem III" on page 306. "In any dynamically determinate galaxy the star density if sufficiently smooth is constant in a first approximation and . . ." In practice one of the outstanding characteristics of most galaxies is the density contrast as one proceeds outwards from the centre. This is so great that all the central regions are normally burnt out on exposures that show the outer parts. The words "sufficiently smooth" are thus included in theorem III to eliminate all galaxies save a very few.

In spite of these shortcomings Ogorodnikov's book is clearly written and translated and is likely to be a standard text for some courses on stellar dynamics. It is a pity that students must be warned against certain passages, and above all it is a pity that the book was not brought up to date before it was translated.

D. LYNDEN-BELL

## GAS DYNAMICS

### Elements of Magnetogasdynamics

By L. E. Kalikhman. Translated by Scripta Technica, Inc. Edited by A. G. W. Cameron. (Saunders Physics Books.) Pp. xvii+366. (Philadelphia and London: W. B. Saunders Company, 1967.) 61s.

MAGNETOHYDRODYNAMICS is the study of the effects of electromagnetic body forces on a moving electrically conducting fluid. When the fluid is an ionized gas, when the processes are relatively slow, and when the energy of the magnetic field dominates that of the electric field a regime of the more general subject is defined by the title of this book. As such, it is of particular interest to astronautical engineers and those working in m.h.d. power generation.

The book is an American translation of a well known Russian text-book first published in 1964 and almost all of its references are to work from these two countries. It is basically a theoretical treatment, but is presented in a manner which the experimenter will find most useful. Russian authors have a good reputation for this engineering science approach and the translation has maintained the original spirit of the work.

This volume is to be recommended to the graduate engineer for the lucid manner in which the physical principles are introduced in the first two chapters; the physicist, generally unversed in fluid dynamics, will appreciate its treatment here. Aspects of steady state plasma flow without dissipation are followed by a discussion on shock waves in plasma. The sections on electrode-adjacent layers and the aerodynamic boundary layer in a plasma are the best collected accounts which I have seen. It is in the region between a solid surface and the body of a plasma that the majority of practical problems are to be found; for example, this region presents some of the critical problems in m.h.d. power generation.

More advanced chapters deal with two dimensional plasma flows in channels, unsteady state flows and hydrodynamic instabilities. A final chapter reviews, quite briefly, the practical applications of plasma propulsion, power generation and flight control, but references to experimental work appear throughout the book.

This book is a valuable contribution; unfortunately the subject has a more limited research effort in this country than it deserves.

J. PAIN



# Cytochrome c and Evolution

by

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A scheme for the evolutionary relationships of living organisms is proposed. It is based on data concerning the reactivities of cytochromes *c* of various organisms with cytochrome oxidase from a bacterium and from the cow; on the assumption that denitrifying organisms are the most primitive and on the similarity of haem *d* to chlorophyll.

ONE approach to the elucidation of the evolution of life on the Earth depends on taxonomic classification which derives from comparative biochemical investigations, especially of enzymes or functional proteins. Investigations of this type have been made by various workers<sup>1</sup>. It is necessary that components involved in such systems should occur widely throughout animals, plants and micro-organisms. Cytochromes are present in all aerobic organisms and in many anaerobic micro-organisms; they function as fundamental components in energy conversion biomechanisms. Among the cytochromes, cytochrome *c* is generally easily extractable by salt solution and the procedures for its purification have been established<sup>2</sup>.

As is well known<sup>3</sup>, there is a distinct biological specificity in the reaction of cytochrome *c* with cytochrome oxidases. This specificity can be used to detect very small differences between cytochromes *c*. It is of interest, therefore, to explore the possibility that interaction between cytochromes *c* and oxidases could provide a comprehensive basis for evolutionary investigations.

Two kinds of highly purified cytochrome oxidases are available: one is *Pseudomonas* cytochrome oxidase, which is isolated from *Pseudomonas aeruginosa* and obtained in a crystalline form<sup>3,4</sup>, and the other is cow cytochrome oxidase (= cytochrome *a*), highly purified from beef heart muscle<sup>5</sup>. *Pseudomonas* cytochrome oxidase reacts very rapidly with *Pseudomonas* cytochrome *c* but reacts very poorly with cow cytochrome *c*, whereas cow cytochrome oxidase reacts very rapidly with cow cytochrome *c* but does not react with *Pseudomonas* cytochrome *c*. Furthermore, cytochromes *c* of various organisms react with the two kinds of cytochrome oxidases at various rates<sup>3,4,6,7</sup>.

In the non-photosynthetic bacteria, cytochromes *c* of the denitrifying bacteria (*Pseudomonas aeruginosa*, *P. stutzeri* and *Micrococcus* species) react with *Pseudomonas* cytochrome oxidase very rapidly, but do not react with cow cytochrome oxidase. In this respect, the denitrifying bacterial cytochromes *c* are very similar to each other. The three kinds of *Micrococcus* cytochromes *c* shown in Table 1 are isolated from the same organisms. Their reactivities with cytochrome oxidases are qualitatively similar to one another, although quantitatively they differ considerably.

*Bacillus subtilis* cytochrome *c* reacts with *Pseudomonas* cytochrome oxidase poorly compared with the denitrifying bacterial cytochromes *c*, and it reacts with cow cytochrome oxidase very slowly. Thus, *Bacillus* cytochrome *c* is very different from the denitrifying bacterial cytochromes *c*.

In photosynthetic bacteria, cytochromes *c* react poorly with both cytochrome oxidases, except for *Chlorobium thiosulphatophilum* cytochrome *c* which reacts very rapidly with *Pseudomonas* cytochrome oxidase. *Chlorobium* is

Table 1. REACTIVITIES OF CYTOCHROMES *c* WITH *Pseudomonas* AND COW CYTOCHROME OXIDASES

	$\alpha$ -Peak (m $\mu$ )	Iso- electric point	Reactivity <i>Pseudo-</i> mono- cyto- chrome oxidase (per cent)	Cow cyto- chrome oxi- dase	Refer- ence
<b>Bacteria</b>					
<i>Pseudomonas aeruginosa</i>	551	4.7	100	0	8
<i>Pseudomonas stutzeri</i> †	552	Acidic	82	0	9
<i>Micrococcus</i> sp.	551	Acidic	47	0	10
<i>Micrococcus</i> sp.	554	3	131	0	10
<i>Micrococcus</i> sp.	548, 554	3.2	108	0	10
<i>Bacillus subtilis</i>	552	Acidic	27	5.7	6, 11
<i>Chlorobium thiosulphatophilum</i>	555	Basic	65	0	12*
<i>Rhodospirillum rubrum</i>	551	6.4	2.0	8.5	13
<i>Rhodospirillum rubrum</i> (dark)§	551		2.2	7.3	13, 14
<i>Rhodospseudomonas palustris</i> (A.T.C.C. 11168)	552	9	1.4	5.6	**
<i>Rhodospseudomonas palustris</i> No. 2137 (van Niel)	551	10.6	0.79	1.2	15
<i>Rhodospseudomonas capsulatus</i>	550	7	0	0	**
<i>Rhodospseudomonas spheroides</i>	551	Acidic	0.71	3.4	**
<b>Algae and related organisms</b>					
<i>Porphyra tenera</i>	553	3.5	15	0.1	16, 17
<i>Navicula pelliculosa</i>	554	Acidic	16	0	18
<i>Euglena gracilis</i>	552	5.5	4.1	0	19
<b>Fungi</b>					
<i>Saccharomyces oviformis</i>	550	Basic	4.9	100	20, 21
<i>Candida krusei</i>	549	Basic	5.0	88	21†
<i>Aspergillus oryzae</i>	550	Basic	5.4	54	21, 22
<i>Aspergillus oryzae</i>	550	Acidic	2.2	0	23
<b>Myxomycetes</b>					
<i>Physarum polycephalum</i>	550	Basic	0.9	7.6	24
<b>Higher plants</b>					
Wheat¶	550	Basic	1.5	26	25
<b>Stipunculida</b>					
<i>Dendrostoma</i> species	550	Basic	1.0	105	
<b>Mollusca</b>					
Oyster	550	Basic	0.54	98	26
Squid	550	Basic	1.2	91	26
<b>Arthropoda</b>					
Prawn	550	Basic	0.95	57	26
Housefly ( <i>Musca domestica</i> )	550	Basic	2.6	74	27
<b>Protochordata</b>					
<i>Styela plicata</i>	550	Basic	2.2	84	28
<b>Pisces</b>					
Tuna	550	Basic	8.7	43	29
Salmon	550	Basic	7.1	94	29
Mackerel	550	Basic	6.7	143	
Bonito	550	Basic	6.4	159	29
Shark	550	Basic	1.5	86	
<b>Amphibia</b>					
Toad	550	Basic	0.73	61	30
<b>Aves</b>					
Pigeon	550	Basic	0.44	70	31
<b>Mammalia</b>					
Horse	550	10	2.5	124	32, 33
Cow	550	Basic	0.53	40	32, 33
Man	550	Basic	0.44	107	34

The reactivities of cytochromes *c* are expressed as relative values; in the reaction with *Pseudomonas* cytochrome oxidase, the turnover number obtained when *Pseudomonas* cytochrome *c* reacted with the enzyme is taken as 100 per cent, and in that with cow cytochrome oxidase the turnover number when *Saccharomyces* cytochrome *c* reacted with the enzyme is taken as 100 per cent. All data on reactivities were determined by the author.

\* Unpublished results of T. Meyer.

† Unpublished results of H. de Klerk and M. D. Kamen.

‡ Unpublished results of H. Okazaki.

§ Previously described as *P. saccharophila*.

¶ Cytochrome *c* is isolated from dark grown cells. All other cytochromes *c* of photosynthetic organisms are isolated from light grown cells.

|| Formerly reported as *S. cerevisiae*.

¶ Cytochrome *c* participates in respiration.

classified among the green sulphur bacteria, whereas *Rhodospseudomonas* and *Rhodospirillum* belong to the group of non-sulphur purple bacteria<sup>36</sup>. *Chlorobium* is very different from *Rhodospirillum* and *Rhodospseudomonas*, also on the basis of the enzyme properties of the associated cytochromes *c*.

There are two kinds of algal cytochromes *c*, derived from *Porphyra tenera* and *Navicula pelliculosa*, and these react with *Pseudomonas* cytochrome oxidase at appreciable rates, but do not react with cow cytochrome oxidase. On the other hand, *Euglena gracilis* cytochrome *c* reacts less with *Pseudomonas* cytochrome oxidase than do *Porphyra* and *Navicula* cytochromes *c*, and it does not react with cow cytochrome oxidase.

Among the fungi, yeast cytochromes *c* are very different from other microbial cytochromes *c* in that they react very rapidly with cow cytochrome oxidase. In this respect, they resemble animal cytochromes *c*, but they definitely differ from mammalian, bird and amphibian cytochromes *c* in that they react fairly rapidly with *Pseudomonas* cytochrome oxidase. On the other hand, although *Aspergillus oryzae* is classified with yeast in the Ascomycetes, its cytochrome *c* is very different from yeast cytochromes *c* with respect to reactivity with cow cytochrome oxidase. *Physarum polycephalum* cytochrome *c* reacts very poorly with *Pseudomonas* cytochrome oxidase, but reacts with cow cytochrome oxidase fairly rapidly.

In higher plants, wheat cytochrome *c*, isolated from the wheat germs, participates in respiration but not in photosynthesis. This haem protein shows reactivity with cytochrome oxidases similar to that of animal cytochromes *c*.

All animal cytochromes *c* are very similar, that is, they possess an  $\alpha$ -peak at 550 m $\mu$ , are basic proteins, react poorly with *Pseudomonas* cytochrome oxidase, and react very rapidly with cow cytochrome oxidase, although they are different from one another in their primary structures<sup>36</sup>. Bony fish cytochromes *c* are, however, distinguishable from other animal cytochromes *c*, because they react with *Pseudomonas* cytochrome oxidase fairly rapidly.

In Fig. 1, the various cytochromes *c* are arranged on the basis of their reactivities with both cytochrome oxidases. Along the abscissa the reactivity with *Pseudomonas* cytochrome oxidase is plotted. This can be assumed to correlate in some fashion with evolutionary time. The ordinate gives reactivity with cow cytochrome oxidase, and this may be assumed to be proportional to the extent of adaptation to oxygen of the organisms from which the cytochromes *c* are isolated. It is evident that cytochromes *c* of primitive organisms are situated in the lower left corner and those of higher organisms generally tend toward the upper right corner. Intermediate cytochromes *c* lie between the two corners. There are some cytochromes *c* in the lower right corner, but it is noteworthy that no cytochrome *c* has been found which is situated in the upper left corner.

Furthermore, we can note an important fact from Fig. 1; all cytochromes *c* that react very rapidly with cow cytochrome oxidase are situated to the right of algal cytochromes *c*. What does this mean? According to the present ideas, the primitive Earth atmosphere contained no molecular oxygen, which appeared on the Earth for the first time as a result of algal photosynthesis<sup>37</sup>. Results of our investigations agree with these ideas, that is, that after algal photosynthesis began, organisms appeared which utilized molecular oxygen.

From Fig. 1, it is suggested that the primitive cytochrome *c*, which reacts very rapidly with *Pseudomonas* cytochrome oxidase but not with cow cytochrome oxidase, could have evolved to the cytochromes *c* which react very poorly with *Pseudomonas* cytochrome oxidase, but react rapidly with cow cytochrome oxidase, and that various cytochromes *c* remain as intermediates in this evolutionary path. What could be a rationale for such evolution of cytochrome *c*? Before I discuss this question, however, I should like to speculate about the evolution of the respiratory system.

As already mentioned, it is believed that there was no molecular oxygen on the primitive Earth<sup>37</sup>. The most primitive organism seems to have appeared on the Earth

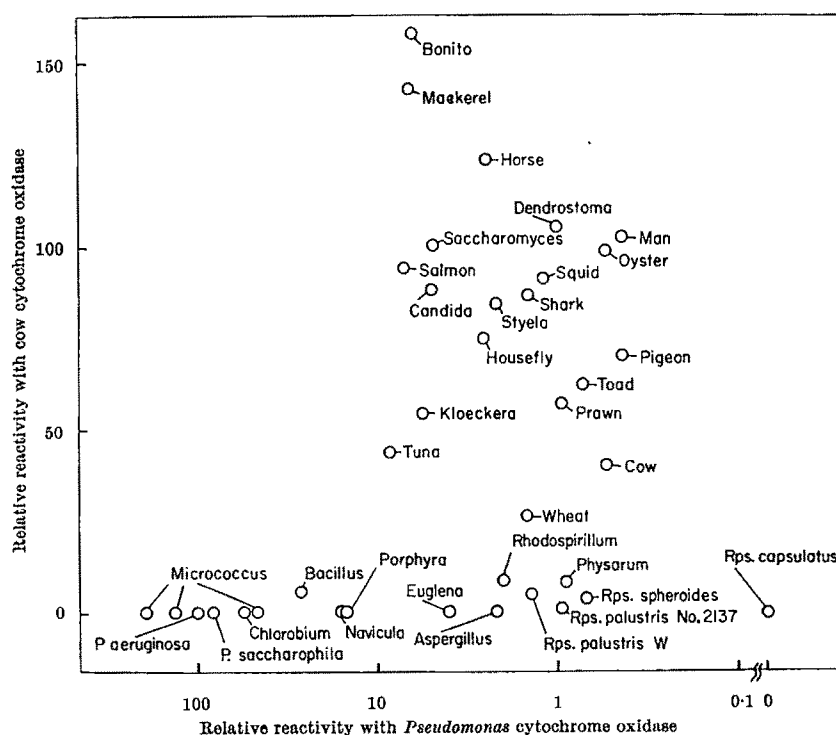


Fig. 1. Evolutionary relationships of organisms on the basis of reactivities of their cytochromes *c* with *Pseudomonas* and cow cytochrome oxidases.

in highly reducing conditions<sup>38,39</sup>. It could have obtained energy for its life processes by eliminating hydrogen from organic substances. The hydrogen was evolved into the atmosphere by means of a hydrogenase system<sup>40</sup>, that is, by oxidation of organic substances. Thereafter, some organisms may have appeared which could reduce molecular nitrogen by hydrogen (nitrogen fixation) instead of allowing hydrogen to be lost into the atmosphere<sup>40</sup>. Meanwhile, some organisms may have appeared which utilized sulphate as the terminal electron acceptor (sulphate respiration)<sup>41,42</sup>, and they could have had a primitive cytochrome system.

In sulphate respiration, however, the energy efficiency is low. When organisms use nitrate in place of sulphate, they can obtain energy more efficiently. Thus, the organisms which obtain energy by nitrate reduction (nitrate respiration)<sup>42,43</sup> could have appeared next, and it has been found that oxidative phosphorylation coupled with nitrate reduction occurs in the cell-free extracts of a few of the denitrifying bacteria in anaerobic conditions<sup>44</sup>. These organisms, however, must have encountered serious trouble in that nitrite, formed as the product of nitrate reduction, is poisonous. Thus, the organisms were required to elaborate a mechanism for elimination of nitrite, so that a nitrite reducing system appeared probably simultaneously with nitrate respiration.

It has been demonstrated that *Pseudomonas* cytochrome oxidase is the same entity as *Pseudomonas* nitrite reductase<sup>4,45</sup>. It is known that the nitrite reducing system of *P. aeruginosa* is composed of a highly developed cytochrome system<sup>4,46</sup>. Because the nitrite reductase functions also as a normal cytochrome oxidase, this cytochrome system is thought to have been able to participate

also in aerobic respiration after molecular oxygen appeared on the Earth.

The cytochrome systems which are observed in various aerobic organisms at the present time may have developed from a cytochrome system such as that present in *P. aeruginosa*. *Pseudomonas* cytochrome oxidase had a haem *d* (formerly called haem *a*<sub>2</sub>)<sup>46</sup> as its prosthetic group<sup>47</sup>. As shown in Fig. 2, haem *d* has two extra hydrogen atoms on one of the pyrrole rings, and in this respect it is similar to chlorophylls. The two extra hydrogen atoms of haem *d* are essential for the function of *Pseudomonas* cytochrome oxidase<sup>51</sup>. Probably because of the presence of two extra hydrogen atoms in haem *d*, *Pseudomonas* cytochrome oxidase is extremely labile in the presence of hydrogen peroxide<sup>52</sup>, and therefore the appearance of molecular oxygen on the Earth may not have been favourable to this cytochrome oxidase (haem *d*-bearing cytochrome oxidase); in aerobic conditions hydrogen peroxide is produced from oxygen by various enzyme systems (for example, by flavo-enzymes). The prosthetic group of the cytochrome oxidase, therefore, may have evolved from haem *d* to haem *a*. The latter is the prosthetic group of cow cytochrome oxidase (and of cytochrome oxidases of various aerobic organisms). The protein moiety of *Pseudomonas* cytochrome oxidase, however, does not function as a cytochrome oxidase with haem *a* as the prosthetic group<sup>51</sup>. The protein moiety which functions with haem *a*, therefore, may have appeared, resulting in the final evolution of haem *a*-bearing cytochrome oxidase. During the evolution of cytochrome oxidase, the protein moiety of *Pseudomonas* cytochrome *c* could have changed, leading to the possibility of reaction with various cytochrome oxidases, which appeared during

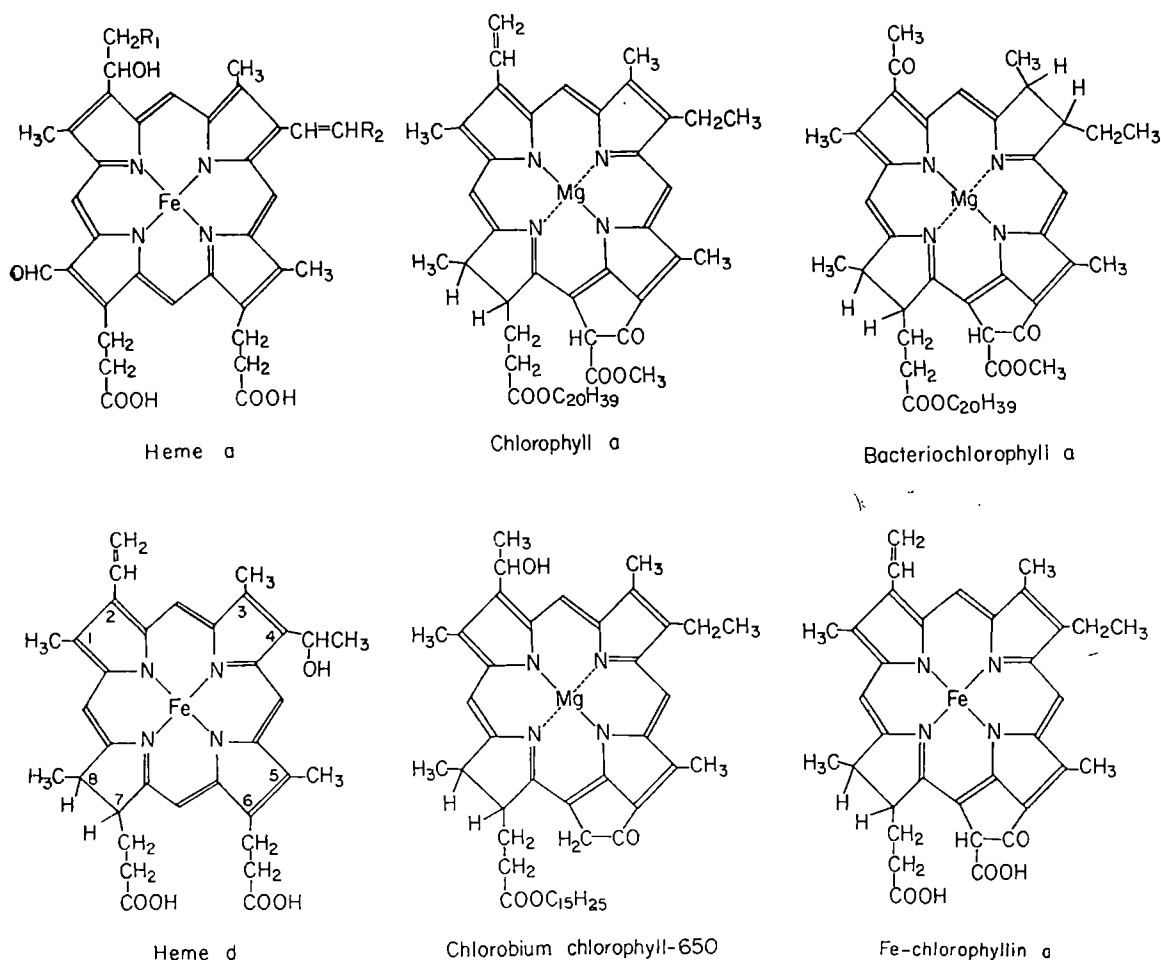


Fig. 2. Haems and chlorophylls<sup>44,46-50</sup>.

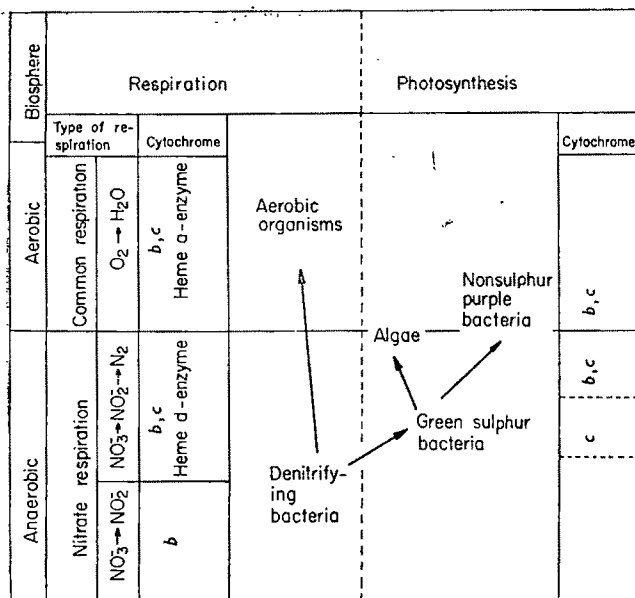


Fig. 3. Diagrammatic presentation of evolution from anaerobic organisms to aerobic organisms. (It has been claimed that a haem *a*-bearing cytochrome oxidase is present in dark grown cells of *R. sphaeroides*<sup>43</sup>.)

the evolution of cytochrome oxidase and which are still present in various organisms. Thus, on this basis, haem *d*-bearing cytochrome oxidase is more primitive than haem *a*-bearing enzymes.

As mentioned in the preceding sections, *Chlorobium* cytochrome *c* is similar to the denitrifying bacterial cytochromes *c* in its reactivities with cytochrome oxidases. On the present hypothesis, non-sulphur purple bacteria are more highly evolved (or more recent) organisms than green sulphur bacteria and algae. Furthermore, as mentioned previously, the structure of haem *d* is very similar to that of chlorophylls (Fig. 2). It is also noteworthy that when iron-chlorophyllin is substituted for haem *d* of *Pseudomonas* cytochrome oxidase, the resulting enzyme shows fairly high activity<sup>51</sup>. Thus, there may be very intimate relationships between the prosthetic group of *Pseudomonas* cytochrome oxidase and chlorophylls, on an evolutionary basis. From the evidence already given, I can present diagrammatically a suggestion as to the evolutionary relationships of organisms, as shown in Fig. 3.

Thus, the reactivities of cytochromes *c* with the cytochrome oxidases seem to provide an important key to elucidate events which occurred during evolution.

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# Quantum Yield of Ferrocyclochrome *c* Photo-oxidation in Chloroplast Particles

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The quantum yield profile of ferrocyclochrome *c* photo-oxidation by chloroplasts subjected to detergent treatment and differential centrifugation appears to represent a partial separation of photosystems into different chlorophyll protein complexes.

THE current hypothesis of two primary photoacts in photosynthesis implies that one photosystem (photosystem I) produces a strong reductant (leading to nicotinamide-adenine dinucleotide phosphate (NADP) reduction) and a weak oxidant, and the other photosystem (photosystem II) produces a weak reductant and a strong oxidant (leading to evolution of oxygen). The two photosystems operate in series, the weak reductant of photosystem II being oxidized by the weak oxidant of photosystem I through an electron transport system involving intermediate electron carriers<sup>1-3</sup>. Abundant evidence indicates that photosystem I is most efficiently sensitized by long wavelength light ( $> 690 \text{ m}\mu$ ) and photosystem II by short wavelength light ( $< 690 \text{ m}\mu$ )<sup>3,4,5</sup>. The two photosystems presumably differ in the composition of their chlorophyll-protein pigment complexes. Accordingly, if it were possible to separate physically a photochemically active particle which contained, for example, only the pigment complex for photosystem I, the action spectrum for a photosystem I assay should reflect this separation when compared with the action spectrum of chloroplasts retaining both photosystems.

Recently, Boardman and Anderson<sup>6,7</sup> separated digitonin treated chloroplasts by differential centrifugation into fractions showing differences with respect to pigment content and photochemical activity. Their results and those from other laboratories<sup>8-10</sup> were interpreted as showing that detergent treated chloroplast particles, the chlorophyll *a/b* ratios of which are increased by the treatment, are "enriched" in the chlorophyll-protein component of photosystem I, whereas particles containing low chlorophyll *a/b* ratios are "enriched" in the pigment component of photosystem II.

The experiments reported here employed preparations of the Boardman-Anderson type and were designed to test whether the quantum yield of photosystem I electron transport activity of these preparations satisfies the requirements for the physical isolation of the pigment complexes of photosystem I imposed by the series hypothesis. The assay chosen for monitoring photosystem I activity was the photo-oxidation of ferrocyclochrome *c* discovered by Nieman and Vennesland<sup>11</sup>. The experiments of Whatley<sup>12</sup> showed that this photo-oxidation reaction was equivalent in these particles to the photoreduction of NADP with the ascorbate-DCIP (dichlorophenol indophenol) couple as the electron donor. Kok *et al.*<sup>13</sup> established the optimum conditions for this reaction by showing that the addition of plastocyanine and a low potential reagent such as methyl viologen ( $E_0' = -0.44 \text{ V}$ ) enhanced the quantum yield of this reaction. They also observed, with detergent treated whole chloroplasts, a long wavelength rise in the quantum yield of ferrocyclochrome *c* photo-oxidation, which indicated photosystem I activity<sup>14</sup>.

Spinach chloroplasts (0.5 mg Chl./ml.), prepared in a *tris*-sucrose medium pH 7.6 as described previously<sup>15</sup>, were treated with 0.5 per cent (w/v) digitonin ( $C_d^0$ ). The resulting particles were separated by differential centrifugation as described by Boardman and Anderson<sup>6,7</sup>. Chloroplast pellets were obtained successively from one preparation at 1,000*g* for 10 min, ( $C_d^1$ ); 10,000*g* for 30 min, ( $C_d^{10}$ ); 50,000*g* for 30 min, ( $C_d^{50}$ ); and 144,000*g* for 60 min, ( $C_d^{144}$ ). Chloroplasts were also subjected to sonic oscillations in *tris*-sucrose medium, pH 7.6, and the resulting particles ( $C_s^0$ ) were either treated with digitonin directly ( $C_s^d$ ) or fractionated to obtain a particle which sedimented between 50,000*g* and 144,000*g*. This particle ( $C_{144}^s$ ) was also treated with digitonin ( $C_{144}^{sd}$ ) and was employed directly after dilution in *tris*-sucrose medium to the appropriate chlorophyll concentration. Total chlorophyll concentration and chlorophyll *a/b* ratios were determined according to Arnon's procedure<sup>16</sup>. Ferrocyclochrome *c* photo-oxidation and ferrocyclochrome *c* photoreduction (catalysed by phenazine methosulphate)<sup>17,18</sup> were monitored with the Kok split beam spectrophotometer which allowed for actinic illumination of the sample while recording absorbance changes. The large extinction coefficient of ferrocyclochrome *c* made it possible to monitor low rates of reaction employing thin chloroplast or particle suspensions and low absorbed light intensities. The actinic intensities used for all systems were such that a two-fold increase in intensity yielded an equivalent quantum efficiency of electron flow. Absolute intensities were measured with a bolometer and chloroplast absorption was measured with an integrating sphere<sup>18</sup>.

The data presented in Fig. 1 indicate that the quantum yield ( $\phi$ ) of ferrocyclochrome *c* photo-oxidation for Boardman-Anderson particles attains a constant maximum of 1 electron equivalent per quantum (1 eq/hv) at long wavelengths ( $> 700 \text{ m}\mu$ ). The particle ( $C_d^{144}$ ), which Boardman and Anderson believe to be enriched in the pigment complex of photosystem I, still retains a long wavelength rise in the quantum yield of electron flow rather than the essentially constant  $\phi$  value as a function of wavelength to be expected from a particle containing principally the pigment complex of photosystem I (Fig. 1, curve 4). At short wavelengths ( $< 680 \text{ m}\mu$ ) a constant  $\phi \approx 0.7 \text{ eq/hv}$  is obtained for ferrocyclochrome *c* photo-oxidation (system I). This  $\phi \approx 0.7 \text{ eq/hv}$  is approximately double that of the maximum quantum yield of the Hill reaction activity of normal chloroplasts (system I + II) as measured by ferrocyclochrome *c* photoreduction when assayed at wavelengths shorter than  $680 \text{ m}\mu$  (Fig. 1, curve 1). Surprisingly, these high  $\phi$  values of unity at long wavelengths for photosystem I activity could only be obtained with the reaction mixture of L. P. Vernon



(personal communication) which contained  $\geq 0.1$  molar phosphate ions. Preliminary experiments seemed to point to a specific effect of high phosphate concentration as other reagents such as sucrose, potassium chloride or sodium chloride suppressed the  $\phi$  values by approximately one half.

The wavelength profile of the quantum yield of ferrocytochrome *c* photo-oxidation obtained with the  $C_{144}^{sd}$  particle (Fig. 1, curve 3) also shows a marked long wavelength rise in  $\phi$  values which attains a relatively constant maximum of 0.8 eq/hv. At short wavelengths, however, ( $< 680$  m $\mu$ ),  $\phi$  values for ferrocytochrome *c* oxidation are essentially equal to the  $\phi$  values for ferricytochrome *c* reduction of normal chloroplasts (Fig. 1, curve 1) and approximately 50 per cent of the  $\phi$  values for ferrocytochrome *c* oxidation of the  $C_{144}^d$  particle. A marked difference between the  $C_{144}^{sd}$  particle and the Boardman-Anderson  $C_{144}^d$  particle is that the  $C_{144}^{sd}$  particle shows a chlorophyll *a/b* ratio of 2.8 whereas the  $C_{144}^d$  particle shows an "enriched" *a/b* ratio of 5.2. This relative enrichment of chlorophyll *a* in the  $C_{144}^{sd}$  particle thus appears to reflect an enrichment of photosystem I activity as suggested by Boardman and Anderson.

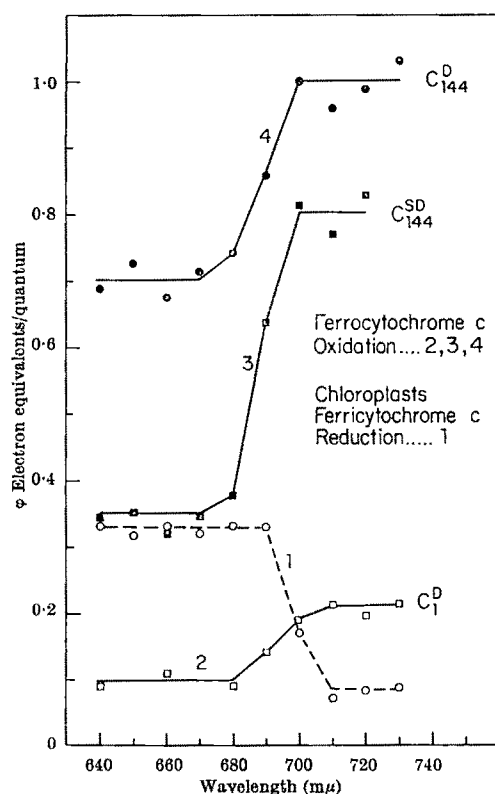


Fig. 1. Quantum yield profile of ferrocytochrome *c* photo-oxidation and ferricytochrome *c* photoreduction in spinach chloroplasts and detergent treated particles. The standard reaction mixture for the assay of ferricytochrome *c* reduction contained the following components in  $\mu$ moles/2.0 ml.: *tris* hydrochloric acid, pH 7.4, 50; magnesium chloride, 15; potassium phosphate (pH 7.4), 10; adenosine diphosphate, 2.5; sucrose, 40; sodium chloride, 35; phenazine methosulphate, 0.02; and ferricytochrome *c*, 0.05. For the assay of ferrocytochrome *c* photo-oxidation the reaction mixture contained ( $\mu$ moles/2.0 ml.): potassium phosphate (pH 6.8), 300; plastocyanine, 0.012; methyl viologen, 0.1; ferrocytochrome *c* (reduced with hydrogen and palladium asbestos), 0.05; and potassium cyanide, 10. All assays were performed with chloroplasts or particles containing either 2.5 or 5  $\mu$ g chlorophyll total (*a+b*)/ml. The absorbed light intensities were less than 15  $\mu$ Einsteins/min at all actinic wavelengths. Not more than 28 per cent of the incident light intensity was absorbed at any wavelength.

1, Ferricytochrome *c* reduction (Hill reaction) with normal chloroplasts, chlorophyll *a/b* = 2.8. Normal chloroplasts do not photo-oxidize ferrocytochrome *c*. 2, Ferrocytochrome *c* oxidation with 1,000g precipitate from digitonin treated chloroplasts, chlorophyll *a/b* = 2.4 ( $C_{144}^d$ ). 3, Ferrocytochrome *c* oxidation with 144,000g precipitate from sonicated chloroplasts, chlorophyll *a/b* = 2.8 ( $C_{144}^{sd}$ ). 4, Ferrocytochrome *c* oxidation with 144,000g precipitate, chlorophyll *a/b* = 5.2 ( $C_{144}^d$ ).

The low  $\phi$  values for ferrocytochrome *c* photo-oxidation obtained with the  $C_{144}^d$  particle most probably reflect the removal by centrifugation of particles enriched in photosystem I activity. The data (Fig. 1, curve 2) show a long wavelength doubling of the  $\phi$  value of electron flow with the  $C_{144}^d$  fraction, however, which suggests that a part of the pigment complex of photosystem I is retained in this fraction.

The data presented in Table 1 compare the photosystem I activity of the  $C_{144}^d$  particle and  $C_{144}^{sd}$  particles with the activity of digitonin treated chloroplasts ( $C_{144}^d$ ) and sonicated chloroplasts which were subsequently treated with digitonin ( $C_{144}^{sd}$ ). Neither the  $C_{144}^d$  nor the  $C_{144}^{sd}$  preparation was subjected to differential centrifugation but simply diluted to appropriate chlorophyll concentrations and assayed for photosystem I activity by ferrocytochrome *c* photo-oxidation. The  $\phi$  values obtained at 710 and 640 m $\mu$  for the  $C_{144}^d$  and the  $C_{144}^{sd}$  preparations show that sonication of chloroplasts increases photosystem I activity. Why the  $\phi$  values for the  $C_{144}^{sd}$  particle are less than half of the  $C_{144}^d$  particle is not clear (Table 1). The  $C_{144}^{sd}$  particle constitutes approximately 23 per cent of the  $C_{144}^d$  mixed particle system as determined by total chlorophyll recovery in the  $C_{144}^d$  pellet. Perhaps the small particles of the  $C_{144}^{sd}$  fraction are more easily accessible to digitonin action or more easily accessible to binding with the plastocyanine which is required for ferrocytochrome *c* photo-oxidation activity.

Table 1. QUANTUM YIELD OF FERROCYTOCHROME *c* PHOTO-OXIDATION AND FERRICYTOCHROME *c* PHOTOREDUCTION BY CHLOROPLASTS AND DETERGENT TREATED CHLOROPLAST PARTICLES

System	$\phi$ electron equivalents/quantum	
Ferrocytochrome <i>c</i> photo-oxidation	640 m $\mu$	710 m $\mu$
$C_{144}^d$ , Chl <i>a/b</i> = 5.2	0.69	0.96
$C_{144}^{sd}$ , Chl <i>a/b</i> = 2.8	0.35	0.77
$C_{144}^d$ , Chl <i>a/b</i> = 2.8	0.11	0.24
$C_{144}^{sd}$ , Chl <i>a/b</i> = 2.8	0.14	0.36
Ferricytochrome <i>c</i> photoreduction		
Chloroplasts, chlorophyll <i>a/b</i> = 2.8	0.31	0.074
$C_{144}^d$ , Chlorophyll <i>a/b</i> = 2.8	0.11	—
$C_{144}^d$ , Chlorophyll <i>a/b</i> = 2.8	0.17	—

Reaction conditions are described with Fig. 1. All assays were performed with 5  $\mu$ g chlorophyll (*a+b*)/ml. The absorbed intensities were 10.8  $\mu$ Ein/min at 710 m $\mu$  and 0.7  $\mu$ Ein/min at 640 m $\mu$ . The experiments were performed in air.

The data (Fig. 1, Table 1) show that a maximum quantum yield of electron flow for photosystem I activity (photo-oxidation of ferrocytochrome *c*) which approaches the theoretical maximum of 1.0 eq/hv in long wavelength light ( $> 700$  m $\mu$ ) can be obtained. The series proposal predicts a maximum quantum yield of unity for a pure photosystem I reaction if the pigment complex of photosystem I were the only absorbing group. Because the Hill activity of chloroplasts as measured by ferricytochrome *c* reduction (Fig. 1 and Table 1) exhibits  $\phi$  values in long wavelengths ( $> 700$  m $\mu$ ) of approximately 25 per cent of the short wavelength maximum (that is, 640–690 m $\mu$ ), the series formulation predicts that at least 12.5 per cent of long wavelength light must be absorbed by the pigment assembly of photosystem II in normal oxygen evolving chloroplasts. It follows that the maximum  $\phi$  value experimentally attainable for photosystem I activity in normal chloroplasts would be less than 1 eq/hv, approaching 0.87 eq/hv as a maximum. At 710 m $\mu$  we observed  $\phi = 0.24$  eq/hv for photosystem I activity (ferrocytochrome *c* oxidation) with the  $C_{144}^d$  particles and  $\phi = 0.36$  eq/hv with the  $C_{144}^{sd}$  particle (Table 1). These values are well below a maximum of 0.87 eq/hv predicted from the Hill activity data at 710 m $\mu$  (Fig. 1, curve 1). The  $C_{144}^{sd}$  particle which retains the *a/b* ratio of normal chloroplasts does, however, show long wavelength  $\phi$  values of approximately 0.8 eq/hv for photosystem I activity (Table 1). Whether the difference between this maximum

Table 2. REDUCTION OF NADP BY CHLOROPLAST PARTICLES AND DIGITONIN TREATED CHLOROPLAST PARTICLES

System	$\phi$ electron equivalents/quantum	
	640 m $\mu$	710 m $\mu$
C <sub>144</sub> <sup>s</sup> , + DCIP	0.12	0.37
C <sub>144</sub> <sup>s</sup> , - DCIP	0	~0.04
C <sub>144</sub> <sup>sd</sup> , + DCIP	0.18	0.49
C <sub>144</sub> <sup>sd</sup> , - DCIP	0.18	0.49

The standard reaction mixture contains the following components in  $\mu$ moles/2.0 ml.: potassium phosphate (pH 6.8), 300; sodium ascorbate, 10; plastocyanine, 0.012; ferredoxin, transhydrogenase, and NADP, 1.0; 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 0.002; and dichlorophenol indophenol (DCIP), 25 (where indicated). All assays were performed with 5  $\mu$ g chlorophyll ( $a+b$ )/ml. The absorbed intensities were 10.3 m $\mu$ E/m at 710 m $\mu$  and 9.7 m $\mu$ E/m at 640 m $\mu$ . The experiment was performed in argon.

$\phi = 0.8$  eq/h $\nu$  and the maximum  $\phi = 1.0$  eq/h $\nu$  for the C<sub>144</sub><sup>d</sup> particle is fortuitous or reflects the difference in the concentration of the system II pigment complex retained by the respective particles is unresolved.

Table 2 presents data obtained in an attempt to equate  $\phi$  values for NADP reduction with ferrocytochrome *c* oxidation. A comparison was made for NADP reduction activity with the C<sub>144</sub><sup>s</sup> and the C<sub>144</sub><sup>sd</sup> particles when supplied with the ascorbate DCIP couple. As shown in Table 2 the quantum yield of NADP reduction at rate-limiting light intensities is greater after treatment of the C<sub>144</sub><sup>s</sup> particle with digitonin (C<sub>144</sub><sup>sd</sup>). It is to be noted that at low light intensities NADP reduction with the C<sub>144</sub><sup>sd</sup> particle requires the ascorbate-plastocyanine couple whereas the C<sub>144</sub><sup>s</sup> particle requires DCIP. Why the absolute  $\phi$

values for NADP reduction are poorer than those for ferrocytochrome *c* oxidation is not resolved (compare Tables 1 and 2).

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## Catecholamine Metabolism in Schizophrenia

by

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Significant differences have been found between the concentrations of free dopamine excreted in the urine of normal individuals and schizophrenic patients.

EVIDENCE suggests that there is a relationship between the metabolism of catecholamines and mental illness<sup>1,2</sup>. Although no definite relation has been established, several theories which relate aberrations in the metabolism of these compounds and schizophrenia have been proposed. In 1954, Hoffer, Osmond and Smythies<sup>3</sup> suggested that the symptoms of schizophrenia result from the abnormal metabolism of adrenaline to adrenochrome and, in turn, adrenolutin. Osmond and Smythies<sup>4</sup> had earlier proposed that schizophrenia results from an abnormal methylation reaction of the catecholamines. More recently, Friedhoff and Van Winkle<sup>5,6</sup> have reported a correlation between the incidence of dimethoxyphenylethylamine in urine and schizophrenia; however, several groups have not been able to confirm this relationship (compare ref. 7).

Recently a spectrophotometric method for the differential estimation of adrenaline, noradrenaline, dopamine, metanephrine and normetanephrine in one urine sample was described<sup>8</sup>. While it was possible to assay only one or two of the biogenic amines in a urine sample, no other approach to the interpretation of the results than a direct comparison of the concentrations of amines was possible. When the concentrations of five of the major biogenic amines, together with their conjugates, are obtained at one time, then it becomes possible to consider

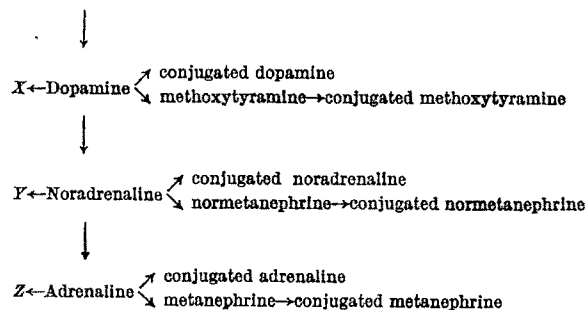
the dynamic aspects of catecholamine metabolism. The application of such an approach to the interpretation of the concentrations of urinary amines in schizophrenia forms the basis of the present article.

Overnight urines were used and the period of the collection recorded. Urines were obtained from acute schizophrenics who were patients in University Hospital, Saskatoon\*, and the normal controls were members of the staff of the Psychiatric Research Unit. The concentrations of free and conjugated adrenaline, noradrenaline, dopamine, metanephrine and normetanephrine were estimated by the method of Mattok *et al.*<sup>8</sup>.

The concentrations of the amines were estimated in urines from twenty-four normal controls and twenty-two acute schizophrenics. Statistical analyses were made using the Mann-Whitney *U* test<sup>9</sup>. Because we did not attempt to predict the direction of the differences in the concentrations of the amines, we calculated two-tailed probabilities for each amine; these values are given in Table 1. The median values of the concentration of each amine assayed are also given in Table 1.

\* University Hospital, Saskatoon, is an active treatment centre. Psychiatric patients participate in a normal amount of physical activity and are maintained on a normal diet. The average length of time which psychiatric patients stay at this hospital is 19 days.

The initial stages in the metabolism of the catecholamines can be represented by the following scheme:



Scheme I.

where X, Y and Z represent the products of unspecified abnormal processes and the precursors are dopamine, noradrenaline and adrenaline, respectively. For the sake of brevity, possible deviations at other stages have not been included. Some alternative pathways which may be associated with schizophrenia, and which would be included by scheme I are: (i) the oxidative melanization of dopamine; (ii) the oxidation of adrenaline to adrenochrome<sup>8</sup>; and (iii) the di-O-methylation of dopamine to dimethoxyphenylethylamine<sup>5,6</sup>.

Table 1. BIOGENIC AMINE EXCRETION FOR NORMAL CONTROL AND SCHIZOPHRENIC SUBJECTS

Amine	Median concentrations in urine and p factors			Conjugated amine excretion*		
	N	S	P	N	S	P
Dopamine	10.3	5.3	0.0028	15.4	18.6	0.5552
Noradrenaline	4.2	5.1	0.1442	1.8	2.7	0.5156
Adrenaline	<0.02	<0.02	0.3898	<0.02	<0.02	0.2420
Normetanephrine	9.0	10.1	0.5532	5.8	6.4	0.8494
Metanephrine	1.6	0.4	0.1600	0.9	2.3	0.0478

N, Normal subjects; S, schizophrenic subjects; P, two-tailed probability.

In general, attempts to correlate the concentrations of biogenic amines with pathological or psychiatric disorders have been based on the absolute concentrations of one or two of the amines and expressed as  $\mu\text{g/h}$  or  $\mu\text{g/mg}$  of creatinine. The usefulness of the creatinine scale has been seriously challenged by Pscheidt *et al.*<sup>10</sup>, who have found that variability in creatinine output is common among mental patients; indeed, a significant correlation may exist between such variations and psychotic status. Some disorders, such as pheochromocytoma, which result in marked elevations of the concentrations of some urinary amines, may be satisfactorily confirmed by measurements of the absolute concentrations of some of the amines present in the urine. An abnormal concentration, however, does not in itself provide any information about the nature of the abnormal reaction or the point at which it first occurs in the metabolic sequence. This kind of information can only be obtained from a consideration of the discriminating features of the metabolic picture in relation to the precursors and metabolites. A given concentration of an amine could result from any of several factors, some of which may not result in an overall harmful effect. For example, a low dopamine concentration could be the result of: (1) synthesis of less than normal amounts of a precursor because of a defective mechanism at some remote stage in the synthesis of dopamine; (2) less than normal decarboxylation of dihydroxyphenylalanine (DOPA); (3) normal formation of dopamine by (2), but subsequently largely conjugated; (4) greater than normal  $\beta$ -hydroxylation of dopamine to form noradrenaline; (5) further metabolism of dopamine by an abnormal process (that is, to form X).

A statistical comparison of the analyses obtained with urines from twenty-four normal controls and twenty-two schizophrenic patients showed that the difference in concentrations of free dopamine in the two groups is highly significant (see Table 1). The median value for urinary free dopamine in the control group was  $10.3 \mu\text{g/h}$  and in the schizophrenic group it was  $5.3 \mu\text{g/h}$ . There is also a significant difference in the concentrations of conjugated metanephrine in the two groups.

Because there are no major quantitative differences in the concentration of dopamine metabolites in normal and schizophrenic subjects, it is likely that normal amounts of dopamine are initially synthesized in schizophrenics. The lower concentrations of free dopamine in schizophrenia are therefore probably the result of increased  $\beta$ -hydroxylation of dopamine (which may arise from a reduction in dopamine binding capacity, thereby increasing the amount of dopamine available for further metabolism), or the intrusion of an abnormal reaction. Alternatively, there may be augmentation of a normally relatively minor pathway in the metabolism of dopamine. An interpretation based on an abnormal metabolic reaction of dopamine, however, would not account for the increased conjugation of metanephrine. Because it is unlikely that two independent aberrations would occur in these two interdependent reactions, the differences in the concentrations of free dopamine and conjugated metanephrine can be assumed to be related. This would be so if there were increased metabolism of dopamine to noradrenaline in schizophrenia. The synthesis of greater than normal amounts of noradrenaline would mean, in turn, greater opportunity for the synthesis of adrenaline and would therefore not necessarily be reflected by urinary concentrations of noradrenaline greater than normal. If abnormally large amounts of adrenaline are synthesized in schizophrenia this would lead to increased conjugation of the biogenic amines, thereby reducing the possibility of the existence of toxic concentrations. The statistical analysis showed, however, no significant difference in the concentrations of total adrenaline (that is, free and conjugated adrenaline and metanephrine) for normal and schizophrenic subjects. The excess (that is, relative to normal) adrenaline synthesized is probably, therefore, metabolized by an abnormal reaction not accounted for by the assay.

The present results therefore indicate that dopamine is synthesized normally in schizophrenia, but is metabolized to give abnormally high concentrations of noradrenaline and, in turn, adrenaline. The increased concentrations of adrenaline may stimulate the processes leading to conjugation and complete detoxification of metanephrine. Because the raised concentrations of adrenaline must be rapidly reduced to more normal concentrations, some of the excess adrenaline is probably removed by an abnormal process.

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## Effect of Feeding Calcium Cyclamate to Rats

by

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Calcium cyclamate (cyclohexyl calcium sulphamate) is present as a sweetener in a number of soft drinks. When it is fed to rats over a long period it can decrease the rate of growth, possibly by affecting the adrenals.

IN recent years the use of artificial sweeteners has increased significantly. The U.S. Department of Agriculture<sup>1</sup> reported that the consumption of sweeteners in 1964 was twice that in 1963 and five times that in 1959. The major part is consumed in drinks containing calcium cyclamate (cyclohexyl calcium sulphamate).

Persons drinking these beverages can take as much as 1 per cent cyclamate in their daily diet. For example, three bottles of soft drinks can contain a total of 4 g of calcium cyclamate. This amount, consumed daily with a normal food intake (400 g dry weight, 2,400 calories) would result in a diet containing 1 per cent calcium cyclamate. Other sources of calcium cyclamate may further increase the proportion of cyclamate in the diet.

Fitzhugh *et al.*<sup>2</sup> fed cyclamate concentrations as high as 5 per cent in the diet to rats for 2 years. No effects were observed with less than 1 per cent sodium cyclamate in the diet. A decrease in the rate of gain was observed at levels of 1 and 5 per cent in the diet. 5 per cent cyclamate caused moderate diarrhoea. Histopathologically, the animals showed no abnormalities.

Richards *et al.*<sup>3</sup> carried out experiments involving feeding cats and dogs with diets containing 0.05, 0.1 and 1 per cent sodium cyclamate. They reported that the body weight of rats receiving 0.1 and 1 per cent sodium cyclamate in the diet tended to remain below that of the control rats. No other pharmacological reactions were noticed. The excretion and distribution of cyclamate labelled with sulphur-35 were studied by Taylor *et al.*<sup>4</sup> 95 per cent of the cyclamate was excreted in the urine and faeces. Small amounts of sulphur-35 were found in all tissues, including the foetus.

The work reported here was carried out using calcium cyclamate, which is now more commonly used as a sweetener than is the sodium salt. Higher levels of cyclamate were used to allow a clearer interpolation to those amounts presently obtained in the daily human dietary. The use of calcium cyclamate in human diet to reduce the caloric intake led us to include feeding trials in which the caloric intake was restricted.

Weanling Sprague-Dawley rats were divided into three groups, twenty-six males and twenty-six females in each group. The animals were housed individually in cages with screen bottoms and given water *ad libitum*. During the first 8 weeks of the experiments all the animals in each group received their respective diets *ad libitum*.

Group 1 rats were fed only on a commercial ground diet containing a minimum of 23.0 per cent crude protein, 4.5 per cent crude fat, a maximum of 6.0 per cent crude fibre, and a maximum of 9.0 per cent ash. Group 2 were given 5 per cent calcium cyclamate in the basal diet; and group 3, 10 per cent.

After 8 weeks on test diets the separate groups were divided into two sections. Rats in section A continued

to receive their respective diets *ad libitum*. Rats in section B received their respective diets at a level equivalent to 60 per cent of the consumption of section A rats of the same group.

In addition to limiting the amount consumed, five females from each section were mated with males from their respective sections, transferred to breeding cages and maintained on the same diet schedule as the non-reproduction animals. Animals with young were allowed to raise them to weaning. After a reasonable rest the females were remated and allowed to raise their young to weaning. After the second litters were weaned, the females were returned to standard screen bottom cages until week 52 when all remaining animals were killed.

Each animal was weighed and its food consumption recorded each week. At selected intervals the individual daily consumption of water was determined on five males and five females from each section of each group. At 6, 9 and 12 months blood and urine analyses were conducted on a representative portion of the animals from each section.

At 90 days two males and two females from each section of each group were killed and examined for gross and histological alterations. At 6 months, three males and three females from each section of group 1 (negative control) and group 3 (10 per cent calcium cyclamate) were killed and similarly examined. At the end of the study all animals were killed and examined.

At 40 weeks, three males and three females from each section of each group were placed in metabolism cages for 3 days. During this period food consumption and faecal and urine excretion were measured. Urine and faeces were analysed to determine nitrogen and calories excreted. Intake and excretion data were then compared to determine amount of nitrogen and calories retained.

The body weights (Figs. 1 and 2) show that the gains in body weight of animals receiving 10 per cent calcium cyclamate were approximately 80–85 per cent of the weight gains noted for animals of the control group. Animals receiving 5 per cent calcium cyclamate gained approximately 85–90 per cent as much weight as the control animals. This difference was apparent throughout the test period and the differences were similar whether limited or *ad libitum* fed groups were compared.

Figs. 3 and 4 give the food consumption converted to basal diet alone, thereby eliminating the quantity of non-caloric calcium cyclamate in the diet. The data show that group 1 (negative control) and group 3 (10 per cent calcium cyclamate) consume essentially equal quantities of basal diet. On the basis of basal diet alone, group 2 animals (5 per cent calcium cyclamate) consumed approximately 7 per cent less than group 1 animals.

Water consumption (Table 1) is significantly increased in animals receiving calcium cyclamate. Addi-

Table 1. AVERAGE DAILY WATER CONSUMPTION AFTER 12 WEEKS

Day*	Ad lib. groups			Limited feeding groups		
	Control	Test-calcium cyclamate 5%	Test-calcium cyclamate 10%	Control	Test-calcium cyclamate 5%	Test-calcium cyclamate 10%
Males (14 rats)						
1	33	47	66	46	60	69
2	34	52	64	21	40	46
3	37	53	63	45	51	54
4	46	60	73	43	28	36
5	35	48	67	28	56	65
6	39	52	70	18	21	33
7	43	58	70	49	59	53
Average	38	53	68	36	45	51
Females (9 rats)						
1	31	45	56	43	57	62
2	34	46	54	30	40	46
3	39	50	53	44	43	52
4	48	58	58	41	25	30
5	33	45	56	25	47	55
6	38	53	62	16	20	32
7	44	54	58	49	51	37
Average	38	50	57	35	40	44

tional water measurements, made periodically during the experiment, also showed this.

The results of the balance studies were calculated on the basis of two assumptions. In one calculation it was assumed that all the calcium cyclamate was excreted in the urine and in the other that all the cyclamate was in the faeces. It is known that calcium cyclamate is excreted by way of urine and faeces, but adequate analytical procedures are not available, and the exact quantity excreted cannot be determined. The available values indicate that animals receiving calcium cyclamate utilized both nitrogen and calories in the feed as well as or better than the animals in the negative control group.

The results of the first litter reproduction study (Table 2) indicated that animals receiving limited ration conceived but were unable to raise their young beyond 5 days.

Animals receiving feed *ad lib.* conceived and were able to raise their young to weaning (21 days). Control weanling rats weighed 42 g and 10 per cent cyclamate weanling rats averaged 50 g in weight; 5 per cent cyclamate weanling rats weighed 32 g.

The results of the second litter reproduction study (Table 3) showed the control animals (limited feeding)

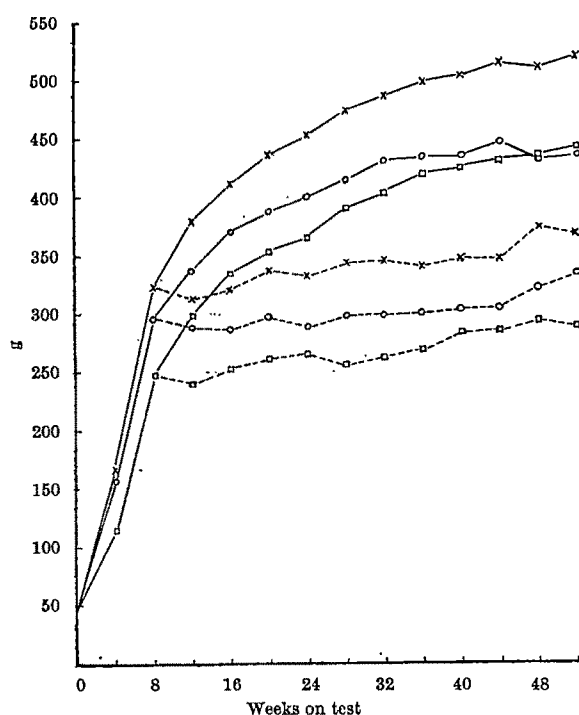


Fig. 1. Average body weight of male rats plotted against time. x, Basal ration; O, 5 per cent calcium cyclamate; □, 10 per cent calcium cyclamate; —, fed *ad lib.*; ---, fed limited diet.

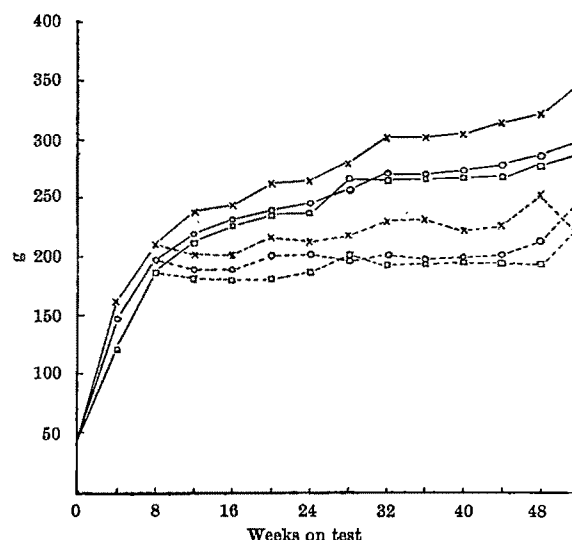


Fig. 2. Average body weight of female rats plotted against time. x, Basal ration; O, 5 per cent calcium cyclamate; □, 10 per cent calcium cyclamate; —, fed *ad lib.*; ---, fed limited diet.

and animals receiving 5 per cent cyclamate (limited feeding) conceived, but that all their young died in 7 days. Animals receiving 10 per cent cyclamate (limited feeding) did not conceive.

Animals receiving feed *ad lib.* conceived and again raised their young to weaning (21 days). Control weanling rats averaged 52 g in weight, 5 per cent cyclamate weanling rats averaged 45 g and 10 per cent cyclamate weanling rats averaged 33 g in weight.

Clinical laboratory examinations showed no remarkable variation between the haematological data of test groups and the negative control groups until the terminal examination, in which a decrease in white cell counts was found in male rats receiving 10 per cent calcium cyclamate *ad libitum*. The nitrogen content of the urine is higher in rats receiving feed *ad libitum* than in rats receiving feed on a limited basis. After 9 months there was an indication that animals receiving the diet containing 10 per cent calcium cyclamate *ad libitum* have significantly higher levels of urine nitrogen than animals receiving the basal diet *ad libitum* (group 1). This was not the case at 12 months.

The animals showed no gross evidence of toxicity nor undesirable results of feeding calcium cyclamate other than loss of weight. The faeces from animals receiving calcium cyclamate were soft, and contained more moisture. At times this condition approached diarrhoea in the animals receiving 10 per cent calcium cyclamate, while faeces from animals receiving 5 per cent calcium cyclamate generally consisted of larger pellets with a higher water

Table 2. REPRODUCTION: FIRST LITTER GROUP VALUES

	Ad lib. feeding			Limited feeding		
	Control	Test-calcium cyclamate 5%	Test-calcium cyclamate 10%	Control	Test-calcium cyclamate 5%	Test-calcium cyclamate 10%
No. of pregnancies	4	5	5	4	5	3
No. of young delivered	44	42	48	44	45	24
Live	38	36	46	44	44	24
Dead	6	6	2	0	1	0
No. of young surviving						
1 day	33	34	41	41	39	24
2 days	31	34	41	38	39	24
3 days	31	34	41	34	38	16
4 days	19	24	30	18	14	3
5 days	18	24	30	8	0	0
6 days	18	24	30	0	0	0
7 days	18	24	30	0	0	0
Weaning	18	23	27	0	0	0
Average weight of young at 4 days (g)	9.8*	10	8.0	5.1	5.5	4.6
Average litter size at weaning	9.6†	11.0	8.8	5.8	4.3	4.0
Average individual weight of young at weaning (g)	6	5.75	5.4	0	0	0
Average total litter weight (g)	49.8	42.3	31.8	—	—	—

\* Average/rat total litter.

† Average/rat of six left in litter.



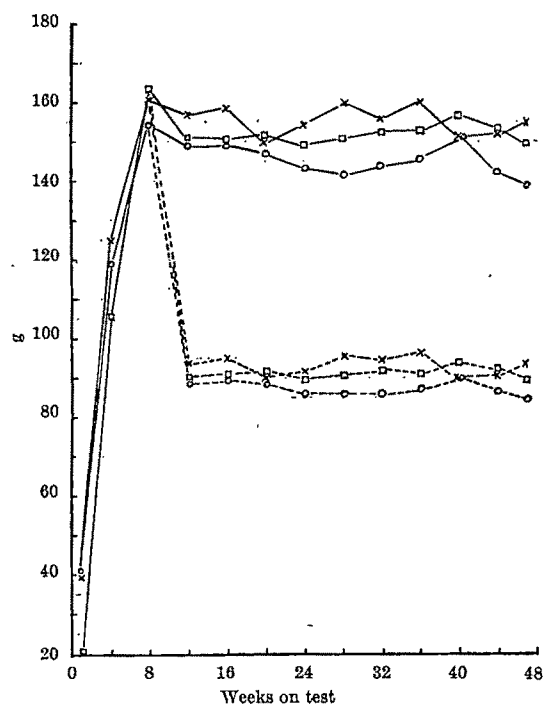


Fig. 3. Weekly values of average basal diet consumed by male rats. x, Basal ration; O, 5 per cent calcium cyclamate; □, 10 per cent calcium cyclamate; —, fed *ad lib.*; ---, fed limited diet.

content. The faecal changes were most marked during the first 8 weeks. The urine of the control animals was found to be different from that of the test animals when allowed to evaporate to dryness. Urine from animals receiving calcium cyclamate left an excessive crystalline residue which was not present in urine from control animals.

The organs of all the animals killed at 1 year were weighed and body weight ratios calculated. The organs weighed were the heart, liver, spleen, kidney, gonads, adrenal and thyroid. Tables 4 and 5 show the average weights of the organs and the percentage of body weight of the *ad libitum* animals and the limited fed animals respectively.

The values for adrenal weights as a percentage of body weights were compared statistically.

The following conclusions can be made with reasonable confidence: (1) The weights of the adrenals of both male and female rats fed 10 per cent calcium cyclamate increased under both feeding regimes. The increase is more marked under limited feeding conditions. (2) The adrenals of male rats fed 5 per cent calcium cyclamate under limited feeding conditions increased in proportion

Table 4. AVERAGE ORGAN WEIGHTS OF ANIMALS KILLED AFTER 1 YEAR

<i>Ad libitum</i> feeding								
	Body wt.	Heart	Liver	Spleen	Kidney	Gonad	Adrenal	Thyroid
Males								
Control (g)	520	1.64	20.0	0.79	4.3	3.1	0.057	0.023
(% body weight)		0.32	3.8	0.15	0.83	0.61	0.011	0.004
5% calcium cyclamate (g)	438	1.39	16.6	0.75	3.7	3.2	0.056	0.019
(% body weight)		0.32	3.8	0.18	0.84	0.70	0.013	0.005
10% calcium cyclamate (g)	414	1.42	16.2	0.67	3.6	3.51	0.065	0.021
(% body weight)		0.34	3.9	0.16	0.86	0.85	0.016	0.005
Females								
Control (g)	343	1.20	12.3	0.66	2.7	0.09	0.060	0.023
(% body weight)		0.35	3.6	0.19	0.79	0.29	0.020	0.006
5% calcium cyclamate (g)	295	1.03	10.7	0.57	2.4	0.80	0.066	0.021
(% body weight)		0.35	3.6	0.19	0.81	0.27	0.022	0.007
10% calcium cyclamate (g)	277	1.02	9.5	0.53	2.4	0.83	0.071	0.023
(% body weight)		0.37	3.4	0.19	0.88	0.32	0.025	0.008

Table 5. AVERAGE ORGAN WEIGHTS OF ANIMALS KILLED AFTER 1 YEAR

Limited feeding								
	Body wt.	Heart	Liver	Spleen	Kidney	Gonad	Adrenal	Thyroid
Males								
Control (g)	359	1.24	11.2	0.502	2.75	3.67	0.053	0.020
(% body weight)		0.35	3.15	0.16	0.77	1.03	0.014	0.005
5% calcium cyclamate (g)	314	1.04	9.48	0.494	2.53	3.31	0.054	0.019
(% body weight)		0.33	3.02	0.16	0.81	1.05	0.017	0.005
10% calcium cyclamate (g)	296	1.00	9.39	0.483	2.45	3.41	0.059	0.016
(% body weight)		0.34	3.17	0.16	0.83	1.15	0.020	0.005
Females								
Control (g)	233	0.991	7.42	0.535	1.90	0.634	0.062	0.021
(% body weight)		0.43	3.18	0.23	0.82	0.27	0.027	0.008
5% calcium cyclamate (g)	216	0.824	6.94	0.473	1.84	0.605	0.060	0.017
(% body weight)		0.38	3.21	0.22	0.85	0.28	0.027	0.007
10% calcium cyclamate (g)	194	0.790	5.90	0.390	1.71	0.365	0.067	0.017
(% body weight)		0.41	3.04	0.20	0.88	0.19	0.034	0.008

to the rest of the body, but this did not occur when the same ration was fed *ad lib.* The weights are significantly higher than those of the control males and significantly lower than those male rats fed 10 per cent calcium cyclamate. (3) Female rats fed 5 per cent calcium cyclamate did not show this rise in relative adrenal weight under any conditions.

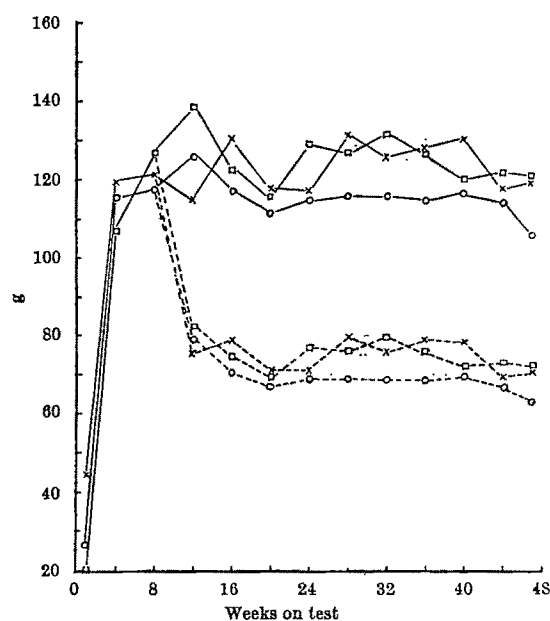


Fig. 4. Weekly values of average basal diet consumed by female rats. x, Basal ration; O, 5 per cent calcium cyclamate; □, 10 per cent calcium cyclamate; —, fed *ad lib.*; ---, fed limited diet.

Table 3. REPRODUCTION: SECOND LITTER GROUP VALUES

	<i>Ad lib.</i> feeding			Limited feeding		
	Control	5% Test-calcium cyclamate	10%	Control	5% Test-calcium cyclamate	10%
No. of pregnancies	4	5	5	4	5	0
No. of young delivered	45	43	43	32	34	—
Live	37	39	41	32	34	—
Dead	8	4	2	0	13	—
No. of young surviving						
1 day	21	37	40	32	34	—
2 days	14	36	39	32	33	—
3 days	13	36	39	32	32	—
4 days*	6	24	30	24	17	—
5 days	6	23	30	18	11	—
6 days	6	23	30	17	10	—
7 days	6	23	30	12	7	—
Weaning (21 days)	6	18	30	0	0	—
Average weight of young at 4 days (g)	11	8	9.4	6	7	—
Average litter size at weaning	6	6	6	0	0	—
Average individual weight of young at weaning (g)	52	45	33	—	—	—

\* Litters cut back to six young.

Histologically, the tissues of rats fed calcium cyclamate showed changes in the testes, adrenals, kidneys and pancreas. The testes of rats fed cyclamate show an increased incidence and severity of atrophy and degeneration of the seminiferous tubules compared with those of control rats. A few atrophic tubules were noted in sections of testes from control rats, and the changes noted in test animals may be an aggravation of a normal ageing process. Severe atrophy of the seminiferous element of the testes was found as early as 6 months in tissue from one rat fed 10 per cent cyclamate. The adrenals of the test rats showed primarily cortical changes. There were subtle alterations of the appearance of the zones in the test rats. In the adrenals of animals fed 10 per cent calcium cyclamate the outer zone of the cortex (zona granulosa) was pale on staining, the cells contained increased cytoplasm and the nucleus was lighter than in controls. The demarcation between the outer zona granulosa and the zona fasciculata was poorly defined. In the adrenals from control animals, cells from the outer zone were more tightly packed and contained a more darkly staining nucleus, growing a darker, better defined zona granulosa. This was particularly evident in tissues from the control female rats. Changes in the appearance of the adrenals were clearer in the females than in the males. The appearance of the kidneys was altered more frequently in animals fed calcium cyclamate than in animals on the basal diet. Concretions were frequently found in or near the renal pelvis. Focal pancreatic atrophy was also more marked in test tissues than in control tissues.

Comparing data on body weight gain and food consumption it is clear that, on the basis of the quantity of basal diet consumed, the calorie intake of the control rats and the rats receiving 10 per cent calcium cyclamate is essentially equal. Also, the 7 per cent decrease in the calorie intake of rats receiving 5 per cent calcium cyclamate compared with the intake of control rats is not equal to the 15 per cent decrease when the weight gains of these same groups are compared. The lack of gain in weight in test animals compared with the control animals cannot be explained by calorie limitations. Calculations of feed efficiencies based on the amount of basal diet consumed also show that animals fed calcium cyclamate utilized the food less well than animals on the basal diet alone. This inability to make effective use of the food was not evident in the calorie and nitrogen balance studies. Examination of the percentage basal nitrogen and percentage basal calories retained shows that the test animals absorb and utilize approximately the same percentage of the calories consumed. The nitrogen retained from the diet was higher for the test groups than for the controls. The possible loss of nutrients as a result of loose stools is therefore small, and is not a major factor in the low weight gain of test animals. These results indicate that nutrients are utilized by animals fed calcium cyclamate in a manner different from that of control animals. These differences may be the result of any one or several effects of calcium cyclamate, including increased metabolic rate, direct interference with cellular utilization of nutrients, and energy utilized to metabolize and/or excrete calcium cyclamate or its metabolites.

The variation in water consumption seems to be a compensation for the increased excretion of water in the faeces of the animals fed calcium cyclamate. Measurements of the total water excreted from urine and faeces do not, however, account for the increased water intake, indicating an increased loss of water from respiration and skin surfaces.

Examination of the urine shows that there is some alteration in kidney excretion. The increased crystalline residue in the urine from animals receiving calcium cyclamate shows that the test material or its metabolites was excreted through the kidney. The variations in urine nitrogen concentrations after 9 months cannot be explained by nitrogen from the calcium cyclamate.

The reproduction experiments involved only small numbers of animals, but essentially the same results were obtained when the experiment was repeated. The relation between the levels of cyclamate administered in these experiments to possible levels consumed by the pregnant or lactating human is not clear. Certainly the effect of nutrition or the effect of curtailment of the growth of the young animal is important. This type of reduction of growth rate is known to be capable of impairing aptitude and accomplishment.

Also, despite the small number of animals observed, the number of young born to each female was reduced when calcium cyclamate was incorporated in the diet. Further experiments have been carried out to confirm this and will be reported later.

The results show a significant increase in adrenal weight in rats fed calcium cyclamate which is more striking when expressed as a percentage of body weight. This indicates an effect either directly on the adrenal or indirectly, possibly by way of the pituitary. We immediately felt that some mechanism was causing an increase in metabolic rate. Preliminary experiments with these animals showed an increase in the uptake of iodine-125, increased consumption of oxygen, change in heart rate, increased irritability, and increased respiration, all indicating an alteration in metabolism.

Tables 4 and 5, which tabulate organ weights, show that as the weight of the animal is reduced, the weights of the heart, liver, spleen and kidneys vary directly with the weight of the animals. In the case of the endocrine organs (gonads, adrenals, and thyroid), however, the organ weights remain the same and the percentage of body weight occupied by these organs increases in the smaller animals.

Histological examination and observations appear to support the results of measuring organ weight in indicating an effect on the testes and the adrenals as a result of feeding calcium cyclamate for 1 year. Because of the significant difference in adrenal weight between test and control groups, the adrenal glands were examined closely for slight changes that could explain the apparent weight variation. The subtle changes in the zones may indicate a tendency to hypoplasia of the zona granulosa of the adrenal in the control animals and a tendency to hyperplasia of the zona granulosa of the adrenals in the test animals. This might explain the increased adrenal gland weights in test animals.

The increased weight of the testes does not appear to result from stimulation of any particular element in testicular tissue, but is probably a general stimulation resulting in increased testicular weight in most cases but occasionally resulting in severe degeneration and necrosis of the seminiferous tubules.

The higher incidence of calcified concretions in kidneys of animals receiving calcium cyclamate may result from an increased intake of calcium from the cyclamate salt or might occur because the comparatively high level of cyclamate salt in the urine changed the solubility of other urine components. The pancreatic changes noted were not unusual for tissues from 1 year old animals.

The results described here indicate that cyclamate has a positive metabolic effect, but these effects appear transitory or take effect only during the time the animals are consuming a diet containing calcium cyclamate. The time of day at which the experiments are conducted is important. The rat is a nocturnal animal, and it consumes most of its food during the night. That the effect on rats receiving calcium cyclamate in the diet is possibly transitory and that its effect may be metabolic necessitate further consideration. Carswell *et al.*<sup>5</sup> have studied *cyclohexylamine*, a possible metabolite of cyclamate, and its properties as a strong base. Audieth and Sveda<sup>6</sup> in their original article on the preparation of the cyclamates draw attention to the properties of *N*-substituted sulphonic acids. Swanson<sup>7,8</sup> compares the action of many aliphatic

amines. Initial excretion work with rats receiving calcium cyclamate shows that the material does break down.

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## Effect of Anti-rat Lymphocyte Antibody on Humoral Antibody Formation

by

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Injections of rabbit and horse anti-lymphocytic sera into hooded rats inhibited the primary humoral antibody response to sheep erythrocytes, but did not have a marked effect on the secondary response. The major part of the immunosuppressive activity was associated with the IgG fraction. Antibody fragments were not effective in suppressing the immune reaction.

INTEREST in the immunosuppressive properties of anti-lymphocytic serum has been stimulated by the observations of Woodruff and Anderson<sup>1,2</sup> that the survival of skin homografts in rats can be greatly prolonged if the recipients are treated with rabbit anti-rat lymphocyte serum (ALS). More recent investigations have revealed that administration of anti-lymphocytic serum can also prolong the survival of skin homografts in mice<sup>3,4</sup> and renal homotransplants in dogs<sup>5,6</sup>, and it appears that anti-human lymphocyte sera may be of therapeutic value in the transplantation of human organs<sup>7</sup>.

Because of the potential practical importance of these observations we have investigated the effect of anti-lymphocytic antibody, and of fragments thereof, on the humoral antibody response of rats to sheep erythrocytes. These investigations were carried out to assess the potency of fractionated anti-lymphocytic serum and in order to obtain further data on the mode of action of this material. Similar investigations have recently been reported in mice using unfractionated antiserum<sup>8</sup>.

The anti-lymphocytic sera used were produced in a horse and in rabbits by the injection of thoracic duct lymphocytes obtained by cannulation of hooded strain male rats. The horse received intravenous injections weekly for 3 weeks and was given a fourth injection 2 weeks later. Each injection contained  $1.07\text{--}1.3 \times 10^9$  lymphocytes ( $> 95$  per cent viable). The horse was exsanguinated 10 days after the last injection. The rabbits received a total of three intraperitoneal injections, each containing  $2 \times 10^8$  lymphocytes at weekly intervals, and were bled 10 days after the final injection. Further bleedings were per-

formed on the rabbits 10 days after re-injection intraperitoneally with from  $1$  to  $2 \times 10^8$  lymphocytes.

'Sephadex G200' gel filtration and reduction with mercaptoethanol (0.2 moles/l.), followed by alkylation, revealed that the antibody produced in horses was distributed in both the 19S and 7S fractions of serum, while the rabbit antibody was predominantly 7S protein. Because of the difficulty of preparing horse antiserum free from rat erythrocyte agglutinin activity, most of this work was performed with a preparation of IgG globulin which was almost devoid of this activity (see Table 1). The limited supply of rabbit antisera prevented extensive work with fractionated material.

The IgG preparation from the horse anti-lymphocytic serum was obtained by repeated (twice) sodium sulphate precipitation (the final concentration of the sodium sulphate was 14 per cent w/v) followed by diethylaminoethyl (DEAE) cellulose batch chromatography on Whatman DE11 exchanger with a capacity of 1.0 m.equiv./g (ref. 8). The product was concentrated by lyophilization and reconstituted in phosphate buffered saline (pH 7.2, 0.06 molar phosphate containing 0.15 molar sodium chloride). Immuno-electrophoretic analysis, using a rabbit anti-horse serum and polyacrylamide gel electrophoresis, indicated that the preparation contained only small amounts of contaminating protein (probably IgT).

The F(ab')<sub>2</sub> portion of the antibody molecule, which contains both antibody combining sites, was obtained by digestion with pepsin<sup>9</sup> at 37° C for 48 h in 0.1 molar acetate buffer, pH 4.0, using 2 mg enzyme for each 100 mg of protein. Degradation of the IgG to the F(ab')<sub>2</sub> product

Table 1. PROPERTIES OF ANTISERA AND ANTIBODY PREPARATIONS

Sample	Protein conc. (g per cent)	Reciprocal titres			Effect on primary response
		Lymphocyte agglutination	Lympho-cytotoxic	Erythrocyte agglutination	
(1) Horse ALS inactivated and absorbed with one volume rat erythrocytes	5.0 (1 g per cent IgG)	512	512	128	Suppression
(2) IgG globulin from horse ALS	1.0	64	256	< 2	Suppression
(3) F(ab') <sub>2</sub> —pepsin digest from 2	1.0	32	8	4	No effect
(4) Fab'—reduced and alkylated sample from 3	1.0	4	8	< 2	No effect
(5) IgG globulin from normal horse serum	1.0	< 2	8	< 2	No effect
(6) Rabbit ALS inactivated and absorbed with a half volume of rat erythrocytes	5.3	512	512	2	Suppression
(7) Normal rabbit serum inactivated	4.9	< 8	< 8	4	Partial suppression

Note: These results are from duplicate assays.

was shown to be complete by immunodiffusion and subsequent cytotoxic analyses (see Table 1). A sample of this material was reduced with 0.1 molar cysteine hydrochloride and alkylated by dialysis against 100 volumes of the phosphate buffered saline containing iodoacetamide (0.02 moles/l.). The univalent antibody fragment obtained (Fab') did not agglutinate lymphocytes *in vitro*, indicating complete reduction (Table 1). Both these preparations were finally equilibrated against phosphate buffered saline as already described.

Erythrocyte agglutinin activity was determined in  $3 \times \frac{1}{2}$  in. test-tubes by adding 0.1 ml. of 2.5 per cent (v/v) erythrocytes to 0.25 ml. volumes of doubling dilutions of the test sample. All dilutions were performed in the phosphate buffered saline used previously. After incubation for 18 h at room temperature (20° C) the contents of the tubes were agitated and then examined macroscopically and microscopically.

Test sera failing to agglutinate sheep erythrocytes were examined for antibodies by the Coombs (anti-globulin technique) using a rabbit anti-rat serum. The non-agglutinating rat sera were incubated with sheep erythrocytes, as in the standard test, after which they were washed (three times) with 3 ml. volumes of phosphate buffered saline. After this the sheep erythrocytes were resuspended in 0.5 ml. of the phosphate buffer and divided into two equal portions. To one sample was added one drop of the rabbit anti-rat serum and to the other one drop of normal rabbit serum. Both sera had been inactivated (56° C for 30 min) and absorbed with equal volumes of sheep erythrocytes. Controls were also performed using normal sheep erythrocytes. The tubes were examined macroscopically after 3 h at room temperature.

formation was achieved only with whole antisera and intact IgG antibody. It should be noted that these samples alone exhibited lymphocytotoxic activity as assayed by *in vivo* and *in vitro* procedures. The preparation of IgG, however, demonstrated only weak cytotoxic activity *in vivo*.

The lymphopenia produced by rabbit antibody was more marked and prolonged than that observed with the horse antiserum. Furthermore, the Coombs anti-globulin test indicated that a greater agglutination was achieved with the rabbit antibody. All the sheep erythrocytes treated with the non-agglutinating sera from rats receiving horse anti-lymphocytic antibody were strongly agglutinated by the rabbit anti-rat serum. In contrast, of the sheep erythrocytes treated with the eleven negative sera from rats receiving the rabbit anti-lymphocytic antibody, only two were agglutinated by the rabbit anti-rat serum. Thus it would seem that rats treated with the horse antibody possessed appreciable amounts of non-agglutinating erythrocyte antibody while this was only rarely detected in rats receiving the more effective rabbit antibody.

The divalent antibody fragment (F(ab')<sub>2</sub>) agglutinated lymphocytes *in vitro* but failed to suppress humoral antibody formation. Thus degradation of the Fc (the complement and cell surface binding) portion of the molecule, with the expected loss of cytotoxic activity, also destroys the immunosuppressive properties of this antibody. The univalent moiety (Fab') did not agglutinate or lyse the lymphocytes *in vitro*, nor did it inhibit antibody formation. Nevertheless, this material was shown by the Coombs technique, using a rabbit anti-horse IgG, to bind to lymphocytes *in vitro*.

Table 2. EFFECT OF ANTI-LYMPHOCYTE ANTIBODY AND ANTIBODY FRAGMENTS ON THE PRIMARY RESPONSE OF RATS TO SHEEP ERYTHROCYTES

Nos. of rats	Treatment	Lymphocyte count day*		Reciprocal erythrocyte agglutination titre											Day of test	Effect on primary response
		-3	0	<2	2	4	8	16	32	64	128	256	512	1,024		
12	No pretreatment			—	—	—	—	—	3	1	4	1	2	1	5	No effect
8	Horse ALS	5,910 (100)	2,180 (37)	6	2	—	—	1	5	4	1	—	—	1	10	Suppression
14	1 g% IgG from horse ALS	5,100 (100)	3,810 (75)	7	—	4	1	2	—	—	—	—	—	—	5	Suppression
8	1 g% F(ab') <sub>2</sub> from above	5,240 (100)	5,320 (101.5)	—	—	—	—	—	—	2	6	—	—	—	10	No effect
10	1 g% Fab' from above	—	7,190	—	—	—	—	1	3	2	4	—	—	—	5	No effect
8	1 g% normal horse IgG	5,600 (100)	4,950 (88.4)	—	—	1	2	—	2	2	3	—	3	—	5	No effect
24	1 g% IgG from horse ALS to 13,560 Wistar rats (line bred)	13,560 (100)	8,860 (65.3)	—	—	—	—	—	—	3	5	15	1	—	5	No effect
4	1 g% IgG from horse ALS to 10,510 Wistar rats (inbred)	10,510 (100)	9,430 (89.7)	—	—	—	1	2	1	3	—	—	—	—	10	No effect
12	Rabbit ALS	5,910 (100)	780 (13.2)	11	—	—	1	—	—	—	—	—	—	—	5	Suppression
6	Normal rabbit serum	5,380 (100)	4,920 (91.5)	—	—	1	3	1	2	1	—	—	—	—	5	Partial suppression

Each animal received 2 ml. of test sample intraperitoneally on days -3, -2 and -1 and was injected (intravenously) with  $1 \times 10^6$  erythrocytes in 1 ml. on day 0.

\* The figures given in parentheses are per cent values based on day -3 count = 100 per cent.

Note: All experiments were performed with hooded rats except where otherwise indicated.

Lymphocyte agglutinins and lymphocytotoxins were determined by procedures described previously<sup>10</sup>. The *in vivo* cytotoxicity of the preparations was assayed by determining their effect on the peripheral blood lymphocyte count.

In most of the experiments, equal numbers of hooded male (210–275 g) and female rats (155–215 g) were used. The quantities of materials administered are indicated in Tables 1–3. A few results obtained with two strains of Wistar rats (one line bred, the other inbred) have also been included in Table 2.

Detailed preliminary investigations revealed that the peak day of primary response in hooded rats to sheep erythrocytes was day 5, and so, in all subsequent analysis of effect on the primary responses, the sera were usually examined on day 5 and day 10. The rat sera were examined 5 days after secondary challenge.

It can be seen from Tables 1 and 2 that effective suppression of humoral antibody (erythrocyte agglutinin)

The preparation of horse antibody IgG to hooded rat lymphocytes failed to inhibit the production of erythrocyte agglutinin in the two other strains of rats tested even though it produced a lymphopenia similar to that observed in the hooded rat. This could be due to a number of factors including the larger size of these animals (260–355 g), their higher initial lymphocyte count and a degree of strain specificity.

The effect of rabbit and horse anti-lymphocytic antibody on the secondary response of hooded rats to sheep erythrocytes can be seen in Table 3. Although only small numbers of rats were used in these experiments, it would appear that the secondary response was suppressed to a slight extent in all the groups receiving anti-lymphocytic antibody (compare rats A with rats B). This suppression was observed irrespective of the treatment before primary stimulation. As has been observed earlier<sup>3</sup>, the inhibition of the secondary response is not so sensitive to anti-lymphocytic antibody as the primary phase. It is possible

that more effective suppression of the secondary response may be achieved by administration of larger amounts of these preparations, or by using stronger (more avid) antisera, such as the rabbit anti-rat lymphocyte material. Monaco *et al.*<sup>3</sup> have already indicated that the degree of inhibition of the secondary response is proportional to the amount of anti-lymphocytic antibody administered. Prolonged pretreatment of this kind might also have prevented the marked primary response observed in the Wistar strain rats.

antibodies (quantitatively and qualitatively), and this may in part explain the reduced humoral antibody production after secondary stimulation.

These results also suggest a number of points of possible practical importance in the production of anti-lymphocytic antibody and its use in transplantation. The preparation of purified IgG possessing anti-lymphocytic activity may in some cases obviate the costly process of absorbing the preparation to remove erythrocyte agglutinins. Furthermore, the use of such preparations makes

Table 3. EFFECT OF ANTI-LYMPHOCYTIC ANTIBODY ON THE SECONDARY RESPONSE OF HOODED RATS TO SHEEP ERYTHROCYTES

Treatment before		Lymphocyte count		Reciprocal agglutination titre												Days after primary challenge
Primary	Secondary	3 Days before secondary	Before injection of antigen	<2	2	4	8	16	32	64	128	256	512	1,024	2,048	
A Horse antibody IgG	Horse antibody IgG	3,250 (100)	2,760 (85)	—	—	—	1	1	1	1	1	—	—	—	—	14
B Horse antibody IgG	Normal horse IgG	2,850 (100)	2,750 (97)	—	—	1	—	3	2	—	—	1	1	—	—	19
A Normal horse IgG	Horse antibody IgG	4,080 (100)	3,500 (88)	—	—	—	—	1	2	1	—	2	—	—	—	14
B Normal horse IgG	Normal horse IgG	4,140 (100)	4,500 (109)	—	—	—	—	2	1	2	—	—	—	—	—	10
A Horse ALS	Horse antibody IgG	6,790 (100)	4,300 (63)	4	—	—	—	—	—	—	1	1	1	—	—	28
B Horse ALS	Normal horse IgG	5,370 (100)	6,430 (120)	4	—	—	—	—	—	—	—	—	—	—	—	33
A Normal horse IgG	Horse antibody IgG	3,830 (100)	2,900 (74)	—	1	1	1	1	—	2	—	—	—	—	—	21
B Normal horse IgG	Normal horse IgG	4,830 (100)	3,300 (67)	—	—	—	—	1	2	1	—	—	—	—	—	26
A Rabbit ALS	Rabbit ALS	4,600 (100)	1,430 (31)	3	—	—	—	—	—	—	—	2	1	—	—	26
B Rabbit ALS	Normal rabbit serum	3,870 (100)	2,000 (52)	3	—	—	—	—	—	—	—	—	—	—	—	14
A Normal rabbit serum	Rabbit ALS	3,700 (100)	1,610 (44)	2	—	1	—	1	2	—	—	—	—	—	—	19
B Normal rabbit serum	Normal rabbit serum	—	—	—	—	1	1	1	—	—	—	—	—	—	—	14
A No pretreatment	Rabbit ALS	2,230 (100)	1,140 (51)	—	1	1	—	—	3	—	—	—	—	—	—	19
B No pretreatment	Normal rabbit serum	—	—	—	1	1	—	—	—	—	1	—	—	—	—	14
				—	—	1	1	—	—	—	—	1	—	—	—	19

All the rats received daily intraperitoneal injections of 2 ml. of whole serum (antibody or normal) or IgG globulin (antibody or normal) on the 3 days immediately before primary or secondary stimulation with  $1 \times 10^6$  sheep erythrocytes. The humoral antibody response was assessed 5 days following secondary stimulation.

These results do not, of course, reveal what mechanisms are responsible for the inhibition of antibody formation with anti-lymphocytic serum. Nevertheless, the observation that antibody IgG suppresses the formation of humoral antibody after primary antigenic stimulation without producing a significant lymphopenia merits further comment. It is possible that the preparation of antibody IgG, which we have found also produces prolonged skin homograft survival, preferentially inactivates a small proportion of cells with a pronounced capacity for producing antibody because of a particular responsiveness to antigenic stimulation. The possibility that there are two sets of immunologically competent cells with differential sensitivity to anti-lymphocytic serum has previously been put forward<sup>4</sup>. The surviving lymphocytes may have been present from the start or may have been produced as the result of lymphoid hyperplasia of a small number of cells. These cells may be intrinsically inactive or else rendered so by coating (blindfolding) with antibody. The failure of antibody fragments which bind *in vitro* to inhibit the primary immune response would, however, suggest that a "blindfolding" mechanism may not be involved, although this should be more clear when fluorescent antibody studies have been performed to determine whether these materials do indeed coat lymphocytes *in vivo*. Moreover, on the basis of the results obtained with the rabbit antiserum it is possible that F(ab')<sub>2</sub> and Fab' preparations from more avid antibody may be capable of inhibiting the primary immune response and experiments are at present being performed to test this hypothesis. As well as the possible effect of ALS on the recognition of antigen, this material might also influence the ability of immunologically competent cells to produce

unnecessary the administration of large amounts of non-antibody protein, part of which possesses anti-enzyme activity which could disturb normal metabolic processes and perhaps influence subsequent graft survival. The few results so far available with rabbit antisera indicate, at least for rats, that this material may be preferable to horse preparations. The erythrocyte agglutinins were readily absorbed from the rabbit antisera and the resultant extremely cytotoxic preparations caused almost complete suppression of humoral antibody formation after primary stimulation. Finally, the anti-lymphocytic activity of the rabbit antisera was located in the 7S region and therefore is readily amenable to isolation.

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## Specific Proteases of the Rat Mast Cell

Labelled inhibitors have been used to study the activity and properties of a chymotrypsin-like enzyme in rat mast cells. In conjunction with light microscopy (this article) and electron microscopy (second article, page 1202), the method yields estimates of the numbers and locations of the enzyme molecules.

AMONG a number of active agents bound in the mast cell are enzymes with both specific protease and esterase actions, which are believed to be important in the activities of these cells. An abundant esterase activity was first detected histochemically<sup>1</sup>; Benditt and Arase<sup>2,3</sup> made extracts showing this enzyme activity and reported that it is inhibited by di-isopropylfluorophosphate (DFP) and hydrolyses esters of *N*-acetylated L-tryptophan, L-tyrosine and L-phenylalanine at relative rates similar to those of bovine pancreatic chymotrypsin. Further evidence for the similarity of the enzyme(s) in rat mast cell extracts to bovine chymotrypsin was reported by Lagunoff and Benditt<sup>4</sup>, based on parallel activities on a series of anilide substrates. The enzyme activity is located in the mast cell granules<sup>5</sup>, and it was shown also to possess endoprotease activity similar to that of chymotrypsin<sup>6</sup>. There have been reports of the presence of another enzyme similar in specificity to bovine trypsin, in the mast cells of the dog, in cat mastocytoma and in human mast cells<sup>6-9</sup>; trypsin-like activity is not, however, detectable in rat mast cells<sup>5</sup>.

This chymotrypsin-like enzyme (or enzymes) stored in the mast cell granules is of great interest. First, knowledge of the distribution and the fate of this enzyme would throw light on a degradative mechanism of mast cells, probably operating in some inflammatory<sup>10</sup>, immunological<sup>11</sup> and other<sup>12</sup> processes. Second, the apparent similarity of specificity to a pancreatic extra-cellular digestive enzyme raises biochemically significant questions. Is it similar in molecular weight, structure, active centre design and mechanism to bovine chymotrypsin, where these features are well known? Can such evidence be related to a common origin of these two enzymes, or has a similar function evolved independently? Is self-digestion prevented in the mast cells by the existence of a zymogen form, similar to chymotrypsinogen? If so, what controls activation? Such parallels, if established in diverse structures, would give considerable enzyme information.

Investigations of the purification of this enzyme are in progress (Kawiak and Barnard, unpublished), but the disruptive isolation necessary inevitably poses some problems about the initial state and properties of the component purified. We have found that much information can be gained by reaction of the protease *in situ* within the granules; such work, reported here, is also preferred for examining the cellular aspects of this enzyme.

The use of the labelled inhibitor technique in cytochemistry<sup>13,14</sup> has made possible new methods for quantitative investigation of some enzymes *in situ*<sup>15-18</sup> and this method was used as the basic tool in the present investigations. The organophosphate-sensitive esterases present were measured by the use of isotopically labelled (tritium or phosphorus-32) DFP, which is known<sup>19</sup> to phosphorylate irreversibly the serine residue at the active centres of these enzymes. The autoradiographic procedure<sup>15-18</sup> was applied to determine the amount of DFP taken up by the individual cells, and this reaction was followed in a variety of conditions to determine some properties of the reactive enzymes.

The basic procedure was as follows. Cell suspensions rich in mast cells were collected with saline (0.17 molar)

from the peritoneal cavities of male Sprague-Dawley rats (weighing 200–300 g). The cells were fixed in suspension in 4 per cent formaldehyde solution (buffered with 0.04 molar phosphate, pH 7.4, in 0.25 molar sucrose) for 2 h at 0° C, smeared and immediately washed. Tritiated DFP and phosphorus-32 DFP were each diluted with unlabelled DFP to a final specific activity of 100–200 mc./mmole and 2.1–4.0 mc./mmole respectively. In all cases this labelled

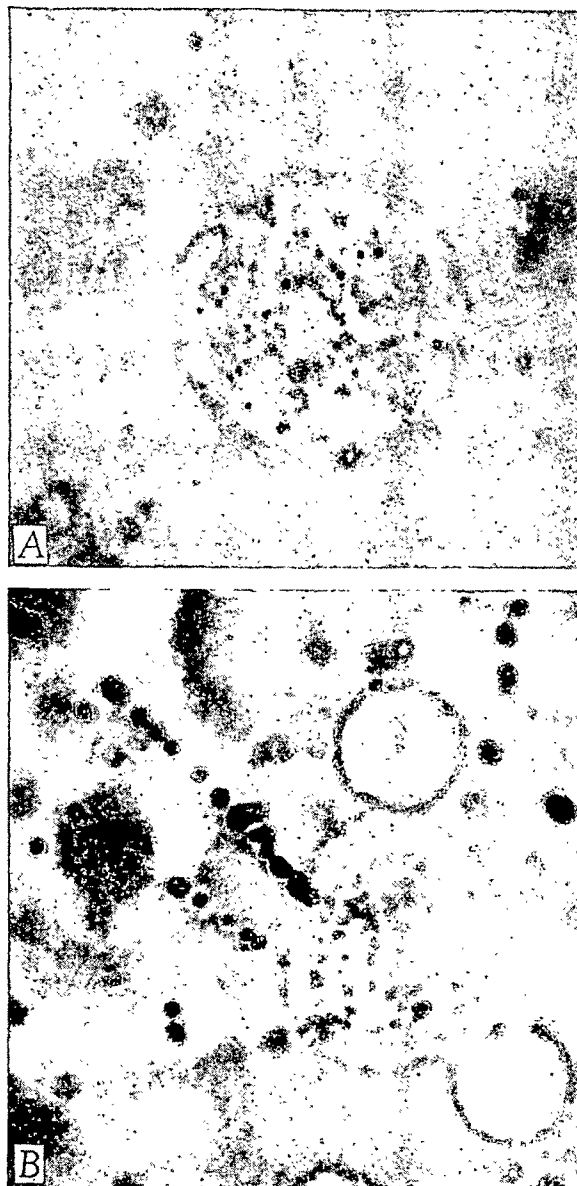


Fig. 1. *A*, Autoradiograph of a mast cell labelled with tritiated DFP (<sup>3</sup>H-DFP) (100 mc./mmole; 24 h emulsion exposure). (Oil immersion,  $\times 750$ .) *B*, A mast cell labelled with phosphorus-32 with  $\beta$  tracks (only two are in focus). (Oil,  $\times 750$ .)

DFP was applied to the smears at  $10^{-4}$  molar in 0.04 molar phosphate buffer, pH 7.4, at room temperature; at the end of all procedures the smears were washed in the buffer, and treated with unlabelled DFP ( $10^{-3}$  molar) for 30 min to remove unincorporated label. After all treatments the smears were post-fixed in ethylene-glycol monoethyl ether overnight at  $0^{\circ}\text{C}$ , which improves preservation of mast cells and completes washing. Autoradiographs were made either with 'AR-10' (Kodak) stripping film (for tritium)<sup>16</sup> or with 'G-5' (Ilford) liquid emulsion<sup>20</sup> ( $60\mu$ ) for phosphorus-32. The labelled material in the mast cells is so abundant that in the tritium autoradiographs (Fig. 1A) the emulsion exposure period was only 24–48 h. The mean of the grain densities over forty cells (after background subtraction) for each slide was used in each result presented.

Alternatively, when phosphorus-32 DFP and thick 'G-5' emulsion was used, the  $\beta$  particle tracks (Fig. 1B) were counted as described in detail for other cases<sup>18,21</sup>; this yields the number of disintegrations/cell/h of exposure, which, with the known specific activity, gives the absolute number of DFP-reactive sites in a cell.

**The reaction of mast cell enzymes with DFP.** This was measured autoradiographically in cells treated with tritiated DFP or phosphorus-32 DFP for various reaction periods from 2–128 min (Fig. 2). During about the first 30 min, the rate of DFP uptake (at  $10^{-4}$  molar) is fast, followed by a slow increase still observable at 128 min. The two slopes seen presumably indicate two different reaction rates with DFP, the first being caused by the reactivity of active centres of the enzymes with a high affinity for DFP, while the other indicates a slow reaction, which it is known could occur at less sensitive esterases<sup>22</sup> or certain other protein sites<sup>23,24</sup>. With tritium, because of the high self-absorption, only proteins in the superficial layers of the cytoplasm are revealed as reactive sites: the similarity in shape (Fig. 2) of this curve with that for total DFP uptake throughout the cell (phosphorus-32) confirms that the penetration of DFP through the cell is not limiting, so that the initial slopes of the curves represent the real rates of DFP reaction with the enzymes.

The mean number of DFP-reactive enzyme sites in a mast cell, that undergo the initial fast reaction, calculated from the track counts (Fig. 2), is  $6 \times 10^8$  (standard error  $0.3 \times 10^8$ ) for these cells, the mean diameter of which was  $16.1\mu$  (standard error  $3.2\mu$ ). To demonstrate the irreversibility of this DFP binding, cells were pre-treated with unlabelled DFP ( $10^{-3}$  molar for 30 min) and after washing were allowed to react with tritiated DFP. Final washes were as in the standard procedure. The very slow rate of tritiated DFP uptake (Fig. 2, lowest curve) then observed confirms the very slight reaction at less reactive sites, these not being fully blocked by only 30 min pre-

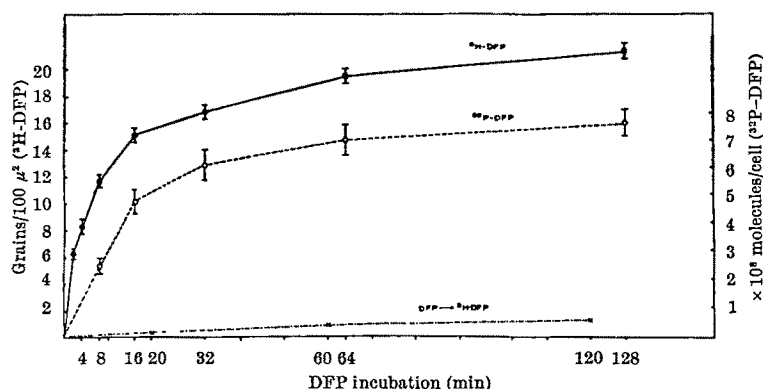


Fig. 2. Dependence of DFP reaction on incubation time (in the standard conditions). Upper line, grain counts over cells reacted with tritiated DFP; middle line, molecules of phosphorus-32 DFP reacting in each cell, derived from track counts; lower line, grain counts over cells pre-treated with DFP ( $10^{-3}$  molar for 30 min) and then reacted with tritiated DFP.

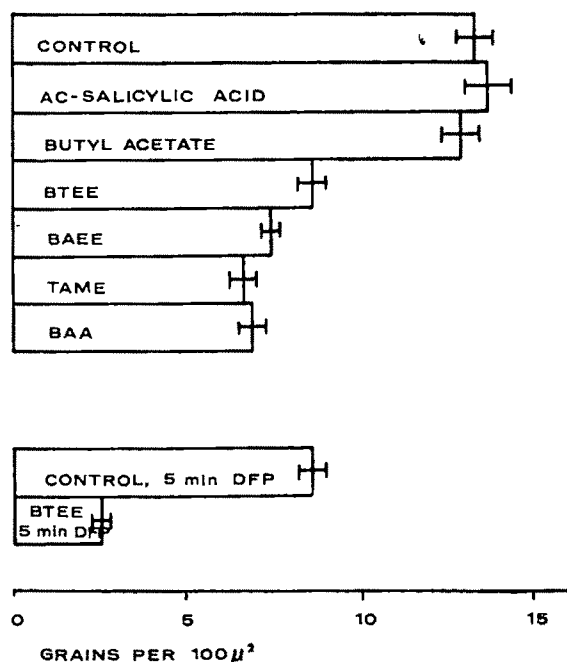


Fig. 3. Grain densities ( $\pm$  standard error) over mast cells after tritiated DFP reaction (upper block) for 16 min in the presence of: no substrate; acetylsalicylic acid; butyl acetate; benzoyltyrosine ethyl ester; benzoylarginine ethyl ester; tosylarginine methyl ester; benzoylarginine amide. All were  $5 \times 10^{-4}$  molar. The lower block shows a similar experiment, using BTEE only, in which the reaction time was 5 min only.

treatment with DFP. The adequacy of the standard washing is also confirmed by these observations.

To estimate the effect of formalin fixation, cells were reacted with tritiated DFP in suspension, either after the standard formalin fixation, or unfixed. The fixed cells showed 15 per cent greater ( $P < 0.01$ ) uptake of tritiated DFP. Thus, the fixation (and smearing) do not inhibit the reaction. The elevation might be caused by removal of an inhibitor, serotonin (see below), in fixation.

To examine the specificity and affinity of the DFP-sensitive enzymes, substrates were included in the tritiated DFP reaction bath. The competition of each substrate with DFP for the enzyme active centres was measured by the rate of inhibition of tritiated DFP uptake in its presence. Before labelling, the smears were equilibrated with the appropriate substrate for 30 min, then tritiated DFP ( $10^{-4}$  molar) was applied for 16 min in solution.

The following compounds were tested, thus, for protection, at  $5 \times 10^{-4}$  molar concentration: *n*-butyl acetate (Fisher), acetylsalicylic acid (Matheson), benzoyl-L-tyrosine ethyl ester (BTEE) (Mann), benzoyl-L-arginine ethyl ester (BAEE) (Mann), *p*-tosyl-L-arginine methyl ester (TAME) (Mann) and benzoyl-L-arginine amide (BAA) (Mann).

The first two substrates were tested to reveal the so-called "non-specific esterases" with very wide specificity for esters<sup>27</sup>, either aliphatic or aromatic or both. BTEE is known to be a highly specific substrate for chymotrypsin<sup>28</sup>. BAEE and TAME are the substrates preferred by trypsin and trypsin-like enzymes<sup>28</sup>. BAA is an amide substrate of trypsin-like enzymes. The results (Fig. 3) show that the two non-specific substrates have no significant effect on the mast cell enzymatic reaction with DFP. The chymotrypsin substrate, BTEE, inhibits uptake of tritiated DFP in these conditions by about 35 per cent, while the three trypsin substrates exert even stronger protection, each about 40 per cent. The substrates are reversibly bound, and so

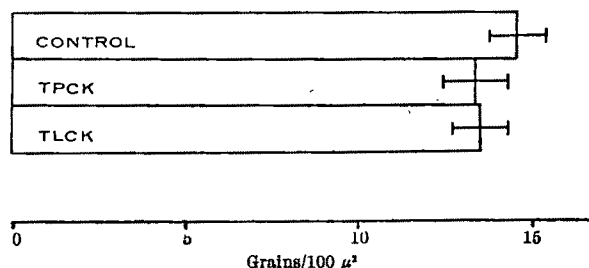


Fig. 4. Grain densities ( $\pm$  standard error) after tritiated DFP reaction (16 min) alone, or after treatment (60 min) with  $3.4 \times 10^{-4}$  molar TPCK or with  $3.4 \times 10^{-4}$  molar TLCK.

protection from the irreversible inhibitor DFP cannot be absolute, but must decline with time at a rate related to the reaction velocity of DFP with enzyme; kinetic investigations, which yield affinity constants *in situ* for substrates, will be presented elsewhere.

We can note here that when a series of concentrations of BTEE is used ( $1 \times 10^{-4}$  molar to  $1 \times 10^{-3}$  molar) and inhibitions are measured at short times (as little as 2 min) of the tritiated DFP reaction, the extent of protection by BTEE can reach a maximum of 85 per cent. Thus, most of the labelled enzymes have a specificity somewhat similar to that of chymotrypsin. The benzoyl group is not essential, because acetyl-L-tyrosine ethyl ester (Mann) used similarly gave about half the protection that BTEE gave. L-Leucinamide gave no protection.

Reaction with specific irreversible inhibitors was examined. A reagent that is extremely specific for alkylation of the active centre of pancreatic chymotrypsin, L-1-tosylamido-phenylethylchloromethyl ketone (TPCK) was designed by Schoellman and Shaw<sup>25</sup>. After this alkylation, chymotrypsin can no longer react with DFP<sup>26</sup>. L-1-Tosylamido-2-leucylchloromethyl ketone (TLCK) reacts similarly with trypsin<sup>26</sup>. In view of the resemblances reviewed above, it is of interest to apply TPCK and TLCK to the mast cell enzymes as probes for the chymotrypsin and trypsin types of active centre.

The cells were treated with TPCK or TLCK ( $3.4 \times 10^{-5}$  molar) in 3 per cent methanol in the pH 7.4 phosphate buffer for 30 and 60 min. (Control cells were exposed to the same medium with the inhibitor absent.) After buffer washes, the standard tritiated DFP procedure was applied. In all cases (Fig. 4) the DFP uptake was normal. The difference (8 per cent) between the control and TPCK-pretreated cells is not statistically significant. Checks made with bovine pancreatic chymotrypsin and trypsin showed full inhibition of these by the TPCK and TLCK media (respectively) used.

The lack of inhibition by TPCK appears to be the first significant difference detected between the chymotrypsin-like enzyme of the rat mast cells and pancreatic chymotrypsin itself. In confirmation, recent experiments in this laboratory (Dr. J. Kawiak, to be published) show that the enzyme, isolated from rat mast cells, that has high activity on the chymotrypsin substrate BTEE, is scarcely inhibited in solution by TPCK (but see note added in proof). The lack of effect of TLCK on the uptake of DFP shows that an enzyme with an active centre similar to that of pancreatic trypsin is also absent in these cells.

Serotonin (5-hydroxytryptamine) and histamine are known to be stored in living mast cells. The effect (reported above) of fixation on the DFP reactivities, whereby fixed and washed cells react faster than unfixed, living cells, together with reports of the inhibitory effects of tryptamine on pancreatic chymotrypsin activity<sup>29</sup> and of serotonin on the autocatalytic activation of trypsinogen<sup>30</sup>, suggested a possible inhibitory function of serotonin on the mast cell proteases. Testing this, the fixed cells were treated for 30 min with serotonin at  $5 \times 10^{-4}$  molar or  $10^{-4}$  molar, in the pH 7.4 buffer (room temperature), and next underwent treatment with tritiated DFP ( $10^{-4}$  molar)

in the presence of serotonin ( $5 \times 10^{-4}$  molar) for 2–64 min (Fig. 5). A marked lowering of tritiated DFP uptake in the presence of serotonin is seen, indicating its inhibition of the enzymes, but a further, interesting phenomenon is observed: DFP reaction decreased progressively (by about 30 per cent) in the cells during the last 32 min of incubation. The phosphorylation is irreversible, and so this is explained as a result of an extraction by serotonin of the labelled enzymes from the cells. (In these serotonin-treated, fixed cells, no degranulation was apparent.)

This interpretation is supported by an unusual spread of the radioactivity into the emulsion from the serotonin-treated mast cells, where a halo 20–40  $\mu$  wide, of dense background labelling, was observed. For further confirmation (Fig. 6, lower block), cells were treated with serotonin ( $5 \times 10^{-4}$  molar) alone (a) for 10 and 20 min after labelling, and (b) for 60 min before labelling (with tritiated DFP alone). In both (a) and (b), a significant decrease of the incorporated label is seen, increasing with the duration of serotonin treatment. Thus, serotonin at low concentrations extracts these mast cell enzymes, both in their original and their phosphorylated forms.

The inhibitory effect of serotonin, independent of extraction, was also measured. Cells were exposed to serotonin ( $5 \times 10^{-4}$  molar) for 5 min and then to tritiated DFP and serotonin together; control cells had the serotonin exposure for 10 min and were then reacted with tritiated DFP (alone) for 5 min. Thus, both groups of cells were exposed to serotonin for 10 min, so that the extractive effects should be identical; from a difference in labelling the binding of serotonin at the enzyme active centres could be separately evaluated. It was seen (Fig. 6, upper block) that serotonin is strongly inhibitory. Further investigations of effects of serotonin on mast cell proteases, including actions on unfixed cells and inhibition of the activity of isolated proteases, will be reported in full elsewhere. Histamine, as another bio-active amine concentrated in mast cells, was tested similarly, but it has failed to exhibit any powers of inhibition or extraction of the enzymes examined here (see Fig. 7).

In conclusion, the reaction of labelled DFP with the proteases of mast cells opens up new analytical approaches to their investigation. Among others, the following points can be established. (1) The absolute number of the organophosphate-reactive protease or esterase molecules in individual mast cells has been determined. Problems of fractionations are avoided. Only mast cells are labelled at this low exposure, and so other cells present do not interfere. It should be noted that this number in each cell is so large that, even if these enzymes are all of molecular weight (in each active centre) of only 20,000, they would constitute at least 10 per cent of the known<sup>31</sup> total dry mass of the rat mast cell (which already includes much heparin, about 30 per cent by weight of the gran-

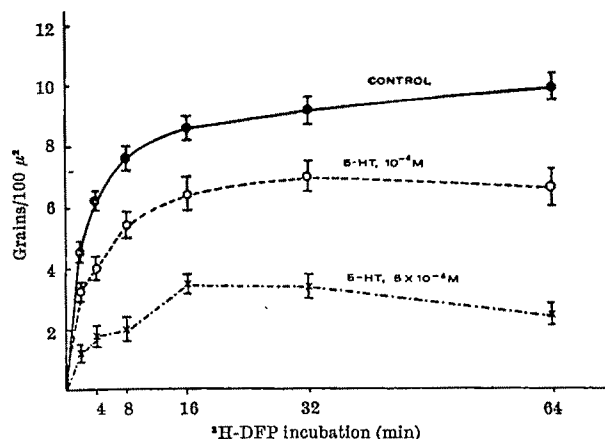


Fig. 5. Rates of tritiated DFP uptake in mast cells, measured by grain density ( $\pm$  standard error), in the presence of serotonin (5-HT) at two concentrations, or in its absence (control).

ules<sup>32</sup>). This enormous enzyme concentration suggests storage for a degradative role in mast cell function. It also indicates that the main enzyme(s) here must be of the order of 20,000 or less in molecular weight.

(2) At least 85 per cent of these enzyme molecules resemble chymotrypsin in affinity for  $\beta$ -aryl structures. The inhibition by BAEE and TAME can also be attributed to the same cause, involving their aromatic substituent; no hydrolysis of these substrates can be found in extracts of these rat cells (Dr. J. Kawiak, unpublished), confirming other reports<sup>32</sup>. The serotonin affinity found also fits a chymotrypsin-like binding site.

(3) This mast cell enzyme has an active centre, therefore, with a binding site similar to that of chymotrypsin and with the activated serine (presumed from the DFP reactivity), but without the active histidine (responsible<sup>28</sup> for TPCK reactivity) (but see note added in proof).

(4) The active centres are fully available in the intact cell, so that a zymogen or precursor protein does not occur. Serotonin (but not histamine) has a high affinity for the active centre of this enzyme; this is indicative, although not conclusive, of a role of serotonin in inhibiting protease activity in the intact mast cell. The slow extraction by serotonin seen later is not inconsistent with this role: the concentration of serotonin may act as a control, restraining protease activity at small concentrations and releasing it in physiological processes when the free serotonin concentration rises.

(5) The location of this enzymatic activity in the mast cell granules<sup>32</sup> can be proved in intact cells by electron microscope autoradiography after tritiated DFP reaction (see succeeding communication); this can also provide useful extensions of these investigations.

(6) The reaction with isotopic DFP can be used to label mast cells for *in vivo* work, to follow the cells and their granules and the fate of the enzyme in functional situations<sup>33</sup>.

This work was supported by grants from the U.S. Public Health Service and from the Damon Runyon Memorial Fund. We thank Miss Eliza Ferby for technical assistance.

*Note added in proof.* These experiments have now been extended (Kawiak and Barnard, to be published). The protease has been isolated in pure form from rat peritoneal

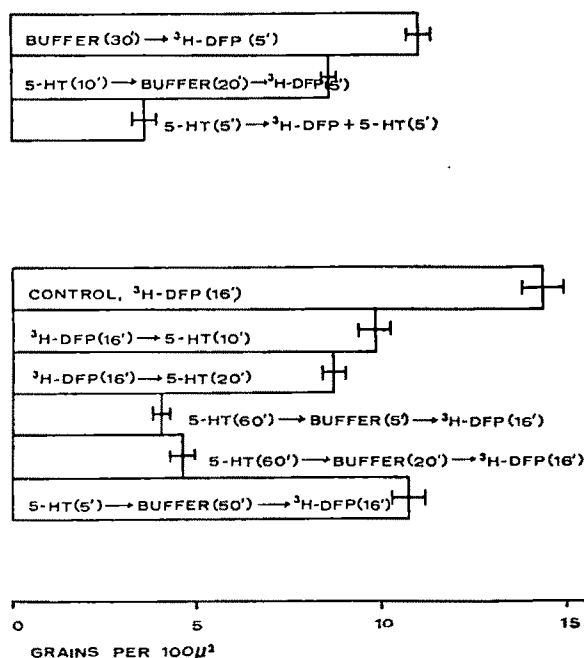


Fig. 6. Upper block: grain densities ( $\pm$  standard error) after tritiated DFP reaction, alone, or after  $10^{-4}$  molar serotonin (5-HT) pre-treatment, or with pre-treatment and in presence of serotonin; lower block: grain densities ( $\pm$  standard error) after reaction with tritiated DFP with various sequences of post-treatment or pre-treatment with  $10^{-4}$  molar serotonin.

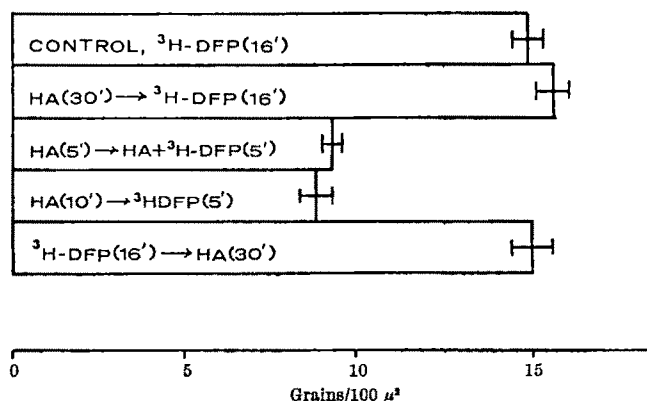


Fig. 7. Grain densities ( $\pm$  standard error) after tritiated DFP reaction alone (control), or with pre-treatment or presence or post-treatment with  $10^{-4}$  molar histamine (HA).

mast cell granules. It has a high activity on BTEE and on casein, and is rapidly inhibited by DFP. It is, however, irreversibly inhibited by TPCK, but at a very slow rate: the half-time for activity loss with  $4 \times 10^{-4}$  M TPCK (a very large molar excess over the enzyme) at pH 7.8, 25°, is 4 h. This is very much slower than the inactivation rate with pancreatic chymotrypsin. Thus our inability to react the protease *in situ* in our conditions is to be expected, from this low reactivity. The difference involved between the mast-cell chymotrypsin-like enzyme and pancreatic chymotrypsin is therefore quantitative rather than qualitative. The active centres must be similar, but nevertheless must differ in some important feature determining this rate.

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### Intracellular Localization of Specific Proteases in Rat Mast Cells

THE possibility of applying radioactive organophosphate inhibitors of high specific activity to localize sites of esterase activity by autoradiography has permitted estimates, in several specific cases, of the number of enzyme active centres present in individual intra-cellular sites<sup>1,2</sup>. For the rat peritoneal mast cell, it has been found (see preceding article) that the absolute number of organophosphate-reactive enzyme molecules with protease (and esterase) activity in individual mast cells can be determined thus, without the problems incurred in fractionating cell components.

While previous investigations by fractionation methods indicated that this enzyme activity is located (at least for the most part) in the specific granules of the mast cell<sup>3</sup>, an *in situ* confirmation of this is highly desirable. Further, an *in situ* analysis should provide a complement to the isolation investigations, for the latter cannot determine whether distribution in a granule is homogeneous or heterogeneous, nor whether the enzyme component occurs in all of the granule population of a cell or only in one fraction of it. Other functional components of the rat mast cell, such as heparin, serotonin and histamine, are also thought to be bound in the granules<sup>4,5</sup>, and it will be essential for an understanding of the activities of mast cells to know whether all occur together with the protease in all the specific granules, or whether there is segregation in different granule types, and, if so, which components are structurally associated. The autoradiographic method, taken to the moderately high resolution attainable in electron microscope autoradiography, should be competent to examine such questions in the case of the mast cell granules.

The reactive enzyme fraction in the mast cell is so abundant that, when using tritiated di-isopropylfluorophosphate (tritiated DFP) at a specific activity of 1 c./mmole (well below the maximum available), adequate labelling for quantitative investigations is obtained over each mast cell in electron microscope autoradiographs (using ultra-thin sections and emulsion layers) after only 6 days exposure to emulsion. We report here such results on rat peritoneal mast cells after 6–50 days exposure, employing methods giving a resolution sufficient to resolve labelling at the level of individual granules. The same approach has concurrently been used on the mast cells of mouse skeletal muscle<sup>6</sup>.

Mast cells were obtained as described in the preceding communication, and fixed in suspension in buffered glutaraldehyde (3 per cent, in 0.04 molar sodium phosphate/0.25 molar sucrose, pH 7.4) for 2 h at 0° C. Using centrifugations, they were, in turn, washed in the buffered sucrose (20 min), treated in suspension with 10<sup>-4</sup> molar tritiated DFP (1.0 c./mmole, in the buffered sucrose, 20 min at room temperature), washed in the buffered sucrose (20 min) and treated with unlabelled 10<sup>-3</sup> molar DFP (in buffered sucrose, 20 min) to remove unincorporated label. The cells were then transferred to ethylene-glycol monoethyl ether for 24–48 h at 0° C. Dehydration was in graded concentrations of ethanol after a treatment in water (1 h) with 1 per cent osmium tetroxide (pH 7.4). Cells were embedded in 'Araldite', sectioned and coated with Ilford 'L<sub>4</sub>' emulsion using the membrane technique<sup>6</sup> (developed in 'D163' or 'Mierodol X' or with Kodak 'NTE' emulsion<sup>7</sup> (developed in 'Dektol').

Both granulocytes and agranulocytes, as well as other cells, also occurred in the samples, but with minor ex-

ceptions only mast cells were labelled. Cytological preservation was very good. Silver grains occurred in a well defined distribution over each mast cell in all autoradiographs after 6 to 50 days exposure. By far the heaviest incorporation was into the specific granules (Fig. 1; Table 1). Two, or possibly three, kinds of granule were recognized morphologically, but the relation of the grains to each type showed no discernible differences. Examination at heavier grain densities (which was undesirable for the initial analysis) will be needed (and feasible) to determine whether smaller quantitative differences exist in this component between types of granule. Most nuclei had only 1–3 grains, which were over the electron-dense

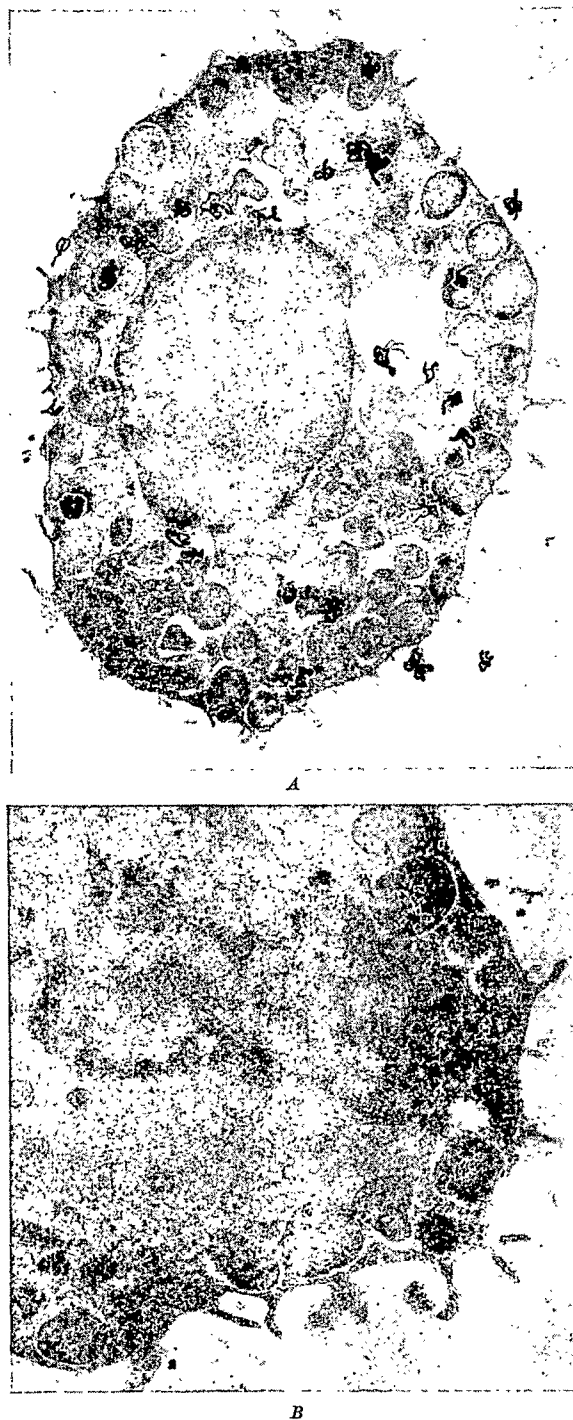


Fig. 1. Electron microscope autoradiograph of a section through a labelled mast cell, showing silver grains associated with the granules. A, 'L<sub>4</sub>' emulsion ( $\times 7,440$ ). B, Another section in higher resolution using 'NTE' emulsion (after 50 days exposure;  $\times c. 12,900$ ).



chromatin, but occasional cells had rather more grains in this region (one such cell was included in the 6 day counts in Table 1).

Table 1. GRAIN DISTRIBUTION OVER PERITONEAL MAST CELLS REACTED WITH TRITIATED DFP

Experiment	Exposure	Total grains	Specific granules	Non-granule cytoplasm	Nucleus
1	6 days	123	100	9	14
2	44 days	116	111	2	3

Counts were made over a number of sections, to the grain totals shown, and these were recorded as occurring over one of the three cell compartments listed. The relative areas of the components were also measured by the point grid method<sup>10</sup> over sections from seven cells: the non-granule cytoplasm occupies 16 per cent by area, but has only 5 per cent (mean) of the total cytoplasmic label. This small cytoplasmic fraction of label outside the granules is taken to be due to spread of radiation from the granules, which must occur in these conditions if only they are labelled; the lower resolution ('L<sub>4</sub>') conditions were used here.

In the conditions we have used, a resolution of at least 0.2 $\mu$  for 'L<sub>4</sub>' emulsion and 0.1 $\mu$  for 'NTE' is predicted<sup>6-8</sup>. In Fig. 2 is plotted the distribution of the developed grains in 'NTE' emulsion over equal areas of granule material, for sections of granules having a radius of 0.4 $\mu$  to 0.65 $\mu$ . Each grain was recorded as a function of its distance ( $d$ ) from the limiting membrane of the associated granule of measured radius ( $r$ ). Limits for  $d/r$  representing a division of the granule into three equal concentric areas were calculated, and each grain was assigned to one or other of these compartments. It was seen that all of a granule is labelled and there is no preferential localization of label in the peripheral or central areas.

Serious errors might be risked if the osmium tetroxide post-fixation could remove some di-isopropylphosphoryl groups, or produce extractions<sup>9</sup> of protein. That this does not occur was established by an experiment using liquid scintillation counting of total extracts of treated cells (Table 2).

We consider that the labelling reveals the sites of the mast cell chymotrypsin-like enzyme(s), on the evidence presented in the previous article. Thus, we conclude that this enzyme is indeed localized in the granules.

That fraction of the label which is definitely not in the granules is in the chromatin of the nuclei. This is a small and variable fraction: some cells had no nuclear labelling. Two possible explanations can be offered. First, the mast cell protease is a strongly basic protein fraction when isolated<sup>3</sup>, and so it can combine with heparin, but also with DNA in mast cell homogenates (Dr. J. Kawiak, unpublished data). It may combine in the mast cell

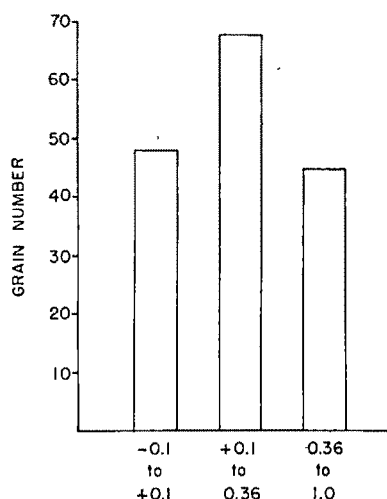


Fig. 2. Histogram showing the distribution of developed grains in 'NTE' emulsion over equal areas of granule material in and around sections of individual granules of radius ( $r$ ) 0.4 to 0.65 $\mu$ . The numbers of counted grains that lie within one or other of three equal concentric areas are plotted. The limits of these areas are defined by the values of  $d/r$  shown on the horizontal axis, where  $d$  is the distance from the grain to the nearest granule boundary. Positive values denote distances within a granule.

nucleus as histones do, either naturally or from a few disrupted granules in those cells (although no discernible damage was associated with nuclear labelling). Second, the chromatin-bound enzyme might be newly synthesized on polynucleotide synthetic centres in these nuclei. This compels further investigation.

Table 2. ABSENCE OF ARTEFACTAL DECREASE OF LABELLING BY OSMIUM TETROXIDE ACTION

Mean counts per min from each sample		Control cells	
Osmium tetroxide-treated cells			
a	3,957 c.p.m.	d	4,189 c.p.m.
b	3,857 c.p.m.	e	3,853 c.p.m.
c	4,268 c.p.m.	f	4,186 c.p.m.
Mean	4,024 c.p.m.	Mean	4,076 c.p.m.

Mast cells were treated by the procedure used for the electron microscope specimens, up to the stage of the unlabelled washing with DFP (but using 5 per cent (w/v) formaldehyde in place of the glutaraldehyde, and with the tritiated DFP specific activity at 120 mc./mmole). They were then efficiently suspended and equally divided in six portions, in 0.04 molar sodium phosphate, pH 7.4, buffer (1.2 ml. total). Samples a, b and c were each treated with osmium tetroxide (2 per cent, in the buffer, 1 h at 0° C), while d, e and f remained in the buffer alone. After buffer washings, each sample was extracted with 0.5 ml. of NCS solubilizer (Nuclear Chicago, Inc.), 16 h. with rapid shaking for 4 h at room temperature, taking all the material into solution. Toluene (15 ml.) containing scintillation materials was added to each, giving clear solutions, which were counted in a Packard 3224 liquid scintillation counter at 7° C. The efficiency was identical in all tubes (and background was 34 c.p.m.); the mean counts, therefore, represent the bound isotope in equal quantities of cells.

It was seen in the light microscope autoradiography described in the previous article that all mast cells in the population contain this enzyme component, and that they are without very marked variations in their content of it. We see here that it is generally distributed among the granules in each cell. We can note that the penetration of DFP to all the sites can be assumed, because the absolute number of reacted sites<sup>2</sup> is large enough to show that all of this enzymatic protein does react. The reaction is also not reduced by previous fixation (see preceding article).

A further analysis of mast cells reacted with tritiated DFP and a detailed series of electron micrographs will be published elsewhere. More extensive labelling and higher resolution are technically feasible. High resolution studies have been pursued on mouse muscle mast cells<sup>4</sup>. It also seems to be possible to extend the electron microscope autoradiographic approach to some other functional components of the mast cell, by suitable incorporations of label (biosynthetically); combination with the protease investigations should then achieve an analysis of the associations of these components in individual granules.

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## Seasonal Variation in the Concentration of Caesium-137 in Grass and Alfalfa

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Analyses of the content of caesium-137 and other radionuclides in grass and alfalfa in Western Slovakia during 1962–65 indicate that such measurements provide a means of determining the rate of radioactive fall-out from nuclear weapon tests, provided certain climatic factors are taken into consideration.

THE accumulation of radioactive caesium-137 as a by-product of nuclear fission is of considerable importance because of its long half-life. Radiocaesium enters plants either by absorption from the soil by the root system or by assimilation of free deposits on various parts of the plant.

The penetration of caesium-137 into plants is frequently studied in connexion with the properties of soils. Essentially soils consist of a polydispersed system of organic and inorganic substances. Clayey mineral colloids and humous organic colloids are interbonded in the soil to form a complex organo-mineral sorbent which is capable of "fixing" such plant nutrients as phosphates, sulphates and water in the soil. The soil solution is very dilute and only a small proportion of the plant nutrients is actually present in it because most of them are bonded to the mineral particles and especially to the humous sorbent complex. The concentration in the soil solution also depends on the sorptive forces between the ions and the soil particles, as well as on the number and nature of the cations and anions present.

Compared with strontium, caesium is firmly bonded with the sorptive soil complex because it has a greater ionic radius and a smaller hydration envelope than strontium. This is one reason why caesium is not washed into the soil solution as easily as strontium and, consequently, is relatively less diffused in the plant. The removal of radiocaesium from the soil by plants depends on the mutual relations of various anions and cations present in the soil. Shone<sup>1</sup> extracted caesium-137 from the soil-type "greensands" in the presence of various electrolytes, and found that calcium chloride is most efficient in reducing the removal of caesium from the soil. A high concentration of calcium makes it more difficult for caesium to be removed from the soil by plants<sup>2</sup>, while a high concentration of sodium and potassium makes it easier.

It is known that up to 70–80 per cent of the caesium-137 from fall-out is fixed and concentrated in the upper organic layer of the soil and remains there in an "unavailable" form<sup>3</sup>. According to Squire<sup>4</sup>, absorption of caesium-137 from the soil by grass represents between a twentieth and a quarter of the strontium-90 taken up—the proportions being determined by the nature of the soil, the pH and the calcium content of the soil.

The relative concentration factor for caesium-137 between plants and the soil is in the region of 0.01–1 (ref. 5), that is, the concentration of caesium in plants is lower than that in the soil provided only the uptake by the root system is taken into account. It is evident from numerous

experiments<sup>6–9</sup> that the uptake of radionuclides from a contaminated soil by the root system is slight compared with the direct contamination in other parts of plants. Wijk and Braams<sup>10</sup>, who followed the removal of caesium-137 by grass over a period of several months, found that in artificially contaminated soil grass absorbed only some tenths of a per cent of the radiocaesium. By means of autoradiographs, Middleton<sup>11,12</sup> showed that leaves are subject to direct contamination by caesium-137 when it is sprayed on to them.

It thus follows that so far as the deposition of radionuclides on plants and foliar absorption are concerned, the most important factor is the direct contamination of the aerial part of the plant or the rate of radioactive fall-out. This supposition is supported by the findings of

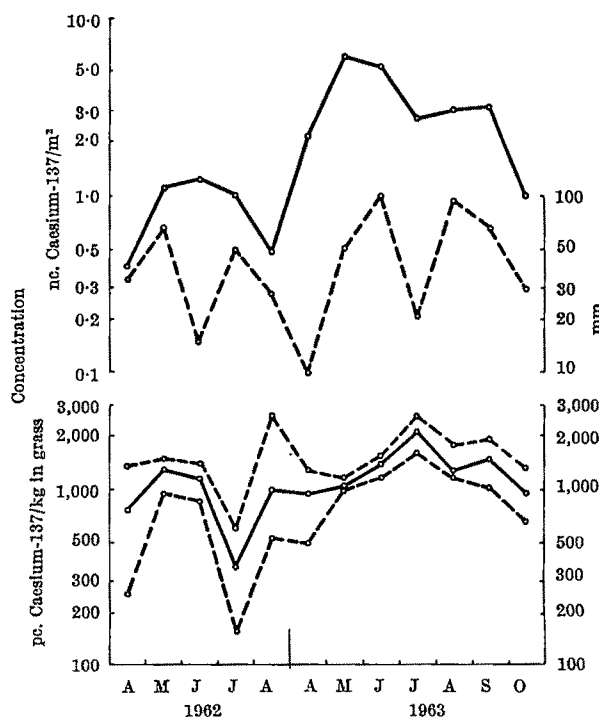


Fig. 1. Comparison of the activity of caesium-137 in fall-out and in grass during 1962–63. Top curves: —, fall-out; ----, rain. Bottom curves: —, mean values; ----, maximum and minimum values.

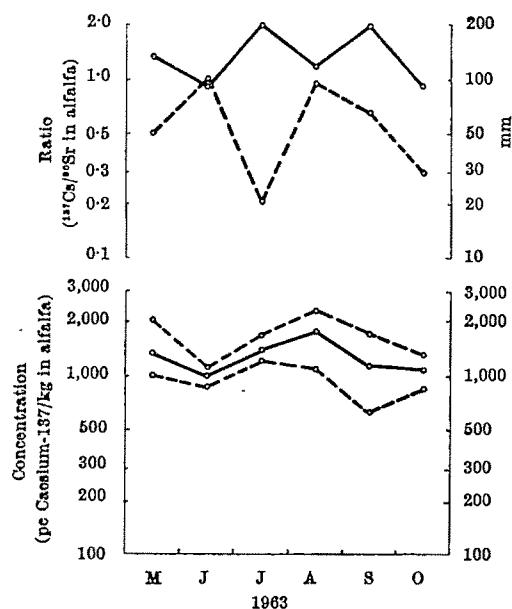


Fig. 2. Comparison of the activity of caesium-137 and the mean ratio of caesium-137 : strontium-90 in alfalfa, in 1963. Top curves: —, ratio  $^{137}\text{Cs}/^{90}\text{Sr}$ ; ---, rain. Bottom curves: —, mean values; ---, maximum and minimum values.

Evans and Dekker<sup>13</sup>, who traced the activity of various agricultural products and found that uncovered alfalfa shows 80 per cent more activity than covered alfalfa.

The radiocaesium content in vegetation varies throughout the whole vegetative period. In order to determine the content of caesium-137 in grass and alfalfa during the period of growth and its accumulation during the vegetative period, samples of these plants were analysed several times a year from 1962 onwards from the regions of Bratislava, Trnava, Jaslovské Bohunice and Piešťany in Western Slovakia. Sampling was on a monthly basis for 2 years and thereafter only twice a year. Caesium-137, in the form of caesium tungsto-silicate<sup>14,15</sup>, was obtained from 100 g of each sample, which had been dried at 105° C and burnt at 450° C, and the  $\beta$ -activity measured.

Fig. 1 shows values for the average activity of caesium-137 obtained from grass samples taken over the period 1962–63 and Fig. 2 gives those obtained from alfalfa. We have also plotted the maximum and the minimum values obtained over separate months at various areas. The graph shows that samples taken on a more frequent basis do not yield results revealing any further marked changes in the content of caesium-137 when expressed per unit of dried matter. There would seem to be a definite relationship, however, between the fall-out, the rainfall and the caesium-137 activity in grass and alfalfa. The activity of caesium-137 in fall-out increases with increased precipitation; however, it drops in grass and alfalfa probably because deposits of caesium-137 on the leaves and flowers are washed into the soil by rain. There are, of course, other climatic factors which influence the level of activity of caesium-137 in the vegetation, the most important of which is the wind. As a result of the action of rain and wind, the soil and the vegetation of some regions may become enriched in radioactive substances,

although in other regions they may become impoverished. Besides the climatic factors, the shape and size of the leaves play an important part in the relationship between plants and radioactive substances. Alfalfa, for example, has a leaf area of 85.6 m<sup>2</sup>/m<sup>2</sup> of soil, while maize has only 2.7 m<sup>2</sup>/m<sup>2</sup>.

The ratio of caesium-137 : strontium-90 provides evidence that the main part of activity of caesium-137, especially in the years after the nuclear bomb tests, comes from fall-out. In alfalfa this ratio is directly related to the fall-out and moves within the limits 1–2 (see Fig. 2).

We followed the activity of caesium-137 in grass and alfalfa obtained at the first and second mowings over a period of 4 years (Table 1). (The first mowing covers the period April–May and the second the period June–August.) The results given in Table 1 for precipitation and for the activity of the caesium-137 in the fall-out and in the vegetation represent cumulative values for the period of active vegetative growth. They show that the activity of radiocaesium in grass and alfalfa is higher in spring than in summer. This indicates the considerable influence which rain has because the fall-out is greater in spring than in the other seasons of the year and this has a direct influence on the intensity of the activity of caesium-137 in grass and alfalfa.

Table 1. COMPARISON OF THE ACTIVITY OF CAESIUM-137 IN GRASS AND ALFALFA DURING TWO MOWING PERIODS IN EACH OF THE YEARS 1962–65

Year	Mow- ing period	Rain- fall (mm)	Deposit $^{137}\text{Cs}$ (nc./m <sup>2</sup> )	K (g/kg)	Grass $^{137}\text{Cs}$ (pc./kg)	$^{137}\text{Cs}/\text{K}$ (pc./g)	K (g/kg)	Alfalfa $^{137}\text{Cs}$ (pc./kg)	$^{137}\text{Cs}/\text{K}$ (pc./g)
1962	(1)*	101	1.51	11.8	1,221.8	103.5	—	—	—
	(2)	94	2.79	6.0	1,000.8	166.8	—	—	—
1963	(1)	62	8.51	13.9	1,088.8	78.3	15.9	1,408.9	88.6
	(2)	218	11.42	21.4	1,225.3	57.2	16.2	1,810.2	111.7
1964	(1)	102	4.72	18.3	786.7	43.0	17.5	940.0	53.7
	(2)	152	5.20	13.5	586.2	43.4	—	—	—
1965	(1)	250	2.45	18.7	655.0	35.0	15.9	317.7	20.0
	(2)	282	2.52	15.1	430.2	28.5	—	—	—

\* (1) Measurements made during the period April–May; (2) measurements made during the period June–August.

There is no substantial difference between the activity in grass and that in alfalfa in any one vegetative season, except that, on average, it is 20 per cent higher in alfalfa than in grass. The potassium content in the plant is relatively constant and, because the activity of radiocaesium in the samples tested shows a tendency to decrease, the ratio caesium-137 : potassium also tends to decrease. A substantially lower content of potassium in grass in 1962 (6.0 g potassium/kg of dried matter) resulted from an early arrest of active vegetative growth due to intense heat and excessive drought in August.

Table 2 gives the annual average content of caesium-137 and strontium-90 in grass and alfalfa, computed for a 4 year period so that a comparison can be made with the amounts in the fall-out. Even the heavy rainfall of 1965 failed to increase the amounts of the two radionuclides in the fall-out. This suggests that to a great extent the radionuclides thrown out by the intensive tests with nuclear weapons during the period 1961–62 have been cleared from the stratosphere. In 1965 the content of caesium-137 in the fall-out dropped by 70 per cent and the content of strontium-90 by 80 per cent as compared with 1963 when the fall-out reached a maximum.

Table 2. CONTENT OF CAESIUM-137 AND STRONTIUM-90 IN GRASS AND ALFALFA IN WESTERN SLOVAKIA DURING 1962–65

Year	No. of samples Grass    Alfalfa	Mean annual rainfall (mm)	Annual cumulative deposit (nc./m <sup>2</sup> )		Caesium-137 (pc./kg)		Strontium-90 (pc./kg)		Mean ratio: $^{137}\text{Cs}/^{90}\text{Sr}$	
			$^{137}\text{Cs}$	$^{90}\text{Sr}$	Grass	Alfalfa	Grass	Alfalfa	Grass	Alfalfa
1962	16	—	583	8.78	12.66	959.0	860.2	—	1.1	—
1963	24	24	558	28.98	22.50	1,365.3	1,335.9	1,000.3	1.0	1.2
1964	7	6	555	15.46	16.86	686.4	869.2	911.1	0.8	1.5
1965	8	13	827	7.75	5.29	535.4	470.4	496.5	1.1	0.8
Mean ratio:										
1965/1962	—	—	1.4	0.9	0.4	0.5	0.6	—	—	—
1965/1963	—	—	1.5	0.3	0.2	0.4	0.5	—	—	—
1965/1964	—	—	1.5	0.5	0.3	0.8	0.5	1.0	—	—

The effects of less fall-out together with high precipitation could be detected in the lower proportion of these two radionuclides present in plants. Compared with 1963, the activity of caesium-137 in grass decreased by 60 per cent and in alfalfa by 70 per cent in 1965. For the same period, the drop in the activity of strontium-90 in grass was 60 per cent and in alfalfa 50 per cent and the ratio of caesium-137: strontium-90 ranged approximately from 1 to 1.5.

These results show that the variations in the content of caesium-137 in grass and alfalfa under natural conditions during the period 1962-65 depended mainly on the rate of radioactive fall-out in a given area. Cumulative deposition of caesium-137 in the soil of the region tested was not markedly manifested by an increased removal of caesium-137 by grass and alfalfa.

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## Persistent Binding of Butter Yellow Metabolites to Rat Liver DNA

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Radioactive metabolites of butter yellow (<sup>3</sup>H-dimethylaminoazobenzene) have been found to bind covalently to the liver DNA of hooded rats. The specific concentration of radioactivity dropped to roughly a half its initial value in the first 7 days and then remained roughly constant for 3 months, which implies an absence of repair. This observation could explain chromosome aberration observed in regenerating liver after treatment with carcinogens and may play a part in the latent period in the carcinogenic process.

AFTER a single intraperitoneal injection (150 mg/kg) of dimethylaminoazobenzene (DAB) labelled with tritium in the primed ring, radioactive drug metabolites were bound covalently to the ribosomal RNA (rRNA), the cytoplasmic and nuclear proteins, and to the DNA of the liver<sup>1</sup>, spleen<sup>2</sup> and kidney<sup>2</sup> of male albino rats. The maximum level of binding to rRNA was 114  $\mu$ moles/g while for DNA it was 14  $\mu$ moles/g. The level of radioactivity associated with rRNA and with the proteins decreased with time, the rate of decay paralleling the rate of turnover of these macromolecules. By contrast, the level of radioactivity bound to DNA remained constant for 12 days<sup>1</sup>.

This work was continued to determine the half life of the radioactivity associated with DNA, and six groups of three male hooded rats weighing about 200 g (Chester Beatty stock) were kept for periods of up to 3 months after a single intraperitoneal injection of 150 mg/kg tritiated DAB (specific activity 1.2 c./mmole). They were maintained on a diet containing 10 per cent protein<sup>1</sup> and allowed just sufficient food to keep weight increases below 20 per cent over 3 months. The groups were killed at 2 days, 1 week, 3 weeks, 6 weeks, 2 months and 3 months. The proteins, rRNA and DNA were isolated from individual animals and purified as previously described<sup>1</sup>. The DNA was purified by treatment with RNase, removal of residual proteins with phenol, and removal of glycogen with methoxyethanol. The DNA was converted to the cetyl trimethylammonium bromide salt, washed well with water, and reconverted to the sodium salt. Finally the DNA was dissolved in a solution containing 2 per cent sodium acetate and 1.5 per cent sodium chloride (0.5 mg/ml.) and spun for 1 h at 20,000 r.p.m. The supernatant was treated with two volumes of 2-ethoxyethanol to precipitate the DNA. DNA prepared in this way contained less than 2 per cent residual protein. For assay of radioactivity the DNA was dissolved in water (1 mg/ml.), and degraded with DNase. Portions (0.1-0.5 ml.) were assayed using a

liquid scintillation spectrometer. The rRNA and the proteins were purified and assayed as previously described<sup>1</sup>. The results showed good reproducibility from animal to animal within each group, the maximum variation being about  $\pm 20$  per cent. Results were expressed as an average for each group.

Radioactivity associated with liver rRNA and with the cytoplasmic proteins is shown in Fig. 1. The half-life of each was between 3 and 5 days and the rate of loss of radioactivity paralleled the rate of turnover of these macromolecules<sup>1</sup>. The levels of binding were similar to

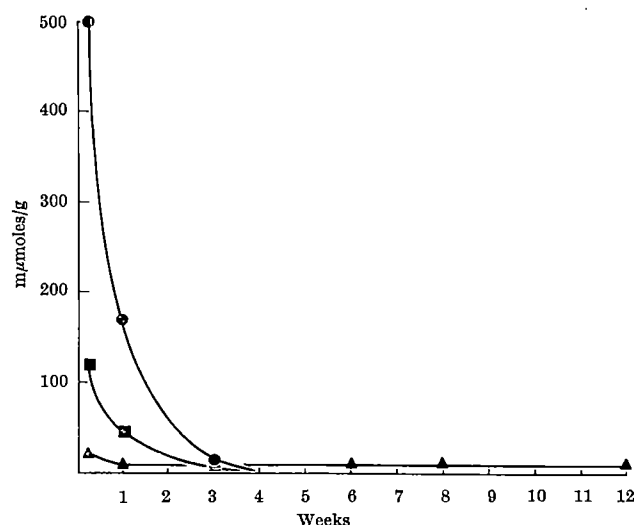


Fig. 1. Binding of radioactive metabolites from tritiated dimethylaminoazobenzene to hooded rat liver DNA, rRNA and cytoplasmic proteins. ○, Cytoplasmic proteins; ■, ribosomal RNA; ▲, DNA.

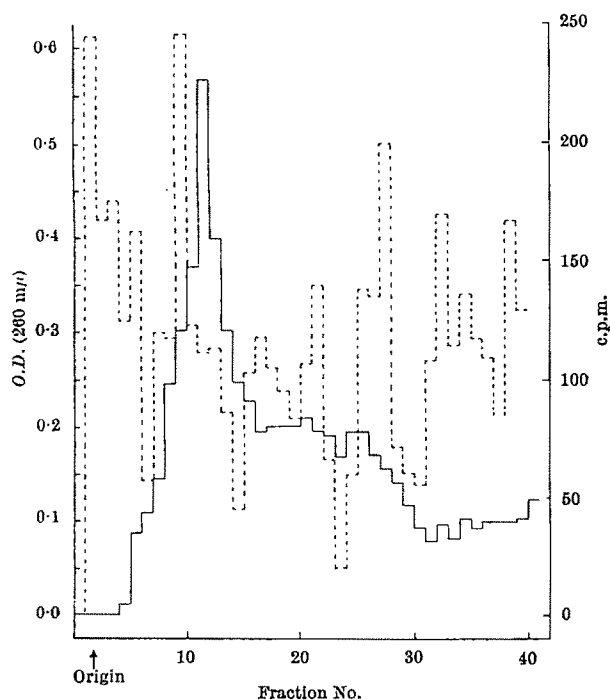


Fig. 2. Hydrolysis of radioactive rat liver DNA with DNase and *Crotalus adamanteus* venom. The solution of hydrolysed DNA (from a rat kept 2 months after injection of tritiated DAB) was submitted to paper chromatography in methanol-ethanol-concentrated hydrochloric acid-water (50:25:6:19) by volume. The chromatogram was cut into 1 cm strips, eluted with normal hydrochloric acid, and the optical density at 260 m $\mu$  (—) and the radioactivity (---) were measured.

those reported earlier for male hooded rats with transplanted hepatomata<sup>1</sup>.

For DNA, however (Fig. 1), the specific activity dropped initially by about half between day 2 and day 7, and the level of binding then remained constant for 3 months. This latter level was of the order of 9  $\mu$ moles/g DNA or 3  $\mu$ moles drug/mole phosphorus. At this time (and for weeks before this) no radioactivity could be detected on the rRNA or on the proteins. The counts associated with DNA were highly significant above background, being about 3,000 c.p.m./mg DNA against a background of about 40 c.p.m.

The radioactivity associated with DNA at all times after injection was due to covalently bound metabolites and not to adsorbed dye, to adsorbed metabolites, or to the spurious incorporation of small metabolites such as tritiated water into the natural bases during synthesis. These points were established by hydrolysing the DNA to nucleosides with DNase I followed by *Crotalus adamanteus* venom, when it was shown that radioactivity could still not be extracted with benzene or ether. Paper chromatography followed by assay of radioactivity and ultra-violet determination showed that radioactivity could not be superimposed over the natural bases (Fig. 2). Furthermore, when DNA was hydrolysed with DNase followed by normal hydrochloric acid at 100° C for 1 h, about 40 per cent of the radioactivity could be extracted from the hydrolysate with benzene. A further 30 per cent was extracted when the solution was made alkaline with solid potassium hydroxide, and the remainder when the pH was adjusted to 7. Similar findings were obtained whether DNA was used from animals 2 days or 3 months after dosing. These experiments establish that some reactive metabolite or metabolites of [<sup>3</sup>H] DAB were covalently bound to liver DNA *in vivo*, and that the binding was labilized by hot normal hydrochloric acid.

Because of the relatively low specific radioactivity of the DNA compared with the proteins at early times after injection of tritiated butter yellow, the possibility that the radioactivity apparently associated with DNA was due to

the presence of up to 2 per cent residual protein could not be completely precluded in earlier investigations<sup>1</sup>. Our investigations make this possibility far less likely unless one postulates the presence of a protein intimately associated with DNA which is reactive towards the drug metabolites and which has a half life far in excess of normal. No evidence is at present available to indicate the existence of such a protein, the rate of turnover of histones for example normally being as high as or higher than that of other proteins<sup>3</sup>.

The mitotic index for liver cells is about 0.005–0.01 per cent<sup>4</sup> and if animals are fed only a maintenance diet as in the present experiment this rate is cut down even further<sup>5</sup>. Hence the cell divisions occurring during the 3 month period would be minimal. The present results therefore support the concept that the DNA in differentiated liver cells not undergoing division is not repaired or is repaired only slowly after modification by covalent interaction with foreign molecules of this kind. In some respects, this is surprising in view of the data of Pelc<sup>6</sup>, who has postulated, on the basis of short-term experiments, a half-life for mouse liver DNA of 13–58 days. On the other hand, Fresco and Bendich<sup>7</sup> incorporated tritiated thymidine into rat liver following partial hepatectomy and found little reduction in the specific radioactivity of the DNA during 3 months, from which they concluded that the DNA of resting rat liver is not being continuously renewed. The results of certain biological experiments are best interpreted on the basis of the latter conclusion, in so far as latent lesions in the form of covalently bound metabolites, or bases altered by the action of radiation, can remain associated with liver DNA for considerable periods of time. Thus Maini and Stitch<sup>8</sup> observed chromosomal abnormalities in the dividing liver cells of partially hepatectomized rats fed six months earlier on a diet containing the hepatocarcinogenic 3'-methyl analogue of butter yellow. In a similar context, Curtis and Crowley<sup>9</sup> and Stevenson and Curtis<sup>10</sup> showed that in mice after a single dose of X-rays many more chromosomal aberrations were present in subsequently dividing cells than in controls and that there was little decrease in the frequency of aberration during several months, indicating the absence of efficient systems for repairing this particular type of lesion in the DNA of non-dividing liver cells.

The implication of the present results is that DNA in the liver of male hooded rats can exist in combination with covalently bound butter yellow metabolites for long periods of time. Only during subsequent cell division does the latent damage manifest itself as chromosome aberrations and hence possibly mutation. This is consistent with the concept of latent period in carcinogenesis, and also with the view that an essential step in chemical carcinogenesis involves a critical level of chemical combination with DNA followed by cell divisions, the latter allowing the necessary processes of selection to occur.

Experiments are in hand to examine in a similar manner other liver carcinogens, such as  $\beta$ -naphthylamine in the mouse, and other reactive non-hepatocarcinogens such as the bifunctional alkylating agents.

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# Structure and Activity in Molluscicides

by

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The search for molluscicides has revealed a variety of simple amides which, although not as toxic in small quantities as other molluscicides, are cheap and not poisonous to people.

THE importance of molluscicides as a means of controlling the part played by snails in the cycle of bilharzia is well established. Recent reviews<sup>1,2</sup> have dealt with various molluscicides and have also emphasized the importance of bilharzia as a parasite disease in man. The selection of compounds for testing as molluscicides has been virtually random, but recently attempts have been made to establish more rational procedures<sup>3,4</sup>.

In a previous study<sup>4</sup>, organic sulphydryl reagents were investigated and the theory used to predict lachrymatory activity was used to predict molluscicidal properties. This line of development was sufficiently promising to lead to a wider interest in compounds showing lachrymatory activity, so that, when Punte and co-workers<sup>5</sup> reported the intense lachrymatory activity of aerosol sprays of pelargonyl morpholide, compounds of this type were included in surveys of molluscicides.

Under the standard conditions described previously<sup>4</sup>, *Bulinus tropicus* was exposed to test solutions for 1,000 min and then allowed a recovery period of 24 h. It was found that pelargonyl morpholide (nonanoyl morpholide) was itself inactive as a molluscicide, but that the dodecanoyl and tetradecanoyl morpholides had considerable molluscicidal activity. Investigation of a variety of amides showed that the unsubstituted amides were inactive, as were those with only one substituent on the amide nitrogen. Amides with two substituents on the amide nitrogen such as dimethyl, diethyl, or the piperidides, morpholides or pyrrolidides have little or no molluscicidal activity when the acid moiety is decanoyl or smaller. The molluscicidal activity increases, however, to a maximum between dodecanoyl and tetradecanoyl and then rapidly disappears with a further increase in chain length. Fig. 1 shows the change in toxicity with increasing chain length for a number of disubstituted amides. Molluscicidal activity is thus a common property to a number of disubstituted amides, but is limited to only a few members of each homologous series.

Initially, only amides from the common even membered fatty acids were tested, but because maximum toxicity was found with either a twelve or fourteen carbon chain, a number of amides of tridecanoic acid was synthesized and found to be slightly more toxic than the amides of the two adjacent acids. The values for the tridecanoyl amides are included in Fig. 1 and it can be seen that they fall on the curves of toxicity for the even membered amide series. It is a common phenomenon for the biological activities of derivatives of homologous fatty acids to reach a maximum in the region of twelve carbon atoms. This has been commented on<sup>6</sup> and exploited commercially.

On investigating further structural variations, it was found that the molluscicidal activity of *N*-ethyl, *N*-dodecyl acetamide ( $LC_{50}$  2.5 p.p.m.) was similar to that of the isomeric *N*-diethyl dodecanoyl amide ( $LC_{50}$  3.0 p.p.m.). Also, the toxicity of the branched chain compound 2-*n*-butyldecanoyl morpholide ( $LC_{50}$  4.5 p.p.m.) was not very different from that of the isomeric straight-chain tetradecanoyl morpholide ( $LC_{50}$  3.0 p.p.m.).

Two series of esters, the methyl esters and the ethoxyethyl esters of the normal fatty acids, were investigated

for molluscicidal properties, but no member of either series had appreciable molluscicidal activity.

All the various results previously given can best be explained by postulating that these molluscicides are toxic because of a physical interference with the properties of membranes or interfaces within the snail and not because of any chemical reaction. For optimum effect, structure, molecular size and solubility relationships would thus be of great importance. The lack of activity of the unsubstituted or mono-substituted amides is probably a result of the influence of hydrogen bonding on the physical properties of the compounds and the lack of toxicity of the esters either to their rapid degradation or to different water solubilities.

The postulate of a physical rather than a chemical mechanism of toxicity for the amide molluscicides is further strengthened by their remarkably reversible action, as repeated washing of moribund snails with fresh water frequently leads to their recovery.

The active amides all have low water solubilities and the snails concentrate the molluscicide out of aqueous solution. Over considerable variations of time, 4–21 h, and of concentration, the exposure time multiplied by the

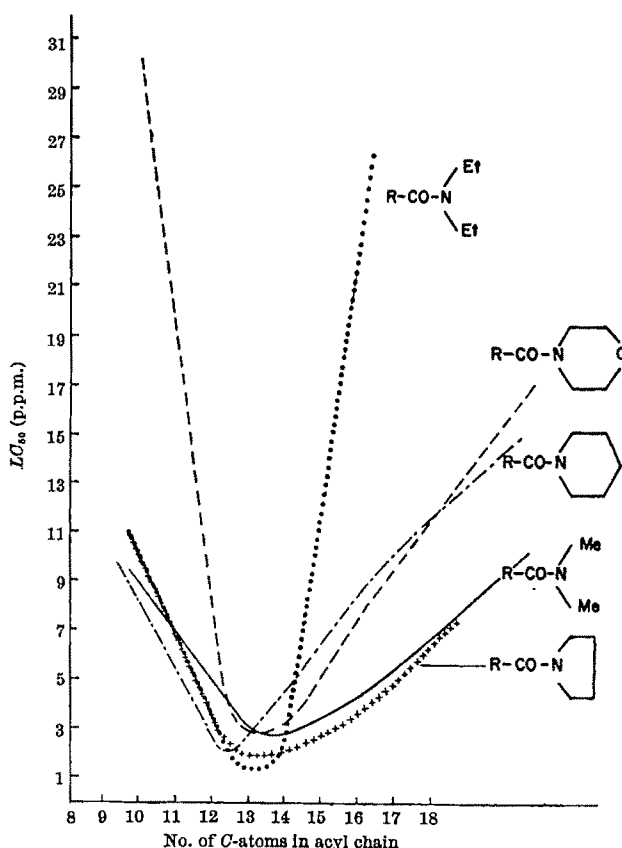


Fig. 1. Variation of toxicity with acyl chain length for a number of disubstituted amides.

$LC_{50}$  is a constant which implies that the uptake of molluscicide by the snail is directly proportional to the concentration in the test solution and that the snail possesses no rapid detoxifying mechanism.

These amide molluscicides can be considered as very water insoluble non-ionic surface active agents with a lyophilic chain of ten to sixteen carbon atoms and a somewhat hydrophilic grouping in the disubstituted amide grouping at the one end.

Berrie and Visser<sup>7</sup> claim to have isolated a substance excreted by a natural population of the snail *Biomphalaria sudanica*. This substance has an inhibiting effect on the growth of the snail and, if its concentration in the water is increased, it becomes lethal. The substance was identified as a mono-hydroxy tricarboxylic acid mono-isodecyl dimethyl ester. It could be expected to have the physical properties of a very water insoluble non-ionic surface active agent and could be very similar in physical properties to the amide molluscicides. Visser<sup>8</sup> recognized the surface active properties of the compound and proceeded to test a wide variety of detergents of all types.

From limited trials conducted with *Tilapia mossambica*, it would seem that the amide molluscicides are toxic to

fish. They have, however, extremely low oral toxicity to rats, as a dose of 2 g/kg body weight elicited no reaction. Pelargonyl morpholide is an extremely irritant oil, but the compounds with molluscicidal activity have no irritant properties at all.

The amide molluscicides are by no means the most effective molluscicides available when judged on a basis of their  $LC_{50}$  values. Because the effect of these molluscicides in a linear function of time and concentration, however, where prolonged dosing is possible very low concentrations can be used. The low cost and negligible mammalian toxicity of these molluscicides, coupled with their ease of manufacture and handling, undoubtedly make them most attractive for further investigation on a large scale.

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## Polarization of Cosmic X-Rays

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The extent to which cosmic X-rays are polarized may provide information about the mechanism producing them, and the region from which they come. In this article the polarized X-rays produced by the bremsstrahlung mechanism are considered.

CONTINUOUS cosmic X-rays may be produced by one or all of three mechanisms—bremsstrahlung (thermal and non-thermal), synchrotron radiation and the inverse Compton effect. The polarization of cosmic X-rays may help to identify which mechanism is responsible, and thus to obtain information about the region from which the radiation comes. Dolan<sup>1</sup> has investigated polarization for the three mechanisms, and concluded that the observation of high polarization of X-rays means that the radiation is either (1) synchrotron, or (2) bremsstrahlung radiation of electrons the velocity vectors of which are non-isotropic. In this article the second possibility is considered; it is probably realized in solar flares, and possibly in some other sources of X-rays.

For a parallel mono-energetic beam of electrons of low energy, the degree of linear polarization is given by<sup>2</sup>

$$\Pi = \frac{\sigma_1 - \sigma_2}{\sigma_1 + \sigma_2} = \frac{B \sin^2 \theta}{B \sin^2 \theta + C} \quad (1)$$

where  $\sigma_1$  and  $\sigma_2$  are the bremsstrahlung cross-sections for the photons polarized perpendicular and parallel to the  $(\vec{p} \vec{k})$  plane, where  $\vec{p}$  and  $\vec{k}$  are electron and photon momenta, with the angle between  $\vec{p}$  and  $\vec{k}$  given by  $\theta$  and  $B$  and  $C$  given by

$$B = (3x - 2) \ln \frac{1 + \sqrt{1-x}}{1 - \sqrt{1-x}} + 6 \sqrt{1-x}$$

$$C = 2(2-x) \ln \frac{1 + \sqrt{1-x}}{1 - \sqrt{1-x}} - 4\sqrt{1-x} > 0 \quad (2)$$

where  $x = E_\gamma/E_K$  and  $E_\gamma$  is the photon energy. Equation (1) is obtained from a Born approximation for an unpolarized beam of electrons, and neglects screening. From equations (1) and (2) it follows that in the region of soft photons ( $x \ll 1$ ),  $B < 0$ , and  $|B| < |C|$ , so that  $\Pi > 0$ , that is, photons are polarized mainly in a direction

perpendicular to the plane  $(\vec{p} \vec{k})$ . In the region of hard photons ( $x \lesssim 1$ ),  $B > 0$  and  $\Pi < 0$ , that is, photons are polarized preferentially in the  $(\vec{p} \vec{k})$  plane. With the increase of photon energy, polarization changes from +1 to -1, passing through zero with  $x \approx 0.12$  regardless of the angle  $\theta$ .

Polarization is zero if the direction of movement of the electron coincides with the line of sight, and it reaches a maximum for electron beams perpendicular to the line of sight.

Increase in electron energy displaces the zero point to the region of harder photons and, at the limit when  $E_K \gg mc^2$ , negative polarization disappears<sup>3-5</sup>. The transition through zero values is a typical peculiarity of bremsstrahlung, which allows this radiation to be distinguished from synchrotron radiation. It is known that polarization of synchrotron radiation depends much less on frequency, and the direction of the predominant electric vector oscillation is always perpendicular to the projection of the magnetic field on the pictorial plane<sup>6</sup>. Under real astrophysical conditions, however, accelerated electrons may possess some energetic and angular distribution. The averaging of these distributions can change both the properties and the value of the polarization.

The simplest way of investigating the influence of the energy distribution on the polarization value may be most simply defined by the power energy spectrum  $E_K^{-\alpha}$ . If the spectrum of electrons extends in the non-relativistic region up to some energy  $E_{K0}$ , for a parallel beam of electrons  $\Pi$  will be defined by equation (1) as before.  $B$  and  $C$ , however, are now the functions of  $x$  and  $x_{K0} = E_\gamma/E_{K0}$  and are expressed by a hyper-geometrical function. With a steep spectrum of electrons ( $\alpha \gg 1$ ),  $\Pi$  is again equal to zero when  $x_{K0} = 0.12$ , and for the limiting values of very soft and very hard photons it reaches 100 per cent for large values of the angles  $\theta$ . As  $x$  decreases, the zero point is displaced towards the region of harder photons, and disappears

when  $\kappa \leq 1$ . This peculiarity of polarization remains in energetic distribution, and is different from the power law one shown, for example, in an exponential distribution (see, for example, ref. 1).

The angular distribution affects the value of the polarization much more than does an energetic one. For a sinusoidal distribution according to  $\sin^n \alpha$ , where  $n$  is a constant and  $\alpha$  the angle between the direction of motion of the electron and the axis of symmetry of the angular distribution (for example,  $H$ ), the degree of linear polarization of non-relativistic electrons is

$$\Pi_{n=2} = \frac{B \sin^2 \theta_0}{4B + 5C - B \sin^2 \theta_0}$$

$$\Pi_{n=4} = \frac{B \sin^2 \theta_0}{3B + 3.5C - B \sin^2 \theta_0} \quad (3)$$

where  $B$  and  $C$  are defined by equation (2) as before and  $\theta_0$  is the angle between the axis of symmetry of the angular distribution and the line of sight. It follows from equation (3) that with the sinusoidal angular distribution, the degree of polarization is much smaller than for the parallel beam of electrons (see equation 1), but increases with the growth of  $n$  (degree of anisotropy), reaching 50 per cent with  $n = 4$ , in the region of hard photons. Soft photons are polarized in the plane, passing through the axis of the angle distribution symmetry and the line of sight, and  $\Pi$ , as before, passes through the zero with  $E_\gamma \approx 0.12 E_K$  ( $B = 0$ ) regardless of the angle  $\theta_0$ . The simultaneous averaging by the angular and energetical distribution makes the maximum polarization even smaller. It is not difficult to investigate such a distribution for separate cases, making use of the assumption that the angular and energy distributions are independent. There is appreciable polarization (with  $\kappa = 6$  and  $n = 4$   $\Pi = 10 - 30$  per cent) only when the energy spectrum is rather steep and reaches the region where  $E_K \ll mc^2$ . The maximum polarization of bremsstrahlung might therefore be expected for parallel beams of accelerated electrons, for which the energy spectrum extends to the region of small energies of up to  $E_{K0} \approx mc^2$  (minimum ionization losses) or even up to the non-relativistic region. These two conditions can be realized primarily in solar flares. Another different case, which is also of interest, occurs when accelerated electrons are caught by a strong regular field in a trap and an anisotropic angular distribution is established in it as, for example, the belts of radiation around the Earth and Jupiter show.

Relativistic electrons accelerated in a flare will simultaneously produce bremsstrahlung, synchrotron and Compton radiation. It is not difficult to see, however, that in the X-ray region of the spectrum there is always a range of photon energies in which bremsstrahlung radiation will be predominant. This region is illustrated in Fig. 1, which shows the power spectrum of  $P/N_e$  radiation, where  $N_e$  represents a whole number of electrons  $\varphi$ . The solid lines are constructed for a spectrum of electrons with a single energy  $E_{K0} = 5 \times 10^6$  eV, and the dotted ones for the power spectrum with  $\kappa = 3$  in the region  $E_K \geq E_{K0}$ , and

$$N_e \propto \frac{1}{(\kappa - 1) E_{K0}^{\kappa-1}}$$

The curves are calculated from the formulae given in ref. 6 for the following values of the parameters in the region of radiation: mean concentration of gas  $n_i = 10^{12}$  cm $^{-3}$ , mean concentration of thermal photons  $n_{ph} = 10^{13}$  cm $^{-3}$  (radiation density  $W_{ph} = 4$  erg/cm $^3$ ,  $T = 6 \times 10^3$  °K) and the magnetic field  $H_1 \approx H = 10^2$  oersted. The maximum of synchrotron radiation occurs in the radiowave region of the spectrum at a frequency  $\nu_s \approx 4 \times 10^{10}$  c/s, the maximum of Compton radiation  $\nu_K$  occurs in the X-ray region at a frequency of about  $4.8 \times 10^{16}$  c/s ( $\lambda \approx 62$  Å), and the spectrum of bremsstrahlung increases logarithmically with frequency up to the energy of photons

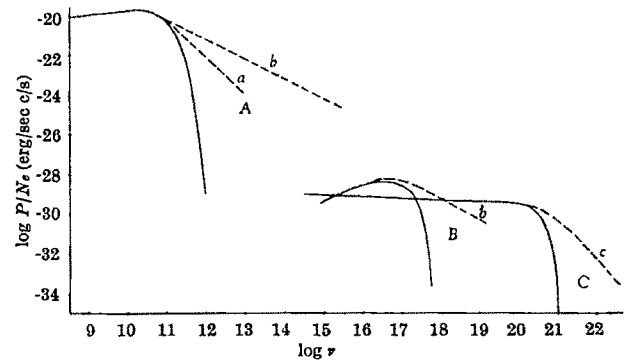


Fig. 1. Power spectrum of  $P/N_e$  radiation, where  $N_e$  represents a whole number of electrons. A, Synchrotron; B, Compton; C, bremsstrahlung; a,  $\nu^{-1}$  ( $\kappa = 3$ ); b,  $\nu^{-2}$  ( $\kappa = 3$ ); c,  $\nu^{-1}$  ( $\kappa = 3$ ).

$E_\gamma = E_{K0} = 5 \times 10^6$  eV, and then falls steeply according to the law  $\nu^{-2}$ .

Fig. 1 shows that synchrotron radiation will predominate in the X-ray region of the spectrum  $E_\gamma \geq 1$  keV only if  $\kappa < 4$ , and the energy spectrum of electrons continues with a constant slope up to very high energies (for  $H = 10^2$  oersted, up to  $5 \times 10^6$  eV). This is not impossible, but it is scarcely probable, because of the rapid increase of energy losses with the increase of energy. Therefore for a steep energy spectrum in the region of large energies, the two other mechanisms will prevail in the X-ray region: for soft photons, the inverse Compton effect; and for hard photons, bremsstrahlung. Using values of the parameters  $n_i n_{ph} \approx 10^{12}$  cm $^{-3}$  and  $\kappa = 3$  the maximum Compton radiation is five times greater than the maximum bremsstrahlung radiation in the range of photon energies 0.01–4 keV.

For  $n_i = n_{ph} = 10^{12}$  cm $^{-3}$  and  $E_{K0} = 5 \times 10^6$  eV the integral total bremsstrahlung radiation is one thousand times greater than the Compton radiation. Because of a sharp maximum in the spectrum, however, there is an excess region of Compton radiation. The objections to the Compton mechanism in ref. 7 are therefore unconvincing.

The location of the three curves in Fig. 1 relative to each other is defined by the physical conditions in the radiating region,  $n_i$ ,  $n_{ph}$  and  $H$ , as well as by the properties of the accelerating mechanism,  $\kappa$  and  $E_{K0}$ .

It is not difficult to imagine the curve distribution using values of the parameters different from those in Fig. 1. The position of the maximum of synchrotron radiation is proportional to  $H_1 E_0^2$ , and its value is fixed by the magnetic field which is proportional to  $H_1$ . The location of the maximum of Compton radiation is proportional to  $T E_0^2$ , and its value is fixed by the mean concentration of thermal photons. If  $E_{K0}$  decreases, the power of the Compton and synchrotron radiation in the X-ray region  $E_\gamma \geq 1$  keV does not increase significantly, while bremsstrahlung radiation increases rapidly in proportion to  $E_{K0}^{(1-\kappa)}$  and becomes predominant throughout the X-ray region of the spectrum.

While the observations are being made, it is desirable to measure the degree of polarization in at least two regions of photon energy, for example with  $E_\gamma = 4$  keV and 20 keV. If the energy spectrum of accelerated electrons falls sharply or shows a break in the region of  $E_{K0} \approx 10^6$  eV, then the direction of polarization should ideally be at the angle  $\pi/2$ . The observation of this peculiarity of X-ray photon polarization will undoubtedly indicate bremsstrahlung radiation, and will enable subcosmic electrons in the atmosphere of the Sun and in other sources of X-rays to be investigated.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Linear Polarization of Radio Sources at a Wavelength of 21.3 cm

THE Cambridge One-Mile radio telescope<sup>1</sup> has recently been used to make high resolution observations of a number of radio sources (refs. 2 and 3, and Macdonald, G. H., in preparation). Observations of the Crab Nebula, Cygnus A and the sources 3C 47, 103, 332, 338 and 4C 29.41 have been repeated with the receiving horns of the telescope set at 90° to their original direction, thus providing maps of these sources at two perpendicular polarizations; although these maps do not allow the unique distribution of polarization across the sources to be obtained, the results are nevertheless of some interest.

For each source observations were made for 12 h periods at either four or eight positions of the moving aerial according to the angular extent of the source<sup>4</sup>; the instrument had a pencil-beam response in the neighbourhood of the source with half power beam-widths of 23" arc in right ascension and 23" cosec  $\delta$  in declination. The receiving horns were set at position angles (measured from north positive through east) of 0° and 90°, respectively; maps of the sources at the two polarizations were then compared enabling information about the distribution of polarization across the sources to be obtained.

The main source of error in these observations is likely to be uncertainty in the calibration of the contour levels in the two maps which may be as great as 5 per cent in some cases; uniform polarization across the whole source can then not be recognized except in those cases where earlier observations have provided values for the total polarization of the source.

(a) *The Crab Nebula.* The overall polarization of the Crab Nebula at  $\lambda = 21$  cm has been found<sup>5</sup> to be 1.6 per cent  $\pm 0.1$  per cent with a position angle of  $86^\circ \pm 2^\circ$ . Because this position angle is very close to one at which measurements were made in the present observations it has been possible to eliminate some of the uncertainty in the calibration of the contour levels by scaling the map of the Crab Nebula at one polarization so that the overall polarization becomes 1.6 per cent when the two maps are compared. The map of the Crab Nebula for position angle 0°, which has already been published<sup>6</sup>, is shown in Fig. 1; the map for position angle 90° is remarkably similar, and in particular the features of emission marked A, B and C in Fig. 1 and which correspond well to similar features in the optical continuum emission from the nebula remain virtually unchanged at both radio polarizations.

By comparing the intensities in the maps at the two polarizations the following deductions can be made: (1) There are no strongly polarized regions of small angular size within the nebula because any region  $\sim 20''$  arc in extent and with a degree of polarization greater than 3 per cent would have been detected; the small mean polarization observed for the nebula is therefore not, as in the optical case, made up of the sum of a number of strongly polarized components. (2) The polarized radiation is confined to an area some 2' arc in diameter near the centre of the nebula over which the polarization is fairly uniform; this conclusion agrees with that previously reached by Davies *et al.*<sup>7</sup>. Over most of the central region

of the nebula the degree of polarization is between 2 per cent and 3 per cent, but the polarization reaches 5 per cent  $\pm 2$  per cent near point A (Fig. 1). (3) There is little correspondence between radio and optical polarization features; in particular there are no anomalous features in the radio polarization in regions where locally there are large changes in the magnitude and direction of the optical polarization. (4) In the outer regions of the nebula no significant polarization is detectable.

It should be noted that Kronberg<sup>8</sup> has observed the Crab Nebula at  $\lambda = 21$  cm with two aerials at a fixed spacing of  $\sim 2,000\lambda$  and concluded that there is a point source with a flux-density of  $(7.5 \pm 1.6) \times 10^{-26}$  m.k.s.  $\sim 1.5'$  arc north-west of the centre of the nebula; this flux-density is about 0.9 per cent of the total from the nebula. This conclusion is clearly incompatible with the present observations which use a range of aerial spacings up to  $7,000\lambda$  and show no evidence in this area for any source having an angular size  $< 15''$  arc and with a flux-density greater than 0.2 per cent of the total from the nebula. Structure  $\sim 30''$  arc is, however, clearly present at several places within the nebula but none contributes more than 0.2 per cent of the total flux-density; furthermore, an upper limit of 0.1 per cent can be put on the flux-density at  $\lambda = 21$  cm of any point source at the centre of the nebula where the low-frequency point source appears to lie<sup>9</sup>. This result is discussed further in the accompanying communication by Bell and Hewish<sup>10</sup>.

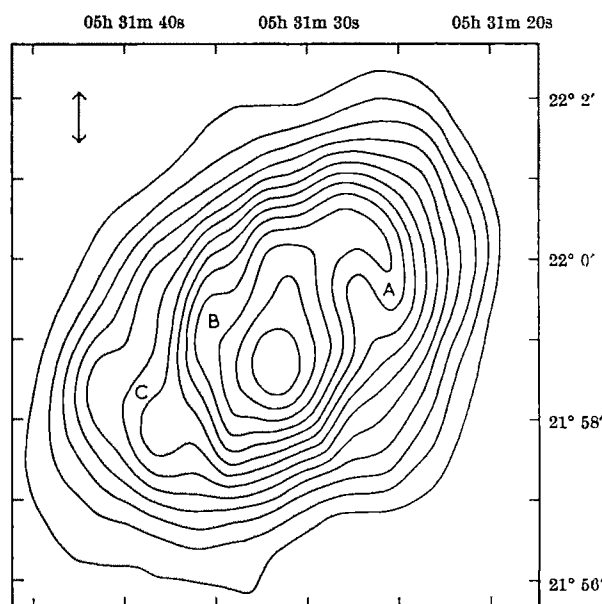


Fig. 1. Map of the Crab Nebula with electric vector in position angle 0°; co-ordinates are 1950.0 and the contour interval is 1,300° K. Regions A, B and C correspond to similar features in the optical continuum radiation from the nebula.

(b) *Cygnus A.* Maps of the source obtained in the present observations are shown in Fig. 2, and cuts along the major axis are shown in Fig. 3. It can be seen that there is virtually no integrated polarization in the source as has previously been shown by Morris and Radhakrishnan<sup>11</sup>; there does, however, appear to be significant polarization in the "bridge" between the two components, the degree of polarization apparently reaching  $\sim 20$  per cent in this region.

It has been suggested<sup>12,13</sup> that for Cygnus A the observed dependence of overall polarization on wavelength results from the effects of differential polarization between the two main components of the source. The present results show that these two components are effectively unpolar-

ized at  $\lambda = 21$  cm. A possible explanation is that Cygnus A has a very large rotation measure and depolarization occurs as a result of differential Faraday rotation over a scale of  $\sim 20''$  arc; such depolarization can presumably only occur in the vicinity of the source, because if it were galactic in origin implausibly large changes in the total rotation would have to occur over very short distances within the Galaxy<sup>12</sup>. An alternative possibility is that most of the polarization previously measured at wavelengths less than 6 cm also comes from the "bridge" region; the depolarization of the source between  $\lambda = 3$  cm and  $\lambda = 21$  cm may then be explained if the polarized "bridge" region had a somewhat flatter spectrum than the source as a whole, although this simple mechanism does not readily account for the pronounced maximum which is believed to occur in the degree of polarization around  $\lambda = 3$  cm (ref. 13).

(c) 3C 47. This source consists of two components separated by  $62''$  arc and the line joining them has a position angle of  $30^\circ$ ; the south-west component is just resolved<sup>3</sup>. Measurements by Bologna *et al.*<sup>14</sup> have shown

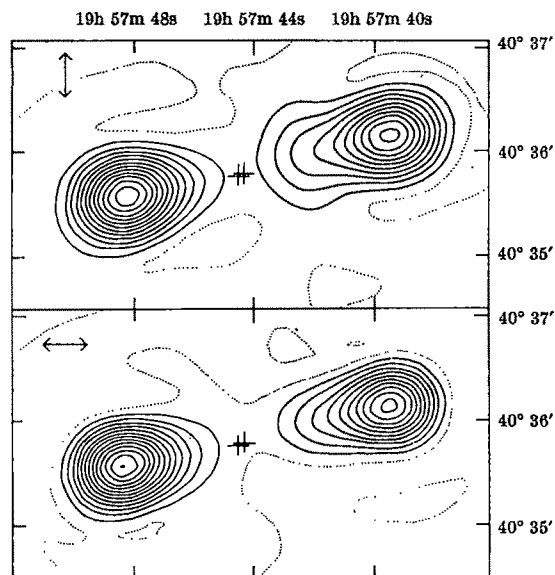


Fig. 2. Maps of Cygnus A at the two polarizations, the position angle of the electric vector being shown in the top left-hand corner of each map; co-ordinates are 1950.0 and for each map the contour interval is 30,000 K. The positions of optical galaxies associated with the source are shown by two crosses.

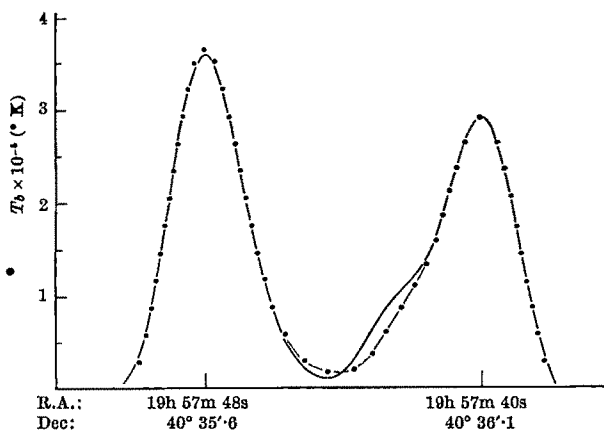


Fig. 3. Sections along the major axis of Cygnus A at the two polarizations; the co-ordinates (1950.0) of the peaks of maximum brightness are indicated. The standard error in the calibration of the brightness scale for each polarization is approximately 4 per cent. Position angle: —●—,  $90^\circ$ ; —,  $0^\circ$ .

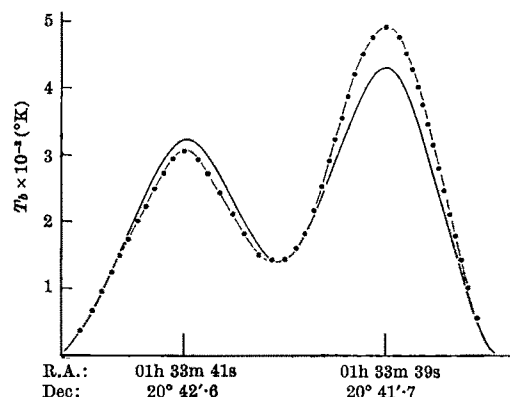


Fig. 4. Sections along the major axis of 3C 47 at the two polarizations; the co-ordinates (1950.0) of the peaks of maximum brightness are indicated. The standard error in the calibration of the brightness scale for each polarization is approximately 4 per cent. Position angle: —●—,  $0^\circ$ ; —,  $90^\circ$ .

that the polarization at  $\lambda = 21.2$  cm is  $2.4$  per cent  $\pm 1.6$  per cent with a position angle of  $4^\circ \pm 21^\circ$ ; the present observations are consistent with these results, and profiles of the source along the major axis at the two polarizations are shown in Fig. 4. The south-west component shows about 7 per cent polarization, and it also appears that the north-east component is polarized but with a significantly different position angle from that of the south-west component.

Source	Structure <sup>3,4</sup>	Components	Polarization of component (per cent)	Position angle of feed for which component has greatest amplitude
3C 47	Two components, the S.W. component being just resolved; separation of the components $62''$ arc.	N.E.	$3 \pm 3$	$90^\circ$
		S.W.	$7 \pm 3$	$0^\circ$
3C 103	Two unresolved components with a separation of $88''$ arc.	N. leading	$1 \pm 3$	$0^\circ$
		S. following	$2 \pm 3$	$90^\circ$
3C 332	Two components each $< 15''$ arc in diameter; separation of the components $54''$ arc.	N. following	$0 \pm 4$	—
		S. leading	$1 \pm 4$	$90^\circ$
3C 338	Two components approximately E.-W. each with complex structure; separation of the components $41''$ arc.	W.	$0 \pm 3$	—
		E.	$0 \pm 3$	—
4C 29.41	Two components, the E. component unresolved and the W. component extended. Separation of the components $50''$ arc.	W.	$5 \pm 4$	$90^\circ$
		E.	$0 \pm 4$	—

(d) 3C 103, 332, 338 and 4C 29.41. Each of these sources consists of two components<sup>3,4</sup>, although in some cases the individual components have resolved features. In no case has significant polarization been detected in the present observations in any part of these sources; details of the measurements made including the results for 3C 47 are given in Table 1. It should be noted that the source 3C 338 has been observed by Bologna *et al.*<sup>14</sup> at  $\lambda = 21.2$  cm to have a polarization of  $3.5$  per cent  $\pm 0.7$  per cent with a position angle of  $57^\circ \pm 6^\circ$ ; this measurement is consistent with the present observations because any polarization with position angle between  $35^\circ$  and  $55^\circ$  is virtually undetectable in measurements at the two position angles used in the present observations.



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### Position of the Low Frequency Radio Source in the Crab Nebula

RECENT radio observations of the Crab Nebula<sup>1-3</sup> have demonstrated the existence of a component of small angular diameter which contributes a significant fraction of the total flux density at frequencies of less than about 100 Mc/s. The high brightness temperature deduced from the interplanetary scintillation of this source at 38 Mc/s (ref. 3) makes it difficult to attribute this radiation to the synchrotron process, and coherent radio emission from a dense plasma associated with the star has been suggested as a possible mechanism. The previously reported position for the small diameter source based on a lunar occultation observed at 26.5 Mc/s (ref. 2) lies about 1.2' arc from the centre of the nebula, however, and is well removed from the centre of the observed expansion. The present observations show that the position of this source in the nebula must be much nearer the centre than the earlier measurement suggests.

The method used here exploits the fact that only the small component of the nebula exhibits interplanetary scintillation. By observing the magnitude of (a) the total signal and (b) the scintillations on the record obtained with a phase switching interferometer, it is possible to determine the difference in phase of the two interference patterns (Fig. 1) and thus the difference in position of the small source and the centroid of the brightness distribution of the nebula. The method avoids the need for correcting for ionospheric refraction and does not involve any other calibration of the instrument.

The measurements were carried out at a frequency of 81.5 Mc/s using the Cambridge interferometer<sup>4</sup>, which has recently been converted to this operating frequency. This aerial system, which could previously observe a source only near its meridian transit, has also been modified to allow its response to track a source over a limited angle. For the present observations this extended the time for which radiation from the Crab Nebula could be recorded from about 5 min to about 20 min per day.

For measurement of position in right ascension the west half of the long cylindrical paraboloid (725 ft. × 65 ft.) was used in conjunction with the shorter movable aerial (190 ft. × 65 ft.) as an interferometer with an east-west baseline of 244λ at 81.5 Mc/s. Two pairs of phase-switching receivers were used in order to record "cos" and "sin" components corresponding to interference patterns in quadrature<sup>5</sup>.

In order to observe the small scintillating component of the signal alone, the receiver output was passed through a filter which removed from the records components with frequencies less than about 0.16 c/s. The rapidly varying scintillations which remained were amplified and recorded separately. The scintillation amplitudes are modulated by the "sin" and "cos" responses to the small diameter source in the usual way and a comparison of the relative sizes of individual maxima on the two records can be used to derive the phase of the envelope (Fig. 1c and d). The direct record (Fig. 1a and b) is little affected by the presence of the small diameter source, which contributes 5-10 per cent of the total signal. Because the source is not appreciably resolved at the baseline used, the relative phases of the direct record and the envelope of the filtered record make it possible to find the position of the compact component relative to the whole source.

From observations made on July 16, 17 and 18, 1966, the right ascension of the small component was found to coincide with the centroid of the total emission to an

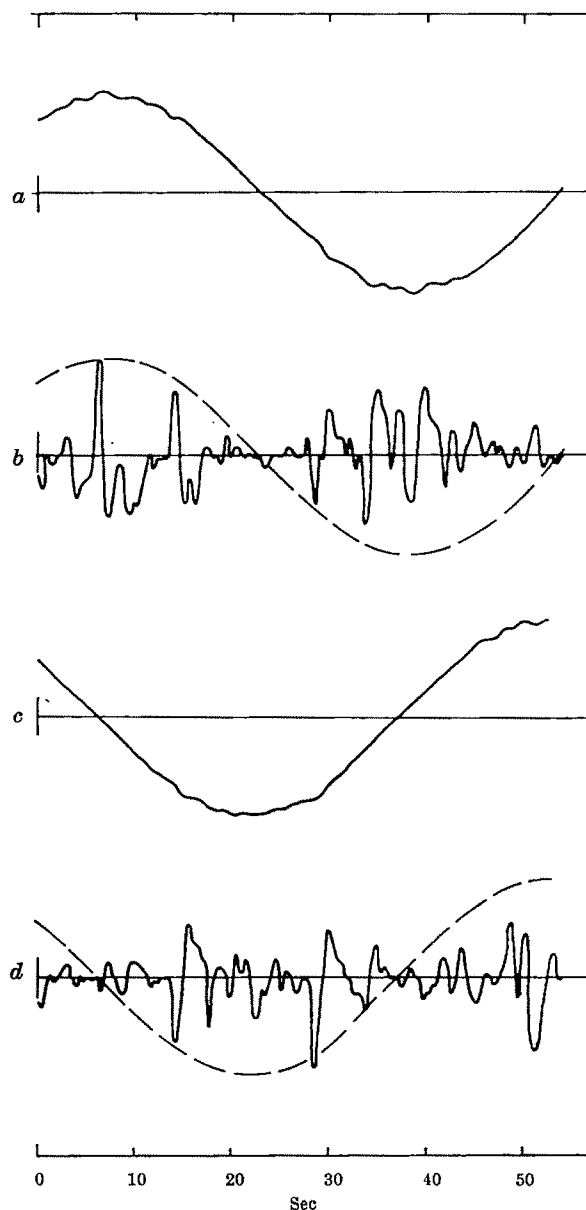


Fig. 1. Receiver output obtained with the 244λ east-west interferometer. Records a and b show the "cos" and "sin" components of the output. The high frequencies present on these records are shown amplified further as c and d.

accuracy of  $\pm 10''$  arc, the average position of the small source being  $1.0'' \pm 10''$  arc earlier.

For position measurements in declination, the west half of the long aerial was again used, this time in conjunction with a small aerial measuring 40 ft.  $\times$  40 ft., which could be moved to give a maximum separation of 840 ft. In this position the centres of the interferometer elements were separated by  $42\lambda$  in an east-west direction and by  $60\lambda$  in a north-south direction when observing at the declination of the Crab Nebula. The same receiving system and measuring technique were used as before. The final position derived from measurements made on July 23 and 24, 1966, was  $0.3' \pm 1.0'$  south of the centroid.

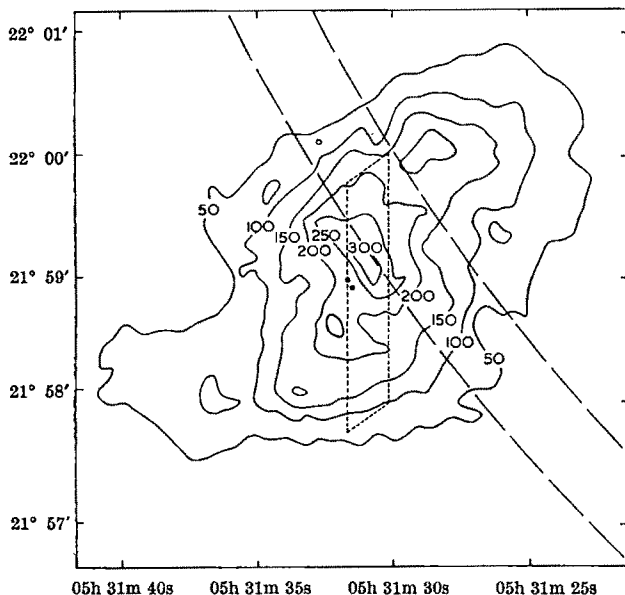


Fig. 2. The measured position of the X-ray source<sup>12</sup> (dotted area) and the low frequency radio source (parallelogram), superimposed on optical isophotes due to Woltjer<sup>13</sup>.

The accuracy of the relative position measurements in the two co-ordinates is limited mainly by the signal to noise ratio on the filtered scintillation records and is correspondingly worse for the declination measurements, which used a smaller effective collecting area as well as a shorter baseline. Position errors as a result of confusion might affect the apparent position of the centroid but not that of the small source. It is estimated, however, that confusion errors should be less than  $2''$  arc.

In order to establish the absolute position of the small component, it is necessary to find the co-ordinates of the centroid of the nebula at 81.5 Mc/s when observed with an interferometer of the resolution used. Recent observations<sup>6,7</sup> have not revealed any variation greater than  $10''$  arc in the position of the centroid for observing frequencies between 60 Mc/s and 456 Mc/s and give a position in good agreement with that derived from observations at 408 Mc/s with the Cambridge One-Mile telescope:

$$05h\ 31m\ 31.0s \pm 0.5s \quad 21^\circ\ 59.1' \pm 0.3' \quad (1950.0)$$

These observations also show that for an interferometer of up to  $500\lambda$  east-west baseline the centroid will be within  $0.2s$  of this position.

Using this position for the centroid, the derived final position for the small component is

$$05h\ 31m\ 30.9s \pm 0.8s \quad 21^\circ\ 58.8' \pm 1.0' \quad (1950.0)$$

This position agrees with that of the pair of  $15m$  stars near the centre of the Crab Nebula. Baade<sup>8</sup> found that the south preceding component of this pair has a proper motion agreeing well with that of the nebula, and a connexion appears likely, although Minkowski<sup>9</sup> found no

definite evidence that this star had passed through a supernoval phase. The position of this star is

$$05h\ 31m\ 31.46s \quad 21^\circ\ 58'\ 54.8'' \quad (1950.0)$$

Shklovsky<sup>10</sup> has already suggested that the low frequency radio source may be associated with the X-ray source which has recently been discovered in the Crab Nebula<sup>11</sup>. The only accurate measurement of the position of the X-ray source<sup>12</sup>, based on observation of a lunar occultation, shows that the source has a diameter of about  $0.5'$  arc to half power and is centred at least  $0.5'$  arc from the star. Measurements were only made at immersion and therefore only one position co-ordinate was determined. The position of the limb of the Moon when the half powers points of the X-ray source were being occulted is shown in Fig. 2 superimposed on the optical isophotes given by Woltjer<sup>13</sup>. The errors for the present measurement of the position of the low frequency source and the position of the pair of stars are also shown.

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### Angular Size and Flux Density of the Small Source in the Crab Nebula at 81.5 Mc/s

THE existence of a source in the Crab Nebula with an angular diameter of less than  $1$  sec of arc has been revealed by observations of interplanetary scintillation at a frequency of 38 Mc/s (ref. 1). The brightness temperature derived from these observations is so great that the synchrotron mechanism may not provide an adequate explanation for the emission. The determination of the spectrum is therefore of great interest.

It has proved difficult in observations at other frequencies, based on lunar occultations and interferometers of high resolving power, to recognize the contribution from this very compact component in the presence of other fine structure within the nebula. Further observations of interplanetary scintillation at 81.5 Mc/s have therefore been made, first to enable the flux density to be determined at this frequency and second to derive a more accurate value for the angular diameter of the source.

The observations used in this investigation were made during about 60 days in the period March–July 1966 by means of one aerial and receiver of the solar wind experiment which has already been described<sup>2</sup>. A pen recorder with a time constant of  $0.3$  sec was used to record the scintillation which amounted to only a few per cent of the total deflexion.

The records were analysed to obtain daily values of the scintillation index  $I'$  (root mean square deviation expressed as a fraction of the mean) and in Fig. 1 the results are plotted as a function of the perpendicular distance ( $p$ )

of the line of sight to the source from the Sun. Each point is an average of observations made during 3 or 4 days. It can be seen that  $F$  reaches a maximum of 0.035 for  $p \sim 0.5-0.6$  A.U. and then decreases as the line of sight moves closer to the Sun. This systematic change of  $F$  with  $p$  is similar to that observed for 3C 138 at 178 Mc/s and the angular diameter can be estimated from the scintillation visibility ( $V$ ) as described by Little and Hewish<sup>3</sup>. In Fig. 2 the observed values of  $V$  (defined as  $F$  (observed)/ $F$  (ideal point source)) are compared with theoretical curves for a symmetrical gaussian source the intensity of which is assumed to be 10 per cent of that of the non-scintillating component. The angular diameter thus derived is found to be  $0.2'' \pm 0.1''$ . This value is somewhat greater than that derived from the earlier observations at 38 Mc/s; however, we believe it to be more accurate because it is based on a direct measurement of the scale of the diffraction pattern at the Earth. Previously, the scale was estimated from the angular spectrum of the scattered radiation assuming multiple scattering, but it is now known that scintillation corresponds to weak scattering beyond 0.5 A.U. at 81.5 Mc/s (ref. 4) and the same is probably also true at 38 Mc/s. A revised estimate of the angular diameter at 38 Mc/s, assuming the pattern to have the same scale at both frequencies, gives a value comparable with our present result. Until more is known about the diffraction pattern at 38 Mc/s there is no evidence that the angular diameter depends on frequency.

The estimated relative intensity of the small source is 10 per cent  $\pm$  1.5 per cent, the greatest uncertainty being caused by day to day changes in the irregularities of the interplanetary medium. The spectrum of the small source derived from the present estimate at 81.5 Mc/s and previous scintillation data at 38 Mc/s is shown in Fig. 3. The uncertainties are large, but at these frequencies the result is consistent with that suggested by Andrew *et al.*<sup>5</sup>. Because the position of the small source is now known to be near the centre of the nebula<sup>6</sup> the 26.5 Mc/s flux derived by Andrew *et al.* cannot be used to derive the spectrum below 38 Mc/s. At frequencies greater than 81.5 Mc/s sensitive measurements are required to detect scintillation and an earlier attempt at 178 Mc/s (ref. 7) was limited by inadequate sensitivity. In the accompanying note Branson<sup>8</sup> has presented his results of high resolution observations at 1,407 Mc/s which show that, in the position determined by Gower, there is no

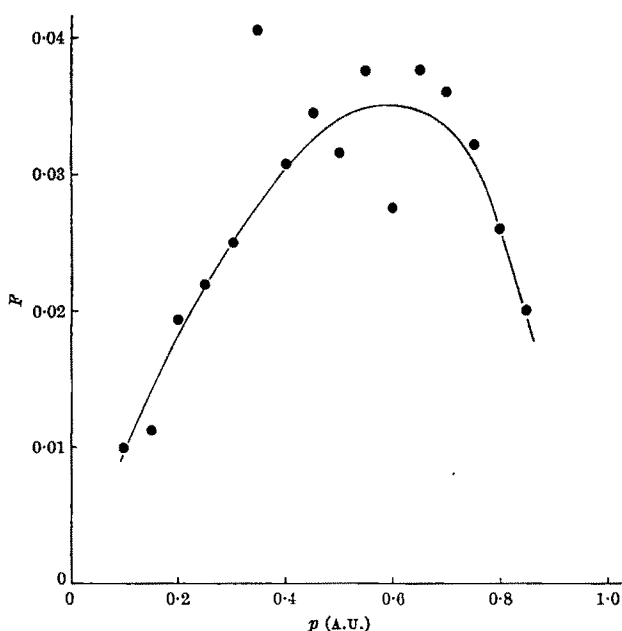


Fig. 1. The scintillation index  $F$  of the Crab Nebula at 81.5 Mc/s as a function of distance from the Sun.

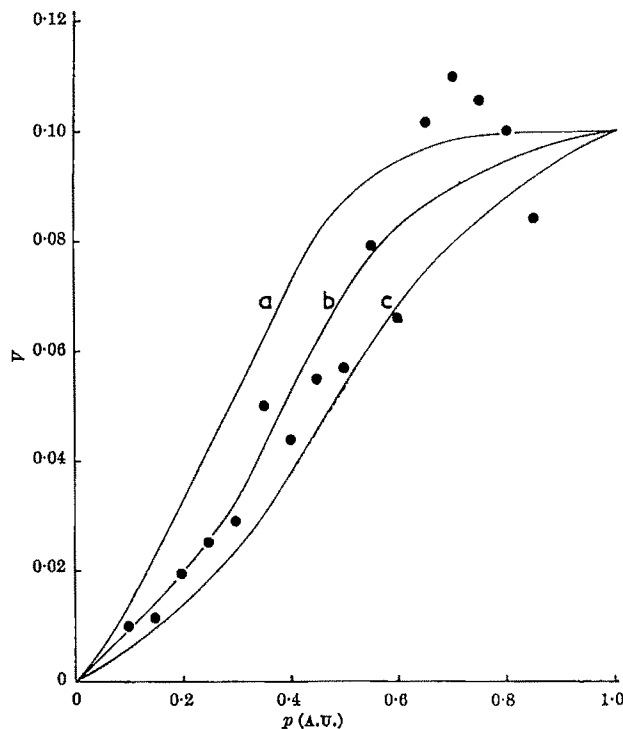


Fig. 2. The observed scintillation visibility  $V$  compared with theoretical curves for a symmetrical gaussian source of diameter: a,  $0.1''$ ; b,  $0.2''$ ; c,  $0.3''$ .

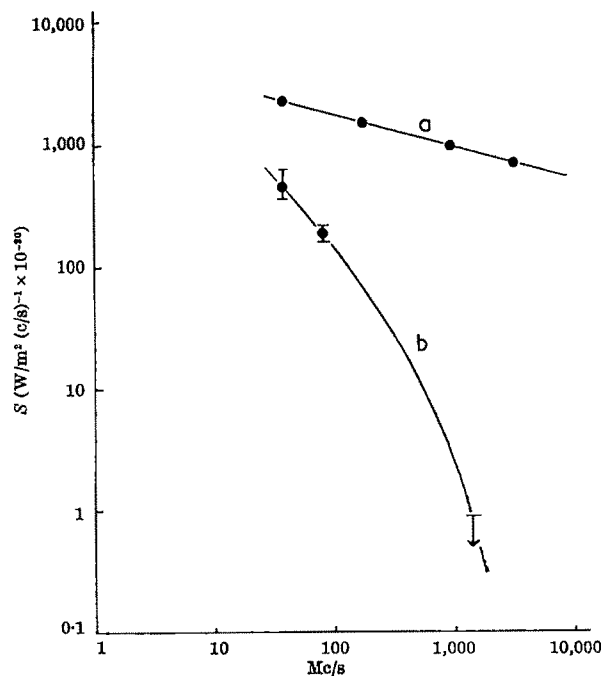


Fig. 3. The spectrum of a, the Crab Nebula; b, the compact source.

source with an angular size  $< 15''$  which contributes more than 0.1 per cent of the total flux from the nebula. This result, plotted in Fig. 3, shows that the spectrum must have a steep slope between 81.5 Mc/s and 1,407 Mc/s and differs from the spectrum derived by Kronberg<sup>9</sup>, who associated the emission from a component of the nebula with that from the compact low frequency source.

Calculations, following the method of Hornby and Williams<sup>10</sup> and using the revised angular diameter, lead to upper limits of  $3 \times 10^{-5}$  G and  $2 \times 10^{-7}$  G at 81.5 Mc/s and 38 Mc/s, respectively, for the field strength in the source if radiation by the synchrotron mechanism is

assumed. The corresponding minimum energies for relativistic electrons alone are then  $\sim 10^{47}$  ergs and  $\sim 10^{50}$  ergs. It is therefore difficult to account for the compact source by synchrotron radiation because this requires both an unreasonably small magnetic field and a minimum particle energy of the same order as that contained in the complete extended source.

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### Changes in Polarization, Light and Colour during the Outburst Stage of the Recurrent Nova *T* Pyxidis

We were notified on December 8, 1966, by Mr. Frank Bateson, of the discovery in New Zealand by Mr. Albert Jones that the recurrent Nova *T* Pyx was at visual magnitude 12.9 on December 7. Previous estimates had placed the nova near magnitude 14.5 for some years. Photometric and polarimetric observations, with the 40 in. and 24 in. reflectors at Siding Spring Mountain, began on the night of December 8-9. The photometric observations during the pre-maximum and maximum phases of the outburst are shown in Table 1 and in Fig. 1. The trends of the variation in visual magnitude, during the gaps in the observations caused by clouds at Siding Spring Mountain, are shown in Fig. 1 by broken curves and are based on visual observations by V. L. Matchett and M. Jones of the Moreton Bay Astronomical Club. A slight adjustment was necessary to bring the visual observations into agreement with the photoelectric results. Previous

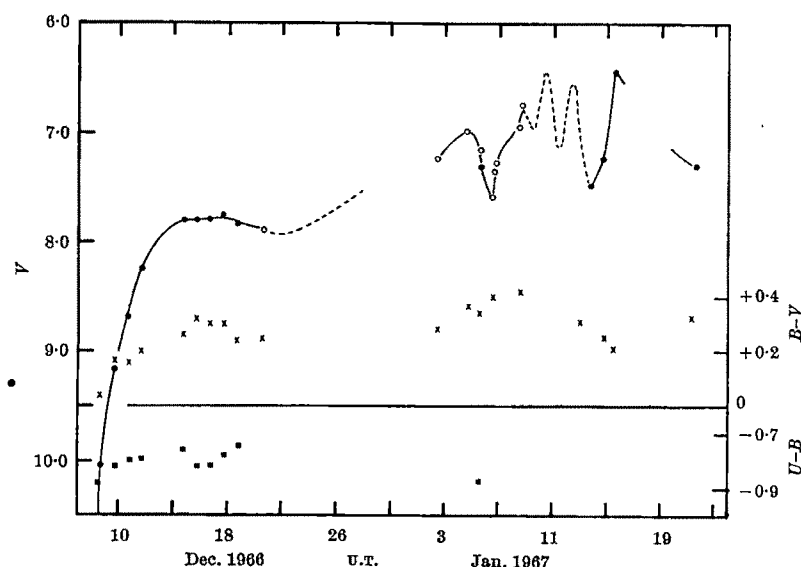


Fig. 1. The light curve for *T* Pyx is drawn using visual magnitudes measured with the 40 in. (●) and 24 in. (○) telescopes at Siding Spring Observatory. The broken sections of the curve were obtained from measurements by V. L. Matchett and M. Jones of the Moreton Bay Astronomical Club.  $B-V$  (x) and  $U-B$  (•) values are plotted at the bottom of the diagram.

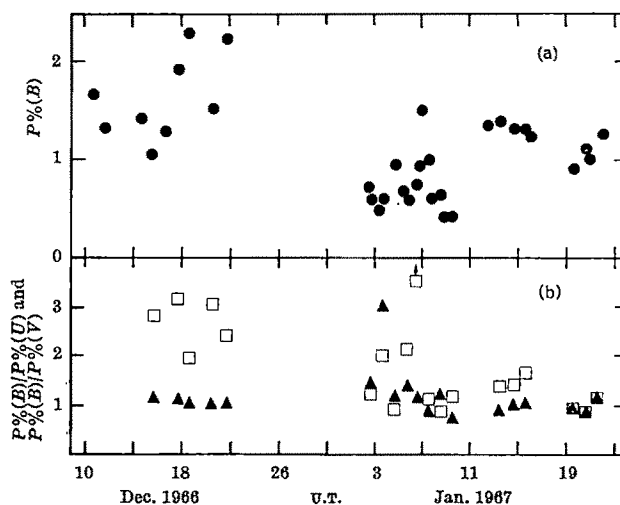


Fig. 2. *a*, The time variation of the percentage polarization,  $P(\%)$ , in the blue is shown for the pre-maximum and maximum phases of the outburst of *T* Pyx. *b*, The ratios of the amounts of polarization in the blue and ultra-violet (□) and in the blue and visual (▲) are plotted.

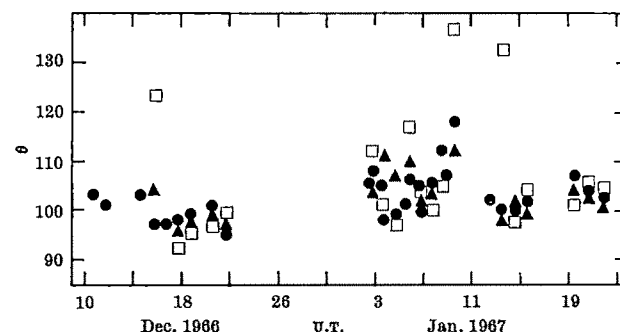


Fig. 3. The time dependence of  $\theta$ , the position angle of the electric vector (measured from north towards east) for the  $U$  (□),  $B$  (●) and  $V$  (▲) system of filters is shown.

outbursts of the nova were in 1890, 1902, 1920 and 1944 and the light curve in Fig. 1 is strikingly similar to those obtained in 1902, 1920 and 1944<sup>1</sup>.

The polarization observations were made with the new rotatable 24 in. telescope which had been put into service only a month before. It is very similar to that described by Hiltner and Schild<sup>2</sup>. A two-channel polarimeter was used with EMI 9524S photomultipliers and  $UBV$  filters.

These observations, which were the first to be made of any active nova, revealed that the light from *T* Pyx was linearly polarized and showed short period fluctuations in the degree, angle, and wavelength dependence of the polarization. The results are presented in Table 2 and in Figs. 2 and 3. A few polarimetric observations made with  $H\gamma$  interference filters show that the degree of polarization is about the same as with the  $B$  filters of the ( $UBV$ ) system and therefore the polarization of emission lines does not seem to be smaller than that of the continuum. This fact suggests that the polarization is produced in the outer shell of the nova, perhaps by scattering of light in the expanding gaseous envelope.

Observations of *T* Pyx are still in progress with the various types of equipment available at Mount Stromlo and Siding Spring Mountain, and we hope to continue them for several months when the nova

Table 1. PHOTOMETRIC DATA FOR  $T$  PYXIDIS

Date	U.T.	V	B-V	U-B	Telescope
December 8	1700	10.03	+0.04	-0.88	40"
9	1700	9.17	+0.17	-0.82	40"
10	1730	8.68	+0.16	-0.80	40"
11	1600	8.25	+0.20	-0.79	40"
14	1630	7.80	+0.26	-0.76	40"
16	1600	7.82	+0.32	-0.82	40"
17	1600	7.81	+0.30	-0.82	40"
17	1600	7.77	+0.30	-0.78	40"
18	1700	7.69	+0.27		24"
18	1400	7.73	+0.24		24"
20	1600	7.84	+0.24	-0.74	40"
22	1400	7.89	+0.25		24"
		7.90			50"†
January 2	1300	7.23	+0.28		24"
	1700	7.30	+0.29		24"
4	1630	6.98	+0.36		24"
5	1200	7.17	+0.34		24"
	1230	7.31	+0.35	-0.87	40"
	1600	7.12	+0.25		40"
6	1730	7.17	+0.32		24"
	1200	7.60	+0.40		24"
	1530	7.35	+0.48		24"
	1750	7.27	+0.32		24"
8	1230	6.94	+0.43		24"
	1750	6.74	+0.40		24"
13	1700	7.49	+0.31		40**
14	1730	7.25	+0.25		40**
15	1430	6.44	+0.21		40**
21	1300	7.32	+0.33		40*†

\* Made by S. C. B. Gascoigne.

† Made by A. W. Rodgers.

‡ Made by J. V. Hindman.

Table 2. POLARIZATION DATA FOR  $T$  PYXIDIS

Date	U.T.	U	B	V
		P% m.e. $\theta$	P% m.e. $\theta$	P% m.e. $\theta$
1966				
December 10	1600		1.68 $\pm$ 0.14 103	
11	1530		1.33 $\pm$ 0.07 101	
14	1630		1.43 $\pm$ 0.21 108	
15	1430	0.37 $\pm$ 0.04 123	1.06 $\pm$ 0.06 97	0.94 $\pm$ 0.07 104
16	1600		1.30 $\pm$ 0.04 97	
17	1700	0.61 $\pm$ 0.13 92	1.93 $\pm$ 0.16 98	1.77 $\pm$ 0.05 96
18	1400	1.18 $\pm$ 0.13 96	2.31 $\pm$ 0.24 99	2.32 $\pm$ 0.11 98
20	1400	0.49 $\pm$ 0.09 98	1.51 $\pm$ 0.04 101	1.44 $\pm$ 0.14 98
21	1630	0.93 $\pm$ 0.17 99	2.23 $\pm$ 0.26 95	2.22 $\pm$ 0.11 97
1967				
January 2	1300		0.76 $\pm$ 0.04 105	
	1530		0.89 $\pm$ 0.06 106	
	1700	0.46 $\pm$ 0.15 112	0.59 $\pm$ 0.03 108	0.44 $\pm$ 0.10 104
3	1500		0.47 $\pm$ 0.14 105	
	1730	0.31 $\pm$ 0.22 101	0.60 $\pm$ 0.14 98	0.20 $\pm$ 0.05 111
4	1630	1.05 $\pm$ 0.19 98	0.94 $\pm$ 0.04 99	0.84 $\pm$ 0.04 107
5	1200		0.68 $\pm$ 0.09 101	
	1530	0.28 $\pm$ 0.09 117	0.59 $\pm$ 0.09 107	
	1730		0.60 $\pm$ 0.06 105	0.44 $\pm$ 0.03 110
6	1200		0.75 $\pm$ 0.03 105	
	1530	0.12 $\pm$ 0.10 104	0.94 $\pm$ 0.07 99	
	1730		1.51 $\pm$ 0.16 100	1.32 $\pm$ 0.11 100
7	1230	0.88 $\pm$ 0.13 100	0.99 $\pm$ 0.03 106	1.14 $\pm$ 0.09 103
	1700		0.60 $\pm$ 0.03 105	
8	1230	0.71 $\pm$ 0.13 107	0.62 $\pm$ 0.11 112	0.51 $\pm$ 0.06 108
	1730		0.40 $\pm$ 0.06 107	
9	1300	0.36 $\pm$ 0.13 137	0.41 $\pm$ 0.11 118	0.56 $\pm$ 0.08 112
12	1300		1.34 $\pm$ 0.13 102	
13	1330	1.03 $\pm$ 0.07 132	1.41 $\pm$ 0.06 100	1.64 $\pm$ 0.03 99
14	1600	0.92 $\pm$ 0.06 100	1.30 $\pm$ 0.06 100	1.31 $\pm$ 0.07 101
15	1500	0.80 $\pm$ 0.07 102	1.31 $\pm$ 0.07 102	1.31 $\pm$ 0.05 99
	1630		1.24 $\pm$ 0.06 102	
19	1230	0.97 $\pm$ 0.03 101	0.90 $\pm$ 0.04 107	1.00 $\pm$ 0.04 104
20	1100		1.11 $\pm$ 0.06 101	
	1200	1.16 $\pm$ 0.08 103	1.02 $\pm$ 0.03 104	1.25 $\pm$ 0.05 102
21	1500	1.09 $\pm$ 0.05 103	1.28 $\pm$ 0.06 103	1.11 $\pm$ 0.06 103
	1730		1.24 $\pm$ 0.06 102	

should have reached its final phase of decline. We then hope to discuss the results in more detail.

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### A Search for the $\Lambda$ -Doublet Transition in the $^2\Pi_{1/2}$ , $J=1/2$ State of OH

RECENT radio astronomical investigations of the 18-cm ground-state  $\Lambda$ -doublet transition of the OH radical have suggested that maser amplification of the radiation is taking place in the interstellar medium. So far, no entirely

satisfactory theoretical explanation of the observations has been published. A number of workers (for example, Cook<sup>1</sup> and Litvak *et al.*<sup>2</sup>) have suggested that optical pumping of the OH molecule is responsible for the apparent population inversion. Another possibility is that the OH molecule is formed in an excited state in the vicinity of HII regions. In either case, an appreciable number of OH molecules may be found in excited levels. If the populations of the excited rotational levels of the ground electronic and vibrational state were sufficiently large, it would be expected that it would be possible to observe  $\Lambda$ -doublet transitions originating in these levels.

According to Dousmanis, Sanders and Townes<sup>3</sup>, the state  $^2\Pi_{1/2}$ ,  $J=1/2$ , which is a pure  $^2\Pi_{1/2}$  state, is weakly coupled to the lowest lying state of the molecule ( $^2\Pi_{3/2}$ ,  $J=3/2$ ), which is composed of a mixture of  $^2\Pi_{1/2}$  and  $^2\Pi_{3/2}$  wave functions. It is possible to estimate the transition time between these two states to be of order 10 sec. This time should be compared with the  $\Lambda$ -doublet transition time in the  $^2\Pi_{1/2}$ ,  $J=1/2$  state. The latter time is determined by the rate of stimulated emission and can be estimated with the aid of the Einstein  $A$  formulae for OH given by Turner<sup>4</sup>. It is found that the  $\Lambda$ -doublet transition time ( $t$ ) due to stimulated emission in the  $^2\Pi_{1/2}$ ,  $J=1/2$  state is  $t \approx 10^8/T_b$ , where  $T_b$  is equal to the brightness temperature at the  $\Lambda$ -doublet line frequency ( $\sim 4,717$  Mc/s) due to both continuum and line radiation. 18-cm OH emission has been observed in the vicinity of HII regions where  $T_b$  is typically  $\sim 100^\circ$  K. The  $\Lambda$ -doublet transition time is therefore a few orders of magnitude smaller than the transition time to the  $^2\Pi_{3/2}$ ,  $J=3/2$  state. Thus it would be expected that the  $^2\Pi_{1/2}$  state would have a considerably lower population than the ground state. Because of the unknown nature of the pumping mechanism in the ground state, however, it appeared worth while to look for the line at 4,717 Mc/s.

We used the 140 ft. telescope of the National Radio Astronomy Observatory (Green Bank, West Virginia) equipped with a parametric amplifier and with the Harvard University 21-channel spectral line radiometer. The total system temperature was approximately 600° K and all measurements were made with a frequency resolution of 30 kc/s. The  $^2\Pi_{1/2}$ ,  $J=1/2$   $\Lambda$ -doublet lines have never been observed in the laboratory. Their frequencies may be calculated by means of the theory of Dousmanis, Sanders and Townes<sup>3</sup>, combined with the values for the hyperfine coupling constants obtained by Radford<sup>5</sup>. The estimated maximum error in the calculated frequencies is  $\pm 6$  Mc/s.

Table 1. SUMMARY OF OBSERVATIONAL RESULTS

Source	Transition	Line frequency (Mc/s)	Frequency range (Mc/s) covered	$\Delta T/T_{1665}$
W3	$F=1 \rightarrow 1$	4,717.2	+6.1 $\rightarrow$ -7.1	0.013
W3	$F=1 \rightarrow 0$	4,702.3*	+4.5 $\rightarrow$ -8.1	0.020
W3	$F=0 \rightarrow 1$	4,807.5*	+9.7 $\rightarrow$ -8.9	0.020
W49	$F=1 \rightarrow 1$	4,717.2	+6.6 $\rightarrow$ -6.6	0.010
W49	$F=1 \rightarrow 0$	4,702.3*	+4.7 $\rightarrow$ -6.1	0.016
W49	$F=0 \rightarrow 1$	4,807.5*	+3.3 $\rightarrow$ -9.9	0.016

\* These frequencies differ slightly from those published by Barrett<sup>6</sup>.

Our observational results are summarized in Table 1. The  $^2\Pi_{1/2}$  lines were searched for, but not detected, in W3 and W49—two radio sources of known 18-cm OH emission. Our measured upper limit to the  $^2\Pi_{1/2}$  line strengths ( $\Delta T$ ) has been compared with the most intense<sup>6</sup> observed 18-cm radiation ( $T_{1665}$ ) from W49 and W3. The quantity of  $T_{1665}$  was obtained by integrating over 10 kc/s the 1,665 Mc/s profiles of Palmer and Zuckerman<sup>6</sup> (W49) and Barrett and Rogers<sup>7</sup> (W3), which were all obtained with the 140 ft. telescope. At 1,600 Mc/s, 10 kc/s in velocity space is equal to 30 kc/s at 4,800 Mc/s. The ratio  $\Delta T/T_{1665}$  is given in the last column of Table 1. In the fourth column in Table 1 we give the frequency range covered for each line relative to  $V = -45.5$  km/s in W3 and  $V = +16.2$  km/s in W49, where  $V$  is radial velocity with respect to the local standard of rest.



The present experiment has placed upper limits on the intensities of the  $^2\Pi_{1/2}$ ,  $J = 1/2$   $\Lambda$ -doublet lines; the upper limits are 1–2 per cent of that of the observed 18-cm  $\Lambda$ -doublet emission lines. If a better understanding of the 18-cm emission should make it possible to predict theoretically that the strength of the  $^2\Pi_{1/2}$  lines is of order of between ten and a hundred times weaker than the upper limits found in our observations, such an intensity should be observable once the  $^2\Pi_{1/2}$  line frequencies are measured in the laboratory.

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## PLANETARY SCIENCE

### Rates of Accumulation of Ferro-manganese Nodules

ALTHOUGH various models have been proposed to explain the origin of manganese nodules (see Goldberg and Arrhenius<sup>1</sup>), two major hypotheses have received extensive attention. One concept suggests that manganese nodules form as the result of interaction between submarine volcanic products and sea water<sup>2,3</sup>. The common association of manganese nodules with volcanic materials constitutes the main evidence for this theory. The second theory involves a direct inorganic precipitation of manganese from sea water. Goldberg and Arrhenius<sup>1</sup> view this process as the oxidation of divalent manganese to tetravalent manganese by oxygen under the catalytic action of particulate iron hydroxides. Manganese accumulation by the Goldberg and Arrhenius theory would be a relatively slow and comparatively steady process, whereas Bonatti and Nayudu<sup>3</sup> believe manganese nodule formation takes place subsequent to the eruption of submarine volcanoes by the acidic leaching of lava.

The measured rates of manganese nodule accumulation seem to support gradual accumulation. The first direct determinations were based on the decay of radium with depth in the nodule and gave values ranging from 0.7 to 65 mm/10<sup>3</sup> yr<sup>4-6</sup>. These measurements have been criticized<sup>1</sup> because much of the radium is probably ionium supported. The rates would therefore have to be lowered to 0.01 to 1.3 mm/10<sup>3</sup> yr<sup>6</sup>. The lower value agrees with the  $^{230}\text{Th}/^{232}\text{Th}$  determination of Goldberg<sup>7</sup> on the "Horizon"

nodule of 0.01 mm/10<sup>3</sup> yr. More recently, Bender *et al.*<sup>8</sup>, using the decay of ionium, determined the rate of accumulation in a north-western Pacific nodule to be approximately 0.003 mm/10<sup>3</sup> yr. Bonatti and Nayudu point out that the difficulty of such slow rates is that the nodules, in their opinion, would become buried because pelagic sedimentation rates are at least 100 times faster.

If the volcanic nuclei commonly found in manganese nodules are dated by the potassium argon method, a lower limit to the rate of manganese nodule growth can be determined. Assuming both a uniform and continuous growth of ferromanganese oxide, this rate is a measure of the growth of the ferromanganese oxide over the entire history of the nodule. We have determined rates by this method (Table 1) and compared them with the differential rates by the  $^{230}\text{Th}/^{232}\text{Th}$  method (Table 2) and the  $^{234}\text{U}/^{238}\text{U}$  disequilibrium method (Table 2). It should be noted that the potassium argon rates are calculated over the entire thickness of the nodules, while the rates calculated from the other two methods cover only the outermost few millimetres of the nodules.

Potassium and argon analyses were made on pure mineral separates from the volcanic cores of manganese nodules. Potassium was analysed by atomic absorption with a sodium buffer, and an isotope dilution analysis of the argon was made with an omegatron mass spectrometer. Thin layers of nodule were sampled with a dental drill for thorium and uranium analyses. Nodule material was dissolved with acidified hydrogen peroxide, and the experimental procedure of Goldberg and Koide<sup>9</sup> was followed for the determination of the  $^{230}\text{Th}/^{232}\text{Th}$  ratio.  $^{234}\text{U}/^{238}\text{U}$  ratios were determined by first coprecipitating the uranium with iron hydroxide. The ion exchange procedure of Korkish and Hazan<sup>10</sup> was then used for isolation of uranium which was measured by alpha-spectroscopy.

The  $^{230}\text{Th}/^{232}\text{Th}$  rates were corrected for uranium support by assuming equilibrium between ionium and uranium at depth in the nodule and a constant uranium concentration in a given nodule. Under these assumptions all of the  $^{230}\text{Th}$  found at depth in the nodule will be from uranium. The method of successive approximations is then used to determine the percentage of equilibrium attained by each sample from that nodule. The correction is then the total amount of  $^{230}\text{Th}$  from uranium multiplied by the fraction of equilibrium attained. The correction when applied to the data presented here reduces the rates of accumulation by a maximum of 25 per cent. The uranium concentration in a nodule may vary by a factor of two<sup>11</sup>. If the uranium concentration is doubled, the correction from uranium can reduce the rate by as much as 50 per cent of the uncorrected rate of the nodules analysed.

The  $^{234}\text{U}/^{238}\text{U}$  data also indicate a slow rate of deposition for ferromanganese nodules. The resulting rates are roughly the same as those determined by the  $^{230}\text{Th}/^{232}\text{Th}$  on the same nodule. It should be noted that the surface uranium ratios are the same within the limits of precision to previously determined ratios in sea water of 1.14 (refs. 12, 13). This, when coupled with normal decay toward equilibrium as one moves toward the centre of the nodule, is evidence for current growth of the nodules.

Table 1. POTASSIUM ARGON DATA FOR MANGANESE NODULES

Sample and location	Material dated	Potassium per cent	$^{40}\text{Ar}$ radiogenic (c.c./g)	Per cent $^{40}\text{Ar}$ radiogenic	Age ( $\times 10^3$ yr)	Rate of accumulation (mm/10 <sup>3</sup> yr)
DWHD 47 41° 51' S. 102° 01' W. 4,240 m	anor	4.70	$1.85 \times 10^{-6}$	70	$9.8 \pm 0.2$	0.5–1
DWHD 47 41° 51' S. 102° 01' W. 4,240 m	amph	0.730	$2.72 \times 10^{-7}$	37	$9.3 \pm 0.3$	0.5–1
"Horizon" 40° 14' N. 155° 05' W. 5,500 m	plag	1.12	$1.80 \times 10^{-6}$	23	$28.9 \pm 1.4$	2–3
Fan BD 20 40° 15' N. 128° 27' W. 4,500 m	glass	2.86	$2.31 \times 10^{-7}$	6	$2.0 \pm 0.3$	2.6–3.5

anor, anorthoclase; amph, amphibole; plag, plagioclase.

Table 2

Lusiad AD4 1020 M Lat. = 6° 03' N. Long. = 32° 22' W.				
Depth in nodule	<sup>230</sup> Th/ <sup>232</sup> Th (uncorr.)	<sup>230</sup> Th/ <sup>232</sup> Th (corr.)	<sup>232</sup> Th p.p.m.	<sup>234</sup> U/ <sup>238</sup> U
0-1	6.05	6.05	66	1.16 ± 0.02
2-3	0.92	0.52	68	
5-6	0.86	0.38	62	
7-8	0.59	0.18	63	
11-13				1.05 ± 0.01
12-13	0.66	0	51	
16-17	0.53	0	63	
18-21				1.06 ± 0.01
20-21	0.84	0	42	
25-27				1.01 ± 0.02
26-27	0.80	0	43	
33-36				1.04 ± 0.02

Rate from <sup>230</sup>Th/<sup>232</sup>Th = 8 mm/10<sup>6</sup> yr.Rate from <sup>234</sup>U/<sup>238</sup>U ≈ 40 mm/10<sup>6</sup> yr.

Carr. 5 3700 M Lat. = 9° 28.5' N. Long. = 113° 16.5' W.				
Depth in nodule	<sup>230</sup> Th/ <sup>232</sup> Th (uncorr.)	<sup>230</sup> Th/ <sup>232</sup> Th (corr.)	<sup>232</sup> Th p.p.m.	<sup>234</sup> U/ <sup>238</sup> U
0-1	88.3	88.3	4.8	1.12 ± 0.01
4-7	8.2	0.0	7.1	
7-9	8.0	5.7	6.4	10.5 ± 0.02
10-13				1.02 ± 0.01
12-14	3.51	0	4.68	
17-19				1.00 ± 0.02
21-23	4.21	0	3.53	
24-26				1.01 ± 0.02

Rate from <sup>230</sup>Th/<sup>232</sup>Th = 17 mm/10<sup>6</sup> yr.Rate from <sup>234</sup>U/<sup>238</sup>U = 24 mm/10<sup>6</sup> yr.

DW 72 920 M Lat. = 21° 31' S. Long. = 85° 14' W.				
Depth in nodule	<sup>230</sup> Th/ <sup>232</sup> Th (uncorr.)	<sup>230</sup> Th/ <sup>232</sup> Th (corr.)	<sup>232</sup> Th p.p.m.	
0-1	39.5	39.5	17.7	
3-4	9.62	8.68	3.11	
5-6	7.58	5.27	1.4	
9-10	9.2	0	0.48	
13-14	8.65	0	0.37	
18-19	14.3	0	1.4	

Rate from <sup>230</sup>Th/<sup>232</sup>Th = 18 mm/10<sup>6</sup> yr.

DWHD 47 4000 M Lat. = 41° 59' S. Long. = 102° 01' W.				
Depth in nodule	<sup>230</sup> Th/ <sup>232</sup> Th (uncorr.)	<sup>230</sup> Th/ <sup>232</sup> Th (corr.)	<sup>232</sup> Th p.p.m.	
0-1	28.0	28.0	11.5	
2-3	2.69	1.59	16.8	
4-5	2.0	0	9.2	
6-7	3.0	0	6.4	
10-11	2.9	0	7.2	

Rate from <sup>230</sup>Th/<sup>232</sup>Th = 6 mm/10<sup>6</sup> yr.MP 26  
1464 M Lat. = 19° N. Long. = 171° W.

Depth in nodule	<sup>230</sup> Th/ <sup>232</sup> Th (uncorr.)	<sup>230</sup> Th/ <sup>232</sup> Th (corr.)	<sup>232</sup> Th p.p.m.
0-1	15.3	15.3	19.0
1-2	7.2	6.0	19.6
3-4	5.4	1.56	8.9
5-6	4.3	1.09	10.8
7-8	4.6	0	8.5
9-10	5.9	0	6.4

Rate from <sup>230</sup>Th/<sup>232</sup>Th = 10 mm/10<sup>6</sup> yr.

Three nodules, each having a volcanic nucleus which appeared to result from a single volcanic event, were selected for potassium argon dating. One of these nodules, the "Horizon" nodule, is among the largest in the Scripps collection. It consists of a 60-100 mm thick layer of ferromanganese oxide covering a predominantly phillipsite nucleus. Sufficient unaltered plagioclase for dating was easily separated from this latter material. The second nodule dated, DWHD 47, consists of a 5-10 mm ferromanganese oxide layer covering a light coloured nucleus. The nucleus has 200-300 micron size anorthoclase and quartz<sup>16</sup>, as well as finer amphibole embedded in phillipsite and smaller amounts of clear glass shards. The anorthoclase, previously dated by Peterson *et al.*<sup>14</sup>, gave an age of  $9.8 \times 10^6$  yr. The third nodule, Fan BD 20, is a slightly altered pumice fragment with about 6 mm of ferromanganese oxide coating. The pumice was crushed and the freshest fragments dated.

If the slow growth model of Goldberg and Arrhenius is assumed, average accumulations can be calculated from the potassium argon ages (Table 1), which are in good agreement with the slow rates of Goldberg<sup>7</sup>, Bender *et al.*<sup>8</sup>, and the <sup>230</sup>Th/<sup>232</sup>Th and the uranium disequilibrium methods presented here. The rates obtained by potassium argon are lower than those obtained by either the uranium

disequilibrium method or the <sup>230</sup>Th/<sup>232</sup>Th method. In only two cases can a direct comparison of methods be made. The 10 mm/10<sup>6</sup> yr rate determined by Goldberg using <sup>230</sup>Th/<sup>232</sup>Th on the "Horizon" nodule compares with the 2-3 mm/10<sup>6</sup> yr potassium argon rate. In DWHD 47 a rate of 0.5-1 mm/10<sup>6</sup> yr by potassium argon compares with the rate of 6 mm/10<sup>6</sup> yr determined by <sup>230</sup>Th/<sup>232</sup>Th on another nodule from the same dredge haul. The rates of accumulation range from 0.5 to 3.5 mm/10<sup>6</sup> yr by the potassium argon method, 6 to 18 mm/10<sup>6</sup> yr by the <sup>230</sup>Th/<sup>232</sup>Th method, and 24 to ~40 mm/10<sup>6</sup> yr by the <sup>234</sup>U/<sup>238</sup>U method. The agreement of the methods is good, especially considering the fact that the methods were applied to different nodules.

The potassium argon ages of the centres of the nodules dated indicate a very old age, and yet these nodules were recovered by surface dredging. These two facts invalidate the argument that very slow rates are impossible because nodules accumulating this slowly would be buried by the more rapid accumulation of pelagic sediments. This, taken with the fact that the systematic decay of <sup>230</sup>Th/<sup>232</sup>Th and <sup>234</sup>U/<sup>238</sup>U in the nodule indicates a continuous growth of the nodule, definitely supports a slow growth model.

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### Origin of Diagenetic Pyrite in the Quilon Limestone, Kerala, India

ALTHOUGH microscopic pyrite from marine and non-marine argillaceous sediments of most geological ages has been extensively studied, its mode of origin has not been clearly understood. Berner<sup>1</sup> and Kalliooski<sup>2</sup> attributed the origin to the reaction of hydrogen sulphide formed by sulphite reduction, with iron-bearing minerals in sediments; but these minerals were not discovered. I have recorded framboidal and other types of diagenetic pyrite in the thin bed of marine, fossiliferous, Quilon limestone<sup>3</sup> of Burdigalian age<sup>4</sup>. Recent examination of the heavy minerals from limestone samples at Padappakara the type area has brought forward some new evidence, which clearly establishes that the fine-grained pyrite was produced by an alteration of detrital, iron-bearing mica, optically identified as biotite.

The soluble part of the limestone was removed by digestion in concentrated hydrochloric acid. The insoluble residue, thoroughly cleaned and dried, was screened using 60, 100 and 200 mesh sieves. The heavy minerals were separated in bromoform and permanently mounted in Canada balsam. For microscopic examination reflected light and a high power magnification of  $\times 315$  were found to be satisfactory. Pyrite was abundant in all the slides irrespective of size. Among the different morphological types recognized were crystal aggregates, microfaunal infillings, pseudomorphs, framboids and their spherical aggregations and scattered idiomorphs. The last three types were restricted to the finer fractions, whereas the rest are almost equally common in all the sizes of fractions. Besides cubes, octahedra and pyritohedra<sup>5</sup>, previously reported by others in argillaceous sediments elsewhere, combinations of cubes and octahedra, with any one of the forms better developed than the other, and interpenetrating cubes, are also reported here for the first time.

The crystal aggregates are tabular or slab-like and made of closely packed euhedra. Microfaunal infillings are characteristically seen in the foraminifers where each chamber is filled with a cluster of tiny pyrite crystals. Some bivalved micro-organisms are represented by pseudomorphs, composed of dull, cryptocrystalline pyrite which may be epigenetic.

Many of the tabular slabs of crystal aggregates have some of their edges straight and smooth, whereas the others appear fractured, embayed or jagged due to unevenly projecting crystals. The jagged edges are partly covered by a translucent brown sheath of biotite, which is evidently a remnant of the parent mineral left after the formation of the enclosed pyrite. Some slabs are completely covered by the sheath, which releases the pyrite contents when the sample is disrupted. Love<sup>6</sup> mentioned sacs or pellicles of organic matter as forming an outer cover around the pyrite. In the case of the material investigated, however, the sheath undoubtedly consists of mica and no trace of any organic matter could be found.

The occurrence of biotite is less frequent in the heavy minerals than might be expected in view of its profusion in the Archaean crystalline rocks of the region. This is so because much of the originally present mica has disappeared by alteration to pyrite. Some of the tabular slabs still retain the original pseudo-hexagonal outline of the biotite.

Dust-like, opaque inclusions are common in the detrital biotite and are found to be pyrite. Some flakes show only a skeletal development of pyrite which may indicate abrupt environmental changes. The grains being sparsely distributed, such flakes enable the observation of genetic details of the pyrite. It can clearly be seen that the pyrite euhedra are formed in more than one generation. The crystals formed earlier are coarser, more widely spaced and are distributed in such a way as to simulate the intersections of a network. These serve as nuclei around which the finer crystals formed later closely adhere. Some of the nuclei seem to give rise to framboids, while others produced crystal aggregates. The geometrical pattern of the network strongly suggests that the atomic structure of the parent mineral influences the mode of development and distribution of the resulting pyrite.

The present investigation has further shown that all iron-bearing minerals are not prone to pyritization though they may be controlled by the same environmental conditions. For example, almandine, which is a major constituent of the heavy minerals, has not been altered in the least. Some ilmenite grains from the assemblage show a regular lamellar structure which results from deep, selective corrosion of alternate layers. The lamellae are thinly coated with pyrite, but this evidence does not conclusively prove its susceptibility to pyritization.

Pyrite is also found as inclusions in almandine and other heavy minerals of the assemblage such as silli-

manite, amphibole and hypersthene. The inclusions are generally in the form of cubes and octahedra, but occasionally framboids are also noted. These have apparently developed by pyritization of the original biotite inclusions in the host minerals. I am not aware of any previous record of a similar occurrence of pyrite as inclusions.

Framboids and morphologically related aggregations are generally regarded as resulting from the crystallization of iron sulphide gel. Whether the single crystals, occurring in between the framboids, as noted in the biotite under alteration, passed through a gel stage or were formed directly by the simple interaction between hydrogen sulphide and iron under favourable physical and chemical conditions is not known. The geometrical pattern of distribution of pyrite in the biotite tends to show that the interaction, including the precipitation of the gel, was restricted to certain points only as determined by the atomic structure of the biotite. The microfaunal infillings seem to represent an influx of extraneous gel which eventually crystallized within the chambers into tiny pyrite euhedra. The inclusions of single crystals and framboids of pyrite found in the associated heavy minerals of the assemblage were probably produced by hydrogen sulphide coming directly into contact with and interacting with the original biotite inclusions. Further work on the hydrogen sulphide susceptible structure of the mica and the mechanism of interaction is in progress.

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## A New Radio-carbon Date for Wales

THIS communication presents the results of a radio-carbon age determination on organic material recently found in fluvioglacial deposits exposed in the workings of the Cardiganshire Sand and Gravel Co. at Banc-y-Warren (Cardiganshire), (SN 202482). This well developed kame-complex was described by Williams in 1927 (ref. 1).

The deposits, which have a minimum thickness of 150 ft., contain "Welsh" and "Irish Sea" erratics including Cambrian grits, Old Red Sandstone, flint, chalk and igneous rocks. Shell fragments are common throughout the succession. In the section examined, stratified sand up to 40 ft. thick was overlain unconformably by bedded gravels, pebbles and cobbles, passing upwards into more stratified sand to a total thickness of approximately 110 ft. The regional stratigraphic relationships were determined by Williams, who found that equivalent fluvioglacial deposits nearby directly overlie stiff blue "Lower Boulder Clay" at Llwyn-llwyd Farm and at Rhos-llyn. Some 70 ft. below the top of the section at Banc-y-Warren, within the gravel layer, a discontinuous horizon of peaty organic mud was found. Approximately 40 g of this was taken for radio-carbon analysis from a point 4 ft. behind the quarry face, which at the time was being actively worked. The sample has been dated by Isotopes Inc., New Jersey (Sample No. 1-2559), as  $31,800^{+1,400}_{-1,200}$  years B.P. The point from which the sample was taken makes it extremely unlikely that it has been contaminated by rainwater percolating downward.

Previous attempts to delimit the extent of the Würm glaciation in western Wales are summarized in Fig. 1, and demonstrate a considerable diversity of opinion. An early attempt was that of Lewis<sup>2</sup>, whose suggestion that the Irish Sea Ice did not cross the Llyn peninsula was rejected by Charlesworth<sup>3</sup>. The latter's "Newer Drift" limit in south-west Wales was in part based on the "conspicuous marginal accumulation" at Banc-y-Warren<sup>3</sup>. Synge regarded the deposit as eroded outwash of Riss (Saale) age<sup>4</sup>. Mitchell, in attempting a correlation between the Welsh and Irish Pleistocene chronologies, suggested that Irish Sea Ice did not impinge on the west coast of Wales during the Würm glaciation<sup>5</sup>. This suggestion has received additional support from Synge who considered that the Brynair moraine, in north-west Wales, marks the maximum extent of the Irish Sea Ice during the Würm<sup>6</sup> (see Fig. 1). By implication, any drifts to the south of Brynair are either pre-Würm in age, or are of a periglacial origin, most probably of Würm age, as proposed by Watson<sup>7</sup>. Shell fragments collected by B. S. John from glacial sands and gravels at Mullock Bridge (SM 811080) and Tre-llys (SM 898349) have previously been dated as  $37,960 \pm 1,700$  years B.P. and  $37,310 \pm 1,515$  years B.P. respectively, providing evidence for "an extensive glaciation of Western Britain later than 38,000 years B.P."<sup>8</sup>. The validity of these dates has been questioned by Bowen, who suggested that the shells may have been contaminated by downward percolating groundwater<sup>9</sup>. Bowen and Gregory did, however, accept the possibility that Weichselian (Würm) ice impinged on a small area in north Pembrokeshire and south Cardiganshire<sup>10</sup>.

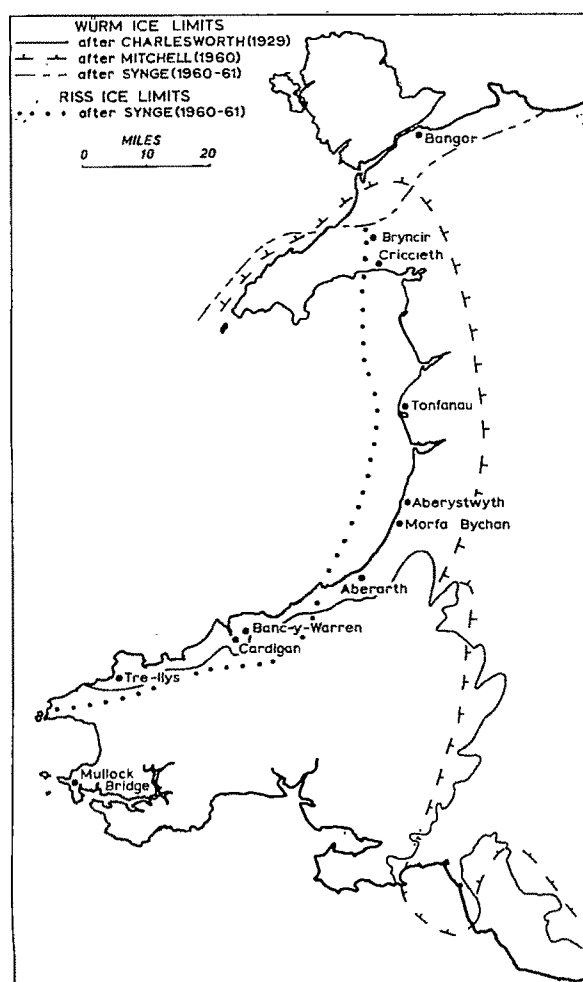


Fig. 1.

By comparison with the known European succession, Boulton and Worsley<sup>11</sup> suggest that a radio-carbon date of  $28,000 \pm 1,800$  years B.P. obtained from shells in fluvio-glacial material at Sandiway (SJ 605708), north of the Ellesmere moraine on the Cheshire-Shropshire plain, indicates a glaciation of Late-Weichselian age, post-dating the Paudorf interstadial (30,000 to 25,000 years B.P.). The date here given for Banc-y-Warren (31,800 years B.P.) would on this basis appear to pre-date the Paudorf interstadial and might therefore be correlated with the "Advance Phase"<sup>12</sup> of the Main Würm of the continental succession.

The Brynair moraine can no longer be considered to mark the southern limit of Würm ice on the eastern flank of the Irish Sea basin, but perhaps it may represent a pause in the recession of that ice-sheet. Alternatively, the Brynair moraine could be the product of a re-advance which post-dates the Paudorf interstadial and hence, on the basis of the shell dates obtained by Boulton and Worsley<sup>11</sup> from Sandiway, be correlated with the Ellesmere moraine.

The presence of numerous north Welsh erratics within the fluvio-glacial deposits at Banc-y-Warren is almost certainly indicative of the contemporaneous existence of Welsh ice.

The age determined for Banc-y-Warren supports John's concept of an extensive Würm glaciation in the Irish Sea basin<sup>8</sup> the limits of which lay well to the south of those proposed by either Mitchell<sup>5</sup> or Synge<sup>6</sup>.

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## THE SOLID STATE

### Determination of the Cation Distribution in the Orthopyroxene Series by the Mossbauer Effect

MEASUREMENTS of the distribution of cations and the detection of order-disorder phenomena in crystal structures provide a potential means of determining the temperature and pressure of formation of minerals. Such data have been obtained from crystal structure analyses of iron silicates by X-ray diffraction techniques (refs. 1-7 and Gibbs, G. V., and Burnham, C. W., personal communications). More rapid and direct techniques, such as infra-red spectroscopy<sup>8</sup> and Mossbauer spectroscopy<sup>9,10</sup>, have since been used to detect cation ordering and to estimate site populations in suites of silicate minerals.

We have used Mossbauer spectroscopy to estimate site populations in a suite of orthopyroxenes derived from regionally metamorphosed rocks<sup>11</sup>. Measurements were made at room temperature<sup>9,12</sup> on five orthopyroxenes of the enstatite–orthoferrosilite series with 14.5–85.9 cation per cent iron (II), and a manganiferous orthopyroxene with 74.8 per cent iron (II) and 9.2 per cent manganese (II). (Fe(II) and Mn(II) refer to the high spin divalent species.) All spectra were obtained using a stainless-steel source; however, duplicate spectra of the 85.9 per cent iron (II) and manganese orthopyroxenes were run with a palladium source and gave better resolution and decreased statistical errors.

Computer plots of three orthopyroxene spectra are shown in Fig. 1. Specimens exceeding 23.1 per cent iron (II) were fitted by computer to four Lorentzian curves, and all parameters were allowed to vary independently. Only the inner two peaks were resolved in the spectra of the 14.5 and 23.1 per cent iron (II) orthopyroxenes, but the widths of these peaks were slightly larger than those of the resolved peaks in the spectra of orthopyroxenes rich in iron. Computer-calculated areas under the peaks are summarized in Table 1, together with standard deviations.  $A_1$  and  $A_2$  refer to the sum of the areas of the outer two and inner two peaks, respectively.

The orthopyroxene structure<sup>4,13</sup> contains two positions of six-fold co-ordination which are designated  $M_1$  and  $M_2$ . Cations in the  $M_1$  positions are co-ordinated to six oxygen ions each linked to one silicon atom. Cations in the  $M_2$  positions are surrounded by four oxygen ions each linked to one silicon atom, and two bridging oxygen atoms which are each shared by two silicon atoms. The octahedron about the  $M_1$  position has a symmetry which is approximately octahedral, but the oxygen polyhedron about the  $M_2$  position is considerably distorted from octahedral symmetry. Average metal–oxygen distances in the two sites in hypersthene<sup>4</sup> are:  $M_1$  site, 2.092 Å;  $M_2$  site, 2.220 Å. Thus, the orthopyroxene  $M_1$  and  $M_2$  sites differ significantly, both geometrically and energetically.

The resolved peaks in the Mossbauer spectra of orthopyroxenes can be assigned as follows: inner two peaks to iron (II) in the  $M_2$  position, outer two peaks to iron (II) in the  $M_1$  position. X-ray diffraction measurements<sup>4</sup> of a 50.0 per cent iron (II) orthopyroxene indicated that iron occurs predominantly in the  $M_2$  position. This may be

correlated with the Mossbauer spectrum of the 48.4 per cent iron (II) orthopyroxene (Fig. 1b), which shows the inner two peaks to be the more intense. Furthermore, the smaller quadrupole splitting of the inner peaks<sup>14</sup> conforms with the occurrence of iron (II) ions in the more distorted co-ordination site<sup>15</sup>.

The proportions of iron in the  $M_2$  and  $M_1$  positions,  $n_2/n_1$ , can be obtained from the ratio  $A_2/A_1$  of the computer calculated peak areas (Table 1) using the relationship

$$A_2/A_1 = C.n_2/n_1.$$

The constant  $C$  can be evaluated precisely from the spectrum of orthoferrosilite ( $\text{Fe}_2\text{Si}_2\text{O}_6$ ). Because synthetic<sup>16</sup>  $\text{Fe}_2\text{Si}_2\text{O}_6$  was not available to us, and naturally occurring orthopyroxenes exceeding 85.9 per cent iron (II) are unknown, we have estimated the constant  $C$  to be 0.90 (ref. 9). For a 35.4 per cent iron (II) cummingtonite, this value of  $C$  gives site populations which are in excellent agreement with those obtained by X-ray diffraction<sup>7</sup>. Because there is close structural similarity between the orthopyroxene  $M_2$  and cummingtonite  $M_4$  sites, and between the orthopyroxene  $M_1$  and cummingtonite  $M_3$ ,  $M_2$  and  $M_3$  sites, a value of  $C$  similar to that chosen for the cummingtonite–grunerite series appears to be reasonable for the orthopyroxene series. Site populations calculated from values of  $C$  of 0.85, 0.90 and 0.95 are summarized in Table 1.

The results summarized in Table 1 show that calculated site populations are relatively insensitive to changes in the constant  $C$ . The quantitative site populations estimated from the Mossbauer spectra show that iron (II) ions are concentrated in the  $M_2$  position of the orthopyroxene structure, and that manganese is enriched more strongly than iron in the  $M_2$  position. The manganese–iron ordering can be gauged from the data for the 85.9 per cent iron (II) and manganiferous orthopyroxenes, which have almost identical contents of iron (II) + manganese (II) + calcium (II), and the 72.7 per cent iron (II) and manganiferous orthopyroxenes (similar content of iron (II)).

Similar results for manganese–iron ordering were obtained by X-ray diffraction<sup>4</sup>. Ghose<sup>4</sup> used a granulite facies hypersthene with 1.00 iron (II), 0.01 manganese (II) and 0.05 calcium (II) per formula unit, and estimated that the  $M_2$  position contained 90 per cent iron (II) and the  $M_1$  position 15 per cent iron (II). The results can be

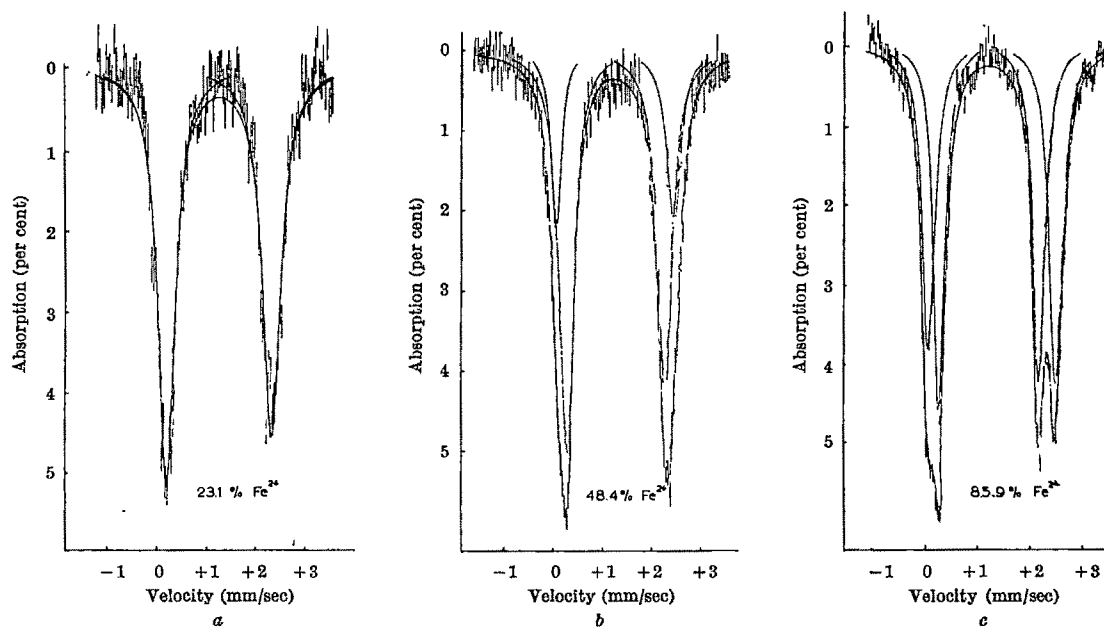


Fig. 1. Computer plots of the Mossbauer spectra of minerals of the orthopyroxene series. a, 23.1 per cent iron (II); b, 48.4 per cent iron (II); c, 85.9 per cent iron (II).



Table 1. DISTRIBUTION OF IRON (II) IONS IN ORTHOPYROXENES FROM MOSSBAUER SPECTROSCOPY

Specimen	Iron (II) (per cent)	Iron (II) per formula unit	Manganese (II) per formula unit	Calcium (II) per formula unit	$\Delta_2$ counts $\times$ channels $\times 10^{-5}$	$\Delta_1$ counts $\times$ channels $\times 10^{-5}$	Iron (II) in $M_1$ and $M_2$ position					
							$C=0.85$ $n_2$	$C=0.85$ $n_1$	$C=0.90$ $n_2$	$C=0.90$ $n_1$	$C=0.95$ $n_2$	$C=0.95$ $n_1$
Bamle Norway	14.5	0.29	0.01	0.01	3.55 $\pm 0.10$	not resolved	$n_2 \geq$		0.27	$n_1 \leq$	0.03	
Howie	23.1	0.46	0.01	0	4.52 $\pm 0.12$	not resolved	$n_2 \geq$		0.43	$n_1 \leq$	0.04	
Howie 68971	48.4	0.97	0.02	0.05	4.24 $\pm 0.35$	1.65 $\pm 0.33$	0.73	0.24	0.72	0.25	0.71	0.26
Howie	72.7	1.45	0	0.04	3.63 $\pm 0.25$	3.41 $\pm 0.25$	0.81	0.64	0.79	0.66	0.77	0.68
Kuno K23	85.9	1.72	0.01	0.04	5.58 $\pm 0.15$	5.81 $\pm 0.15$	0.91	0.81	0.89	0.83	0.86	0.86
Henry Cambridge 190553	74.8 (9.2 per cent manga- nese (II))	1.50	0.18	0.07	6.50 $\pm 0.14$ 8.28 $\pm 0.12$	10.04 $\pm 0.15$ 4.30 $\pm 0.12$	0.65	0.85	0.63	0.87	0.60	0.90
							0.71	0.79	0.69	0.81	0.67	0.83

compared with those obtained in the present investigation for a charnockitic hypersthene with 48.4 per cent iron (II) (0.97 iron (II), 0.02 manganese (II) and 0.05 calcium (II) per formula unit), which indicate that about 72 per cent and 25 per cent of the  $M_2$  and  $M_1$  positions, respectively, are occupied by iron.

Because the quadrupole splitting of the two doublets approaches a similar value at low iron concentrations<sup>14</sup>, resolution of the outer doublet is impossible in the 14.5 per cent and 23.1 per cent iron (II) orthopyroxenes, and statistical errors are rather large in the 72.7 per cent and 48.4 per cent iron (II) orthopyroxenes (> 15 per cent). In the cummingtonite-grunerite and anthophyllite series, the statistical errors were considerably smaller<sup>9,10</sup>. Statistically acceptable  $\chi^2$  (less than 400 for a 400 point spectra) were obtained for the 14.5 per cent, 48.4 per cent and 85.9 per cent iron (II) samples. Higher values for the other three samples suggests that there are very small contributions to the spectra from other iron atoms (for example, iron (III), or iron (II) in the  $M_1$  position in the 23.1 per cent iron (II) orthopyroxene).

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## PHYSICS

### Thin Silicon Solar Cells for Large Flexible Arrays

THE past year has witnessed increasing interest in solar-powered, electrical propulsion systems for spacecraft in both the United States and the United Kingdom. In the United States the main interest is in the use of electrical propulsion for missions to Mars and Jupiter, where there is a marked payload advantage over chemically powered spacecraft which are limited by their energy rather than by their power. The incentive for the United Kingdom to initiate development in this field was provided by Burt<sup>1</sup> who theoretically showed that it was possible to change independently all the elements of a spacecraft's orbit by means of small continuous thrusts from an electrical propulsion unit. In particular, he showed that the ability of the unit to change the inclination of an orbit made it possible for a communications satellite launched from a non-equatorial site to attain a geostationary orbit.

The power required for such a mission would be from 1 to 2 kW, and this would give a tangential acceleration of about 0.1 cm sec<sup>-2</sup>. With this acceleration, it would be possible to climb from a circular orbit of 550 km to a synchronous altitude in about 50 days.

Silicon solar cells offer a promising approach to the problem of providing kilowatts of power over periods of several years on account of their reliability, adaptability and advanced technological status. The most important requirements for large arrays are low specific mass—20 kg kW<sup>-1</sup> for the mission already mentioned—and the ability to stow and to support the assembly in a small volume for launching.

Specific mass can, of course, be reduced by improving the conversion efficiency obtained by expressing the maximum cell output power as a percentage of the input power (that is, the mean solar irradiation above the Earth's atmosphere multiplied by the active cell area) and which at present stands at about 11 per cent for an *n-on-p* cell at 300° K. The theoretical limit for a planar *p-n* junction at this temperature (assuming that 10<sup>-3</sup> recombinations of electron-hole pairs are radiative) is 22 per cent, so an increase to the theoretical maximum performance would halve the specific mass of an array and, with it, the specific area. From past experience, actual achievements in this direction during the next few years are likely to be marginal.

A much more significant saving can be made by using a thin plastic substrate instead of the conventional rigid support and reducing the thickness of the solar cells. Such assemblies are sufficiently flexible to permit large areas to be wrapped around the body of a spacecraft or around a separate drum, and thus meet the requirement for stowage and support during launch. A reduction in the thickness of the cell from 400 to 100  $\mu$  has already been achieved in pilot production.

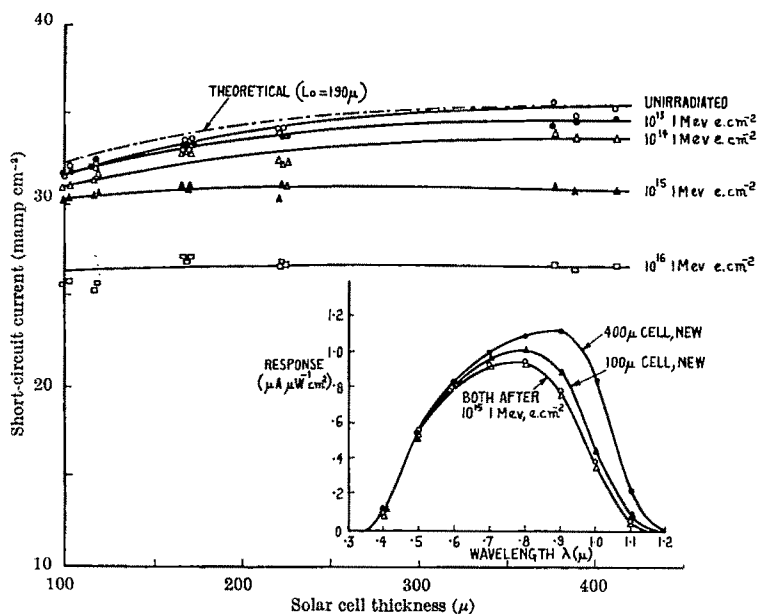


Fig. 1. Short-circuit current of 100–400 $\mu$  silicon solar cells irradiated with electrons.

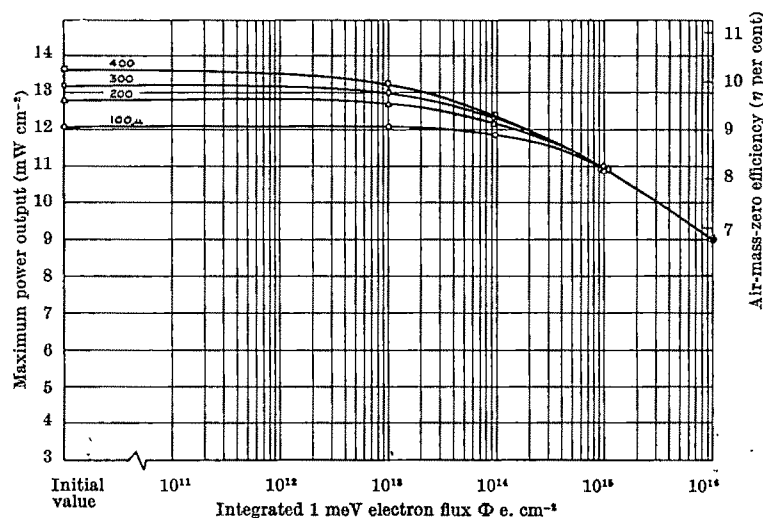


Fig. 2. Power output of 100–400 $\mu$  silicon solar cells irradiated with electrons.

Silicon cells cannot be thinned to this extent without some loss of response to red and infra-red light (Fig. 1, insert). Because of the predominance of indirect transitions in silicon, photons in the red and infra-red wave bands are absorbed at an appreciable depth. For example, at a wavelength of  $1\mu$  the absorption coefficient is  $100\text{ cm}^{-1}$  and this gives a depth of penetration of  $100\mu$ . In order that the photo-generated minority carriers (electrons in the case of a  $p$ -type base region) at this depth can contribute to the output of the cell, a long diffusion length is necessary for minority carriers. The diffusion length  $L$ , or mean distance moved by an electron or hole during its lifetime, determines the effective thickness of the cell. In thin cells, there is an additional loss because of the proximity of the rear contact which covers most or all of the back surface of the cell and facilitates a very high surface recombination rate for minority carriers.

Wolf and Ralph<sup>2</sup> have computed the variation of short circuit current as a function of the thickness of a solar cell. They found that at thicknesses of cells of less than  $300\mu$  the measured short circuit currents inexplicably decreased at twice the rate of the theoretical curves.

The results of our experiments on 100–400 $\mu$  shallow junction,  $10\Omega\text{ cm } n\text{-on-}p$  silicon cells are shown in Figs. 1

and 2. The good agreement with theory (Fig. 1) has been achieved, we believe, by accurate measurement and the elimination of work damage at the rear silicon surface during fabrication of the cell. Estimates of the specific mass of flexible arrays show that the poorer initial performance of the  $100\mu$  cell is more than counter-balanced by its smaller mass when compared with cells of conventional thickness.

Another interesting feature of thin silicon cells is their radiation resistance. Electron or proton irradiation produces lattice defects in the crystal which behave intrinsically, or in combination, with other impurities as recombination centres, reducing the diffusion length of minority carriers according to the relation

$$L^{-2} = L_0^{-2} + K\Phi$$

where  $L_0$  is the initial value of the diffusion length,  $L$  the value after an integrated flux  $\Phi$  and  $K$  the damage coefficient.

The degradation of diffusion length reduces the effective thickness of a conventional cell and results in a reduction in the response to red and infra-red light similar to that caused by physically thinning the cell.

Investigations of radiation damage carried out at the Royal Aircraft Establishment have shown that, after a flux of  $10^{15}$  electrons  $\text{cm}^{-2}$  at 1 MeV, the spectral responses (Fig. 1) and consequently the performance (Fig. 2) of 100 and  $400\mu$  cells are very nearly the same. The performance degradation after  $10^{15}$  electrons  $\text{cm}^{-2}$  corresponds to a reduction of the diffusion length of minority carriers from an initial value of  $160\mu$  to  $30\mu$ . The damage coefficient  $K$  is  $8.89 \times 10^{-11}$  electrons $^{-1}$ . Recent experiments with 70–140 MeV protons have shown a similar effect.

The superior radiation resistance of thin cells will be of particular value when a spacecraft has to traverse the Van Allen proton belt. The recent work by R.C.A. towards the development of a lithium doped  $p\text{-on-}n$  silicon cell resistant to radiation<sup>3</sup> promises a further bonus in this context.

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## CHEMISTRY

### Some Electrical Properties of Bimolecular Phosphatidyl Inositol Membranes

THE formation of "black", bimolecular lipid membranes in aqueous solutions was first reported by Mueller *et al.*<sup>1</sup> in 1962 and has since been investigated by several research groups<sup>2-7</sup>. Most of the work has been carried out with phosphatidyl choline (lecithin) from natural sources, although "black" films could also be obtained with diglycerides<sup>5</sup>, sphingomyelin<sup>7</sup> and synthetic dioleoyl phosphatidyl choline<sup>7</sup>. A common property of all these com-

pounds is that the net charge of the molecule is zero. It is of interest that several types of negatively charged lipids, for example, phosphatidyl serine or phosphatidyl inositol, are found in cell membranes. Because of speculations on the biological functions of these lipids<sup>8</sup> it was of interest to investigate the electrical properties of artificial bimolecular membranes bearing negatively charged groups.

"Black" membranes were obtained from a 0.5 per cent (w/v) solution of phosphatidyl inositol in *n*-decane. The membranes were formed at 35° C on a circular aperture in the wall of a 'Teflon' cell which was immersed in an aqueous salt solution<sup>7</sup>. With salt concentrations of  $c \geq 10^{-3}$  moles/l. the membranes were stable for several hours. The phosphatidyl inositol was purchased from Sigma (grade II, from bovine brain) and further purified on a DEAE cellulose column<sup>9</sup>.

The electrical capacitance of membranes of phosphatidyl inositol was found to be  $0.29 \pm 0.03 \mu\text{F}/\text{cm}^2$ , which is slightly lower than the value observed with membranes of phosphatidyl choline<sup>3,7</sup>. Using the same assumptions as in ref. 7, a thickness of 80 Å is calculated for the membrane of phosphatidyl inositol.

In solutions of potassium chloride the electrical resistance of these membranes is very high and almost independent of the salt concentration—the values for individual membranes varied between  $5 \times 10^7$  and  $5 \times 10^8 \Omega \text{ cm}^2$ . Resistances of the same order of magnitude were observed with phosphatidyl choline membranes in potassium chloride solutions<sup>3,7</sup>. It seems that only the highest resistance values are true resistances of the black film, and that the lower values result from border leakages<sup>10</sup>. By replacing the chloride ions in the solution by iodide ions it was possible to reduce the resistance of the phosphatidyl choline membrane by a factor of about one thousand<sup>7</sup> and to demonstrate that the membrane resistance in iodide solutions is a true property of the black film<sup>7</sup>. With phosphatidyl inositol membranes the effect of the iodide ions on their resistance was much lower but could be greatly enhanced when a large excess of an "indifferent" salt, such as potassium chloride, was added. This can be seen in Table 1 in which the resistances of a phosphatidyl inositol membrane at 35° C are summarized. The observed resistance values vary from membrane to membrane. The effect of the iodide ion on the resistance of a membrane is always reversible: if the ( $10^{-2}$  molar potassium iodide + 2 molar potassium chloride) solution was replaced by 2 molar potassium chloride at the end of the experiment, the membrane resistance returned to a very high value.

In a further series of experiments the electrical resistance of membranes prepared from mixtures of phosphatidyl choline and phosphatidyl inositol was investigated (compare Fig. 1). When the composition of the solution was gradually changed from pure phosphatidyl choline in *n*-decane to pure phosphatidyl inositol in *n*-decane, the membrane resistance increased by a factor of about 300 from  $0.049 \times 10^6$  to  $16 \times 10^6 \Omega \text{ cm}^2$ . It is emphasized, however, that the molar ratio in the black film is not necessarily the same as in the decane solution.

The interesting observation that the resistance of a phosphatidyl inositol membrane in  $10^{-2}$  molar potassium iodide can be reduced by a factor of about one hundred by adding a large excess of potassium chloride even though the resistance in a solution of potassium chloride alone is very high can be explained by the fact that the surfaces of the membrane are ionized. If the area occupied by a molecule of phosphatidyl inositol in the membrane is assumed to be  $A \approx 40 \text{ Å}^2$  (a value suggested by monolayer investigations<sup>11</sup>) and if each molecule is ionized, then the surface charge density of the membrane is  $q = -e_0/A \approx$

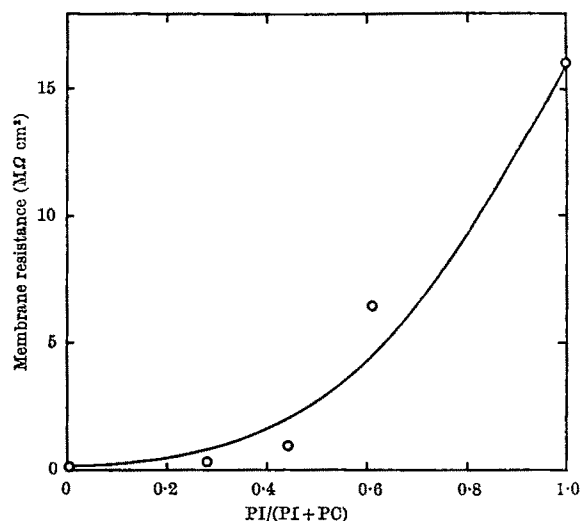


Fig. 1. Electrical resistance of mixed phosphatidyl choline/phosphatidyl inositol membranes in  $10^{-2}$  molar potassium iodide as a function of the composition. PC and PI are the concentrations (mg/ml.) of phosphatidyl choline and phosphatidyl inositol, respectively, in the solution from which the membrane was formed.

$-4.0 \times 10^{-5}$  coulomb/ $\text{cm}^2$  ( $e_0$  is the elementary charge). The negative charge of the membrane is electrically neutralized by a diffuse layer of opposite charges in the solution. From the theory of the diffuse double layer<sup>12</sup> the following relation between the charge density  $q$  and the electrical potential  $\phi_s$  at the surface of the membrane is derived (for a 1 : 1 electrolyte solution):

$$q = \sqrt{\left(\frac{2}{\pi} \epsilon c RT\right)} \sinh\left(\frac{\phi_s F}{2RT}\right) \quad (1)$$

Here  $\epsilon$  is the dielectric constant of water,  $c$  is the total salt concentration,  $R$  is the gas constant,  $T$  is the absolute temperature, and  $F$  is the Faraday constant. This equation should be considered as only approximate. In more concentrated electrolyte solutions a part of the membrane charge may be screened by a layer of closely associated ions of opposite charge (the "Helmholtz layer"). In this case  $q$  should be replaced by an effective charge density  $q_{\text{eff}}$  with  $|q_{\text{eff}}| < |q|$ ;  $\phi_s$  is then the potential at the outside of the Helmholtz layer. For the sake of simplicity we assume  $q_{\text{eff}}$  to be independent of the concentration of the electrolyte. Previously, it was found that the membrane conductivity in iodide solutions is a function of the concentration of the iodide ions and independent of the cation<sup>7</sup>. It is therefore feasible to describe the membrane conductivity by the following equation

$$\frac{1}{R_m} = \text{constant} \times (c_1)_s \quad (2)$$

where  $R_m$  is the resistance of  $1 \text{ cm}^2$  of the membrane and  $(c_1)_s$  is the concentration of the iodide ions at the membrane surface. Because the electrical potential  $\phi_s$  at the membrane surface is negative with respect to the solution, the concentration  $(c_1)_s$  is smaller than the concentration of the iodide ions  $(c_1)_0$  in the bulk solution by the Boltzmann factor  $\exp(\phi_s F/RT)$

$$(c_1)_s = (c_1)_0 \exp(\phi_s F/RT) \quad (3)$$

so that equation (2) assumes the form

$$R_m = R_0 \exp(\phi_s F/RT) \quad (4)$$

Substitution of  $\phi_s$  from equation (1) leads to

$$R_m = \frac{\alpha R_0}{1 + \alpha - \sqrt{1 + 2\alpha}} \quad (5)$$

$$\alpha \equiv \frac{\epsilon c RT}{\pi q^2} \quad (6)$$

For small values of  $\alpha$ , equation (5) can be replaced by the approximation

Table 1. ELECTRICAL RESISTANCE OF AN INDIVIDUAL PHOSPHATIDYL INOSITOL MEMBRANE IN DIFFERENT ELECTROLYTE SOLUTIONS

2 molar KCl	$1.1 \times 10^8 \Omega \text{ cm}^2$
$10^{-2}$ molar KI	$1.9 \times 10^7 \Omega \text{ cm}^2$
$10^{-2}$ molar KI + 2 molar KCl	$1.5 \times 10^6 \Omega \text{ cm}^2$

$$R_m \approx \frac{2R_0}{\alpha} \quad (5a)$$

With  $q = -4 \times 10^{-6}$  coulomb/cm<sup>2</sup>,  $\alpha = 0.091$  for an aqueous solution of  $c = 2$  moles/l. The approximation (5a) is therefore valid for the whole range of concentrations. If the membrane resistance were measured in two solutions with identical values of  $(c_1)_0$  but different total concentrations  $c_1$  and  $c_2$ , according to equations (5a) and (6) the resistance ratio would be expected to be

$$\frac{R_m(c_1)}{R_m(c_2)} = \frac{c_2}{c_1}$$

In our experiments with  $c_1 = (c_1)_0 = 10^{-3}$  moles/l. and  $c_2 \approx 2$  moles/l., a resistance ratio of 130 was found which agrees approximately with the calculated ratio of 200. It can therefore be concluded that the influence of an added indifferent electrolyte on the iodide conductivity of the membrane can be explained by an electrostatic double layer effect.

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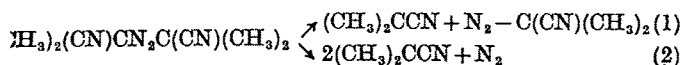
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### Pairwise Trapping in Solid State Photo-eliminations

UNDER certain circumstances radicals may be trapped in pairs which are so well separated that there is no chemical bond between them, but sufficiently close that there is a magnetic coupling between the unpaired electrons. If the trapping is sufficiently precise this may be revealed in the electron spin resonance spectrum which will be characteristic of a triplet-state species<sup>1,2</sup>.

Photolyses or radiolyses sometimes involve the elimination of small stable molecules such as nitrogen or carbon monoxide, and if this occurs in a rigid medium the resulting radicals would seem to be ideally placed for the production of such weakly coupled systems with the precision required for detection. We have recently established that  $\gamma$ -irradiation of crystalline diaryl carbonates, which involves formation of carbon monoxide, gives pairs of aryloxy radicals separated "magnetically" by about 6 Å (ref. 3). The purpose of the present communication is to call attention to the solid state ultra-violet photolysis of azobisisobutyronitrile, which results in a species with an electron spin resonance spectrum which is also characteristic of a triplet.

The reaction was originally written as (1) (ref. 4),



(2)

rather than (2), but the spectrum has exactly the form required for radical-pairs (Fig. 2 of ref. 4). The separation of about 280 G between the outer features corresponds

to an effective or "magnetic" separation between the radicals of 5.8 Å which is very close to expectation if the two  $(\text{CH}_3)_2\text{CCN}$  radicals simply become planar on the extrusion of a nitrogen molecule. The hyperfine coupling, which is probably dominated by the methyl group protons, is about half that assigned to the protons in separated  $(\text{CH}_3)_2\text{CCN}$  radicals<sup>4</sup> as would be expected for pairs undergoing rapid spin exchange<sup>5</sup>.

This result is of considerable importance because it proves that nitrogen is either extruded in a one-step process or, less reasonably, that the radical  $\text{N}_2\text{C}(\text{CH}_3)_2\text{CN}$  loses nitrogen spontaneously even at 77° K.

The alternative interpretation<sup>4</sup> that a single radical,  $\text{N} = \text{NC}(\text{CH}_3)_2\text{CN}$ , was responsible for the spectrum is improbable because this radical is expected to be bent at the inner nitrogen atom, with the unpaired electron in a  $\sigma$ -orbital<sup>6</sup>. This is expected to give a spectrum quite different from that detected.

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### Voltages produced by Oxygen in Platinum-Sodium Chloride Crystal Cells

IT has been found that single crystals of sodium chloride annealed in nitrogen under mild compression between platinum electrodes produce electrical potentials when treated with traces of oxygen at 700°–730° C.

Specimens cleaved to blocks 10 × 10 × 3 mm were placed with the two large surfaces in contact with flat platinum electrodes which rested against recrystallized alumina disks for electrical insulation. The assembly was slightly compressed by springs adjusted to finger tightness. The crystal holder and silica cell were essentially the same as those described in detail by Allnatt and Jacobs<sup>1,2</sup>. The cell was evacuated to a pressure of 10<sup>-6</sup> mm of mercury overnight at room temperature and filled with "pure" nitrogen gas (10 p.p.m. of oxygen according to the manufacturer) which had been passed over hot copper turnings. All gases entered the cell through a methanol-solid carbon dioxide trap. The gas flow rates in the experiments mentioned here were all approximately 20 ml./min. When the temperature was initially raised above 500° C a variable voltage of several millivolts was observed similar to that described for

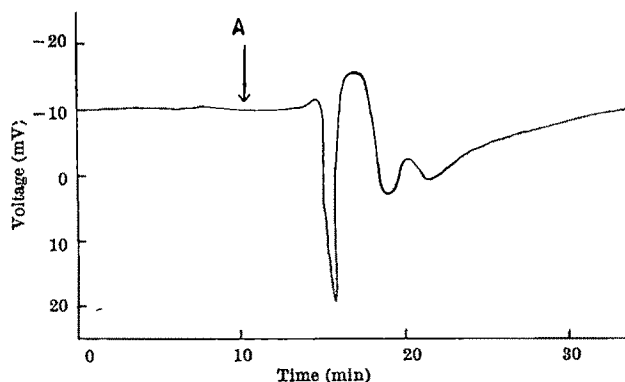


Fig. 1. Voltage-time relation after entry of first portion of oxygen at A. Results are independent of absence or presence of the small thermoelectric voltage at  $t = 0$  in the graph.

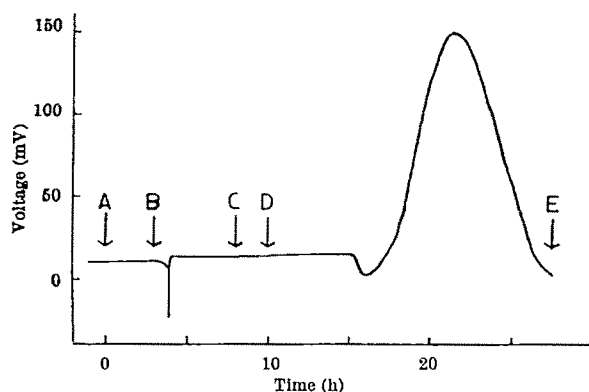


Fig. 2. Voltage-time relation for oxygen treatments described in text.

potassium chloride and other crystals<sup>2</sup>. This vanished after annealing at 750° C for 1 h and various thermoelectric and a.c. conductivity measurements were made over a period of several days. Comparison of conductivity with values in the literature showed that the initial annealing perfected the electrode contact. The "knee" of the conductivity curve<sup>3</sup> was below 450° C. The potential between the electrodes as a function of time was next measured using an electrometer and a recorder.

In one run 50 ml. portions of 0.5 per cent oxygen in nitrogen were flushed through the cell with "pure" nitrogen. The first two portions, separated by 1 h, produced similar voltage oscillations after 5 and 6 min respectively (Fig. 1). A third portion of gas 25 min after the second produced no effect in 5 h. A series of experiments showed that the sensitivity to further gas samples was restored by annealing for periods of 1–12 h at the same temperature (700° C) or higher. The amplitude of voltage oscillations varied between 10 and 100 mV with four or five reversals of sign often occurring.

Fig. 2 summarizes another experiment at 730° C on a different specimen. Between A and B a stream of nitrogen not purified over hot copper was passed. The oxygen content was too small to produce an effect in 200 min. From B to C 0.5 per cent oxygen in nitrogen was passed and the usual voltage oscillations appeared after 50 min. From C to D a static atmosphere of the same gas was maintained; from D to E purified nitrogen was passed. Note the 140 mV peak, the relative slowness of its production and decay, and the absence of rapid oscillations. Crystals treated with several gas samples produced similar peaks in overnight anneals, but the peak voltages were only 10–20 mV, presumably because of the smaller amounts of oxygen passed.

Similar results to those shown in Figs. 1 and 2 were obtained for a crystal in which the surfaces in contact with platinum had been coated with graphite, and for a crystal containing  $2.3 \times 10^{-4}$  mole fraction of strontium chloride. The main features recur in each experiment, but the details are not very reproducible. For example, the voltage oscillation after the first gas portion in an experiment appeared from 5–50 min after first admission of gas.

Voltages can be produced by plastic deformation of alkali halide crystals because of the motion of charged dislocations<sup>4</sup>, but detailed studies have all been carried out near room temperature. The plasticity of such crystals near the melting point is also familiar and the method of perfecting the electrode contact already described, and used by other workers<sup>5</sup>, depends on this. Changes of thickness of up to 1 per cent occur. Embrittlement of sodium chloride by oxygen gas at room temperature is also well known<sup>6</sup> and may occur because gas atoms at the surface, or diffused into the surface layers, impede the motion of dislocations. The initial rapid voltage fluctuations are presumably connected with the flow of

dislocations during the early stages of embrittlement by oxygen in the surface layers and the variability in details from experiment to experiment is connected with slight differences in the amount and uniformity of compression inevitable in such a simple cell. By contrast the broad peaks, like that between D and E in Fig. 2, are formed after oxygen has had time to diffuse into the bulk of the crystal, presumably in the form of  $O_2^-$  ions observed by paramagnetic resonance<sup>6</sup>. This, together with the relative slowness of the voltage production, suggests a different but related process. Speculation on the details of the processes involved is of little use without experiments with more sophisticated apparatus designed for the study of mechanical properties at high temperatures.

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### Study of Electronically Excited Iodine Atoms $I(5^2P_{1/2})$ by Time Resolved Emission

Donovan and Husain<sup>1-7</sup> have recently carried out a number of investigations on electronically excited iodine and bromine atoms,  $I(5^2P_{1/2})$  and  $Br(4^2P_{1/2})$ , by kinetic spectroscopy in absorption in the vacuum ultra-violet after flash photolysis of a number of gaseous halides. The long mean radiative life-times of these excited atoms<sup>8</sup> arising out of the electric dipole forbidden transitions to the ground states,  $I(5^2P_{3/2})$  and  $Br(4^2P_{3/2})$ , have facilitated such studies in absorption. This communication describes the study of the time resolved emission  $I(5^2P_{1/2}) \rightarrow I(5^2P_{3/2}) + h\nu$  ( $1.315\mu$ ) following the flash photolysis of gaseous trifluoroiodomethane ( $CF_3I$ ), monitored by means of a lead sulphide photoconductive cell placed at the exit slit of a high aperture grating monochromator (Bausch and Lomb,  $f$  4.4) and displayed on the screen of an oscilloscope. The concentrations of excited iodine atoms produced on photolysis<sup>2</sup> are sufficiently high to overcome the low value of the Einstein coefficient for spontaneous emission, and adequate intensities are thus obtained. A typical trace for the decay of the emission is shown in Fig. 1. The decay is slower for increasing pressures of argon in accordance with a process primarily controlled by diffusion to the walls of the reaction vessel<sup>2-4</sup>.

The rate of decay of  $I(5^2P_{1/2})$  outside the region of photolytic initiation can be written

$$-d[I(5^2P_{1/2})]/dt = (\Sigma k_i[M_i] + \beta'/p + A_{nm})[I(5^2P_{1/2})]$$

where  $\Sigma k_i[M_i]$  is the sum of the first order collisional deactivation rate coefficients by species  $M_i$ ,  $\beta'$  is the part of the rate coefficient for diffusion to the walls of the reaction vessel independent of pressure ( $p$ ), and  $A_{nm}$  is the Einstein coefficient for spontaneous emission. The term in induced emission is not included because, although a population inversion is readily observed in this system<sup>2</sup>, this is in the absence of laser action because these experiments do not make use of a laser cavity. The intensity of the emission detected ( $I$ ) is given by

$$I = \phi A_{nm}[I(5^2P_{1/2})]_t \\ = \phi A_{nm}[I(5^2P_{1/2})]_{t=0} \exp(-(\Sigma k_i[M_i] + \beta'/p + A_{nm})t)$$



using the rate equation.  $\phi$  is the fraction of the emitted light detected. Thus the slope of the semilogarithmic plot of the intensity against time yields the overall first order decay coefficient ( $k$ ) for the rate equation. These plots are linear over a range of the order of milliseconds and eventually depart from linearity due to rapid quenching by  $I_2$  formed by atomic recombination. The plot of  $k$  against  $1/p$  is, in turn, of slope  $\beta'$  and intercept  $A_{nm} + k_{CF,I}[CF_3I]$  for a trifluoroiodomethane/argon mixture. This is shown in Fig. 2 for mixtures containing trifluoroiodomethane at a pressure of 1 mm of mercury. The decay rates at the different pressures of argon are corrected for quenching by oxygen present as a trace impurity at a concentration of 0.4 p.p.m. using the value obtained by Donovan and Husain for deactivation by this molecule<sup>4</sup>. The measurement of small differences in decay rates due to small differences in diffusion rates at these relatively high pressures of argon, necessary to prevent a significant temperature rise on flashing, involves relatively large errors. We consider the form of Fig. 2 to be a satisfactory confirmation of the mechanism of the decay of  $I(5^2P_{1/2})$  described by the rate equation given here. Any possible contribution from collisionally induced emission could not readily be detected with the present experimental arrangement. This effect would be observed primarily in the magnitude of the intercept for the semilogarithmic plot of the emission intensity against time; the main contribution from such an effect to the slope would be included in the overall value of  $k$ . The plots of  $k$  against  $1/p$  were made for mixtures using different pressures of trifluoroiodomethane (0.5 mm, 2.0 mm and 10 mm (441 J)). The value for  $k_{CF,I}$  taken from the intercept for  $p_{CF,I} = 10$  mm of mercury, and attributed solely to collisional deactivation of  $I(5^2P_{1/2})$  by this molecule as the contribution by  $A_{nm}$  is relatively small, was found to be  $3.5 \pm 0.6 \times 10^{-16}$  c.c./molecule/sec, in accordance with a previous estimate of this quantity from vacuum ultraviolet measurements<sup>2,4</sup>. There was limited reproducibility in the slopes  $\beta'$  which should be equal, and the error in estimating  $A_{nm}$  from the intercepts ( $1/p = 0$ ) is large as the spontaneous emission coefficient is the small remainder of the sum of faster kinetic processes. The magnitude of  $\beta'$  combined with the solution of the diffusion equation for a cylinder<sup>9</sup> leads to a value for the diffusion

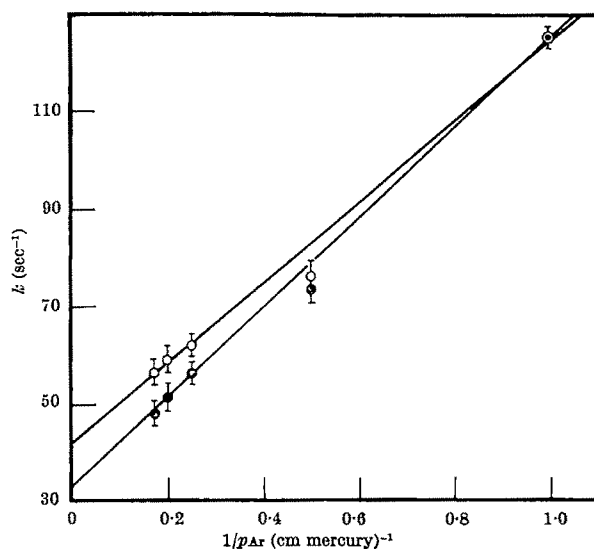


Fig. 2. First order decay coefficient ( $k$ ) for the emission from  $I(5^2P_{1/2})$  as a function of pressure of argon.  $p_{CF,I}$ , 1.0 mm; energy, 1,767 joules. O,  $k$  observed; ●,  $k$  corrected for quenching by 0.4 p.p.m. of oxygen in argon after ref. 4.

coefficient of  $I(5^2P_{1/2})$  in 1 atm. of argon of  $0.41 \pm 0.05$  cm<sup>2</sup> sec<sup>-1</sup>, which is in reasonable agreement with estimates made by Donovan and Husain<sup>2-4</sup>, and is some four times faster than the diffusion of xenon in argon, calculated on the basis of the first approximation Chapman-Enskog equation<sup>10</sup>. The value of  $A_{nm}$  was found to be  $22 \pm 6$ /sec, which can be compared with Garstang's<sup>8</sup> calculated value for this quantity of 7.8/sec. Investigation of collisional quenching has been carried out by monitoring the spontaneous emission in the presence of various added gases, and this work will be presented in a later publication.

Polanyi *et al.*<sup>11</sup> have investigated the steady spontaneous emission from  $I(5^2P_{1/2})$  in the classical photolysis of hydrogen iodide. Pimentel and his co-workers<sup>12,13</sup> have studied the stimulated emission from  $I(5^2P_{1/2})$  in the flash photolysis of a number of iodides.

We thank Professor E. A. Abrahamson for laboratory facilities and many stimulating discussions. One of us (J. R. W.) wishes to thank the U.S. National Science Foundation for a graduate traineeship during the tenure of which this work was carried out, and the other (D. H.) the United States-United Kingdom Educational Commission for a Fulbright Travel Award. This work was supported by a contract of the United States Atomic Energy Commission.

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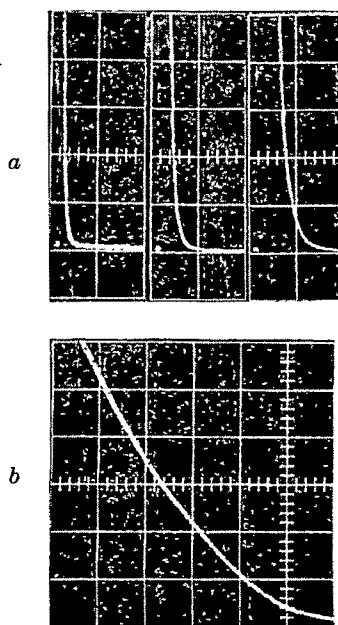


Fig. 1. Decay of the emission from  $I(5^2P_{1/2})$  in argon. a, Time response of apparatus. Photoflash on empty reaction vessel. Sensitivity 10 mV/d. Time scales left to right 2 msec/d, 1 msec/d and 0.5 msec/d. Energy, 1,767 joules. b, Emission from  $I(5^2P_{1/2})$ ;  $p_{CF,I}$ , 1.0 mm;  $p_{Ar}$ , 20 mm; energy, 1,767 joules; sensitivity 2 mV/d, time scale 5 msec/d.

## PHYSIOLOGY

## Motoneurone Depression by Norepinephrine

Dahlström and Fuxe have shown histochemically that norepinephrine (NE) is localized in a pathway from the brain stem to the spinal cord with terminals predominantly in the dorsal and ventral horns<sup>1</sup>. Biochemical investigations demonstrating the release of NE from the spinal cord by stimulation of descending tracts<sup>2</sup> and almost complete disappearance of NE below a spinal transection<sup>3</sup> provide additional evidence for a descending NE pathway. A pharmacological investigation of the sensitivity of neurones in the spinal cord had previously reported<sup>4</sup> that interneurons were not sensitive to the electrophoretic administration of NE in barbiturate anaesthetized cats (the effect of NE on motoneurons was apparently not investigated). Because it is known that barbiturate anaesthesia can reduce or abolish pharmacological responses in the spinal cord<sup>5,6</sup>, we reinvestigated this problem in decerebrated cats and a few cats anaesthetized with ether and found that Renshaw cells and a number of interneurons are sensitive to NE (refs. 7 and 8). Similar findings have now been reported by other investigators<sup>9,10</sup>. During the course of these investigations, the extracellular action potentials of single motoneurons were only occasionally recorded; the investigation of these cells revealed that NE depressed the firing of some motoneurons.

The experiments were performed in the sixth lumbar to first sacral segments of the cat spinal cord. Five-barrel glass micropipette electrodes with tip diameters of 2–8  $\mu$  were used to record extracellular neuronal action potentials and to administer drugs by electrophoresis at the site of recording. Details of the experimental procedures have already been reported<sup>8</sup>. Motoneurons were identified by antidromic invasion following stimulation of the central end of transected ventral roots. Because  $\alpha$  motoneurons did not fire spontaneously under these experimental conditions, the effect of NE was tested on antidromic action potentials, neuronal firing induced by an excitant amino-acid, and injury discharge. In eleven of twenty-five  $\alpha$  motoneurons thus investigated, neuronal firing was depressed by electrophoretically administered NE.

In contrast with our findings on Renshaw cells<sup>7</sup>, which were more often depressed but occasionally excited by NE, none of the  $\alpha$  motoneurons so far investigated showed any facilitation of firing during the administration of NE.

When the effect of NE was investigated on extracellularly recorded antidromic spikes, it was found that in some motoneurons the *A-B* inflexion<sup>11</sup> on the rising phase of the spike became more prominent. After the increased *A-B* break, a blockade of the antidromic invasion of the *B* spike was observed, as illustrated in the second column of Fig. 1, which indicates that NE decreased the excitability of the somedendritic membrane<sup>12</sup>. Consistent *A-B* invasion returned within 5–30 sec after the administration of NE, but recovery was accompanied by a prominent *A-B* break (third column of Fig. 1) before the spike returned to the control configuration. The bottom two records in Fig. 1 illustrate controls and these show that current of the same magnitude applied through a sodium chloride barrel of the same micropipette has no appreciable effect on the antidromic spike.

When smaller (negative) antidromic spikes were recorded, we investigated the effect of NE on the tonic firing induced by the iontophoretic ejection of the excitant amino-acid—DL homocysteic acid—from another barrel of the microelectrode. In some cases, the electrophoretic administration of NE reduced the frequency of the firing induced by the amino-acid. The upper portion of Fig. 2 shows the antidromic action potential of a single motoneuron on the negative field potential generated by groups of these cells; the stimulus was adjusted to threshold for the neurone so that the all-or-none spike can be seen. The lower portion of Fig. 2 shows a polygraph record of the amino-acid induced firing of the same motoneuron and the depression of this firing produced by the electrophoretic administration of NE with 75 n.amp of current. The same magnitude of current through a sodium chloride barrel of the electrode had no effect. Norepinephrine also depressed the injury discharge of some motoneurons, but because of the short duration of this discharge, there was never sufficient time after the pharmacological test to test for the possibility of electrotonic effects of current.

In addition, acetylcholine and serotonin were administered to two of the motoneurons sensitive to NE, with

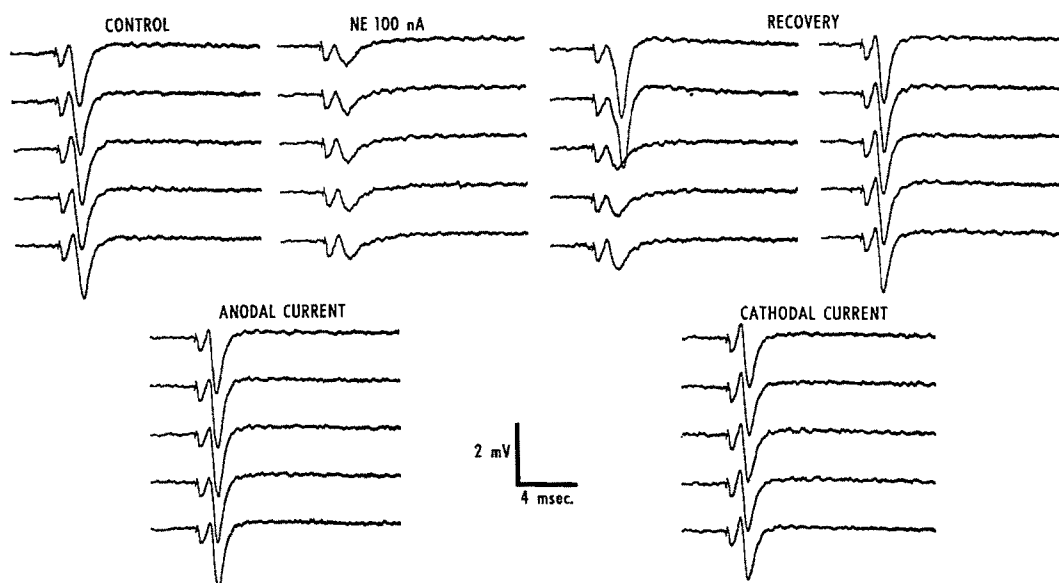


Fig. 1. Effects of electrophoretically administered norepinephrine (NE) on the extracellularly recorded antidromic spike of an  $\alpha$  motoneuron. In each column, successive oscilloscope sweeps progress from bottom to top; each sweep is one second apart. Negativity of the microelectrode gives an upward deflexion. Top. The sweeps in the second column were recorded during the administration of NE ejected by a cationic current of 100 n.amp, the record beginning 12 sec after the onset of the current. Sweeps in the third and fourth columns begin 26 sec and 45 sec, respectively, after the end of the electrophoretic current. Bottom. The effect of 100 n.amp of anodal and cathodal current applied through a sodium chloride barrel of the same micropipette.

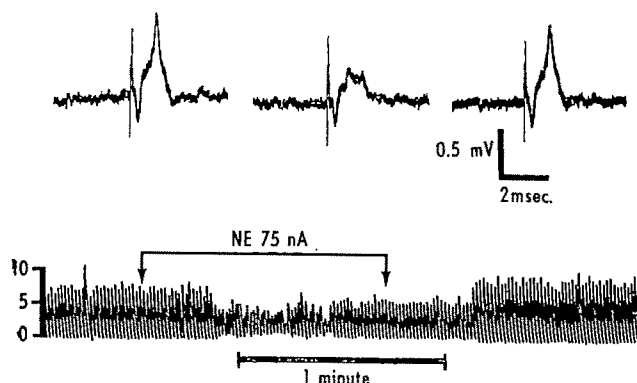


Fig. 2. *Top*. Antidromic action potential of a motoneurone recorded extracellularly; the stimulus was adjusted to threshold so that all-or-none spikes can be seen. *Bottom*. Polygraph record of the tonic firing of the same motoneurone induced by the electrophoretic ejection of DL-homocysteic acid from one barrel of the microelectrode. Norepinephrine was administered for the period between the arrows by an electrophoretic current of 75 namp. Calibration scale at the left is in impulses per sec.

similar magnitudes of electrophoretic current, but no effect was observed; however, this sample is too small to draw any conclusions regarding the possibility of sensitivity of motoneurons to these substances.

Dahlström and Fuxe<sup>1</sup> used retrograde degeneration to establish whether the cell bodies of  $\alpha$  motoneurons receive NE terminals; they found that NE terminals form close contacts with the cell bodies and processes of some  $\alpha$  motoneurons. Although several criteria must be satisfied for transmitter identification<sup>13</sup>, the presence of NE terminals on motoneurons and the demonstration of the sensitivity of motoneurons to NE suggest that NE may function as a transmitter for at least some  $\alpha$  motoneurons.

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### Motor Effect of Adrenaline on the Rat Uterus

ADRENALINE has an inhibitory effect on the rat uterus in all stages of the reproductive cycle, and this suggests that the rat uterus contains only  $\beta$  adrenergic receptors. It was of interest, therefore, to test whether adrenaline reversal could be obtained in this organ, because such a reversal would imply the presence of  $\alpha$  adrenergic receptors.

The phenomenon was investigated in rats treated with stilboestrol, and in ovariectomized rats treated with progesterone. Mature female Wistar rats weighing about 200 g were used. Stilboestrol was given subcutaneously in a single dose of 0.5 mg dissolved in arachis oil, and the animals were used 40 h later. Ovariectomy was

carried out by the dorsal route under ether anaesthesia; 0.5 mg of stilboestrol was then given, and for the following 5 days progesterone was given intraperitoneally dissolved in arachis oil in a dose of 2.5 mg/kg, and the animals were used on the day after the end of treatment.

The animals were stunned by a blow on the head and bled out; the uterus was removed and suspended in a 25 ml. organ bath containing mammalian Ringer-Locke solution at 36.5° C, and through which air was passed in a continuous stream. Readings were made on a smoked drum with an isotonic lever. Normal contractions appeared within 5 min, and the preparation was left to equilibrate for a further period of 30–60 min.

Adrenaline reversal was investigated under the influence of isoprenaline, in accordance with the method used by Butterworth<sup>1</sup> in studies of the cat blood pressure. Isoprenaline in a concentration of 0.1  $\mu$ g/ml. caused an immediate and total inhibition of the intrinsic rhythm for a period of 7–14 min. Thereafter the uterus contracted irregularly at about 10–30 per cent of the original extent. The further addition of isoprenaline at this stage did not inhibit these contractions. A typical experiment showing this escape from the action of isoprenaline is shown in Fig. 1. Escape was obtained in uteri from rats treated with stilboestrol, but in rats treated with progesterone inhibition remained complete for periods exceeding 60 min. The escape did not result from the breakdown of isoprenaline into a substance with motor activity because bath fluid from a preparation where escape had occurred caused maximum inhibition of a fresh preparation; nor could it be ascribed to changes in pH.

During the inhibition and escape from the action of isoprenaline the effect of adrenaline in a concentration of 0.1  $\mu$ g/ml. showed that during most of the quiescent phase adrenaline was without effect. The addition of adrenaline towards the end of the quiescent phase, or during the recovery period, produced a motor response which increased in magnitude as the addition of adrenaline was delayed, and the maximum contraction amounted to 80 per cent of the original intrinsic rhythm when the adrenaline was added after 50 min. The results are shown in Fig. 2, curve A.

In rats treated with progesterone no response was obtained to the addition of adrenaline, even when it was added 60 min after the onset of inhibition. The results are shown in Fig. 2, curve B, and are represented by a straight line along the axis of the abscissa.

These results therefore show that adrenaline reversal can be obtained under the influence of isoprenaline in the

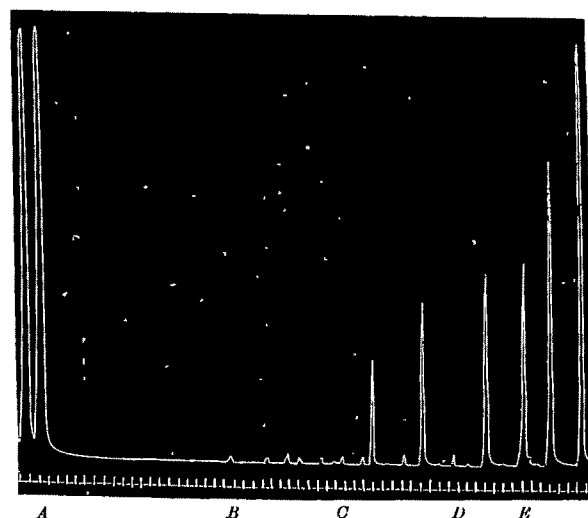


Fig. 1. Uterus from a rat treated with stilboestrol. A, Isoprenaline added to a final concentration of 0.1  $\mu$ g/ml.; B, onset of escape; C and D, repetition of addition of isoprenaline; E, wash. Time marker, 30 sec.

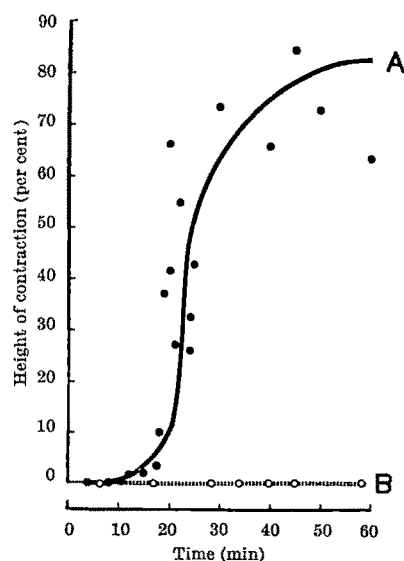


Fig. 2. Motor response of rat uterus to adrenaline under the influence of isoprenaline. *A*, Animals treated with stilboestrol; *B*, animals ovariectomized and treated with progesterone. Ordinate, height of contraction produced by adrenaline as a percentage of the height of the original intrinsic contraction; abscissa, time in minutes after onset of isoprenaline inhibition. Each point represents a result obtained with one horn of a uterus.

rat uterus in animals treated with stilboestrol, and not in animals treated with progesterone.

According to the receptor hypothesis, these results mean that  $\alpha$  receptors are present in the rat uterus in animals treated with stilboestrol, but are absent or masked in animals treated with progesterone. If the  $\alpha$  receptors are anatomical entities, it seems very improbable that they could appear and disappear in this manner; if receptors are closely related to enzymes, however, then treatment with stilboestrol for 40 h would afford sufficient time for such an enzyme to be induced. In this case the enzyme would probably not be induced in the presence of progesterone.

The form of curve *A* in Fig. 2 may be explained by postulating that a substance with motor activity accumulates in the uterus during the period of isoprenaline inhibition, and is liberated by the addition of adrenaline; the amount liberated, and therefore the height of contraction, would be related to the time of addition of adrenaline during isoprenaline contact.

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### Islet Formation in the Cortex of the Human Thymus

MORPHOLOGIC units consisting of a central macrophage-like cell surrounded by several lymphocytic or plasma cells, and termed islets, are seen in lymph nodes<sup>1</sup>, and in cultures of lymph node cells<sup>2</sup>, blood lymphocytes<sup>3,4</sup> and thymic cells<sup>5</sup>. Islets formed by lymphocytes and (or) eosinophilic and neutrophilic leucocytes are observed surrounding degenerating mononuclear cells containing antigens<sup>6</sup>.

It is the purpose of this paper to emphasize that a phenomenon similar to islet formation is frequently seen in the cortex of thymuses obtained at autopsies of infants and young children. In such thymuses, there is an obvious, though not very marked, destruction of cells in the thymic cortex. Pycnotic cells and small haematoxylin-positive particles of chromatin are seen scattered in the cortex.

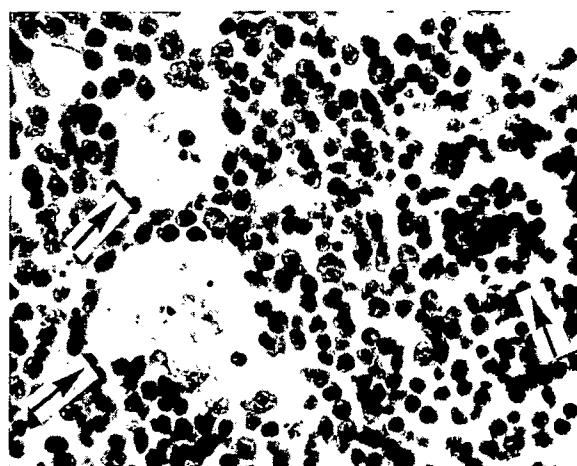


Fig. 1. Islets in different phases of development in the cortex of a human thymus. Haematoxylin and eosin section,  $\times 160$ . On the right is an islet formed by several deeply stained small and medium-sized lymphocytes. On the left are two islets in an early stage of development. In the upper islet there is a large cell with a pale nucleus and abundant cytoplasm containing a small lymphocyte and two haematoxylin-positive nuclear particles. The lower islet shows lymphocytes just beginning to invade the islet.

Besides these there are, in most of the cases, peculiar oval areas containing homogenous masses of dead cells and often a large cell with a large pale nucleus and abundant cytoplasm (Fig. 1). These areas are infiltrated by lymphocytes resulting in the formation of islets (Fig. 1). The number of the cells in these islets varies greatly. Morphologically, the cells invading the islets are similar to small and medium-sized lymphocytes (thymocytes). Occasionally these cells stain with haematoxylin more intensely than the other lymphocytes. Some islets seem to be demarcated from the surrounding tissue by a thin membrane.

The hypothesis that RNA (ref. 7), breakdown products of DNA or other agents mediating information are obtained by the lymphocytes in the area of islets seems attractive, but definite evidence for such a theory is lacking. It is possible, at least in the thymus, that the lymphocytes which infiltrate the islets are destroyed; the unknown fate of thymic lymphocytes under stress conditions might so be explained. Whatever the significance of the islets is, the observations presented seem to indicate that the thymus is not principally different from the other lymphoid organs in the capacity to form islets.

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### Mechanisms of the Crayfish Tail Flick

THE escape reaction of the crayfish consists of a rapid backward swimming movement brought about by powerful flexion of the abdomen. In the course of a primarily anatomical re-investigation (as yet unpublished) of the musculature responsible for this flexion in the crayfish *Procambarus clarkii*, it was observed that some 40 per cent of the cross-sectional area of the main flexor musculature is contributed by three muscles which both arise and

insert largely or entirely dorsal to the inter-segmental hinge. These are the central, transverse and dorso-lateral muscles, in the terminology of Daniel<sup>1</sup>. The expected "direct" action of such muscles would be extension rather than flexion; that so large a proportion of the flexor musculature is apparently "indirect" in its action was considered to be of a sufficiently general physiological interest to be presented separately from the main body of the work.

The anatomy of the main flexor muscles is shown diagrammatically in Fig. 1; the superficial flexors have been omitted from this discussion because they have been shown<sup>2</sup> to be purely tonic in action and thus not involved in the mechanism of the very rapid tail flick. The paired central muscles (*C.*) arise on the ventral surface of the transverse membrane (*Tr. mb.*) which crosses the segment at this level; they then twist ventro-laterally through the dorsally directed loop formed by the muscles in the anterior oblique "series" (*A i, ii* and *iii*; see also Fig. 3) and divide into two heads. Of these, the larger axial head (*C. ax.*) inserts on the underside of the next posterior transverse membrane, while the smaller transverse head (*C. trans.*) forms a mid-line inscription with the fibres of its opposite number on the other side of the body. The transverse muscles (*T.*) arise laterally on the under side of the transverse membrane and insert, with the transverse head of the central muscle, at the mid-line inscription (shown cross-hatched in Figs. 1A and 2A) which appears to be a ventral extension of the medial part of the transverse membrane. The three heads of the dorso-lateral muscle (*D-L*, Fig. 1B) also run transversely but arise and insert on the dorsal side of the transverse membrane. Each anterior oblique "series" was considered by Schmidt<sup>3</sup> to be a single muscle extending over three segments; however, the existence of clear inscriptions within each "series" shows that each consists of three separate segmental muscles connected end to end. Within any one segment three of these muscles (*A i, ii* and *iii*) are found, corresponding to the component muscles of three consecutive "series". In this figure only one "series" is shown. While the inscription between *A i* and *A ii* is not laterally connected to any skeletal part, the other two inscriptions appear to provide functional attachments for the *A* "series", the inscription between *A ii* and *A iii* being firmly attached ventrally and the

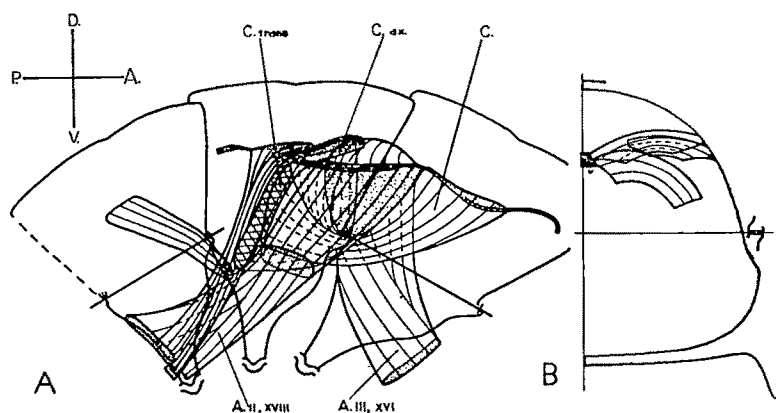


Fig. 2. The crayfish abdominal flexor musculature in the flexed position. A, Medial view of the second, third and fourth abdominal segments. B, Anterior view of the third abdominal segment.

more dorsal inscription between *A iii* and *A i* being continuous with the transverse membrane. The posterior oblique muscles (*P.*) run ventrally from the transverse membrane to insert with the *A ii* muscles at their inscription with the *A iii* muscles of the next posterior segment. The auxiliary muscles (*Aux.*) arise immediately lateral to the *A iii* muscles of the same segment and, at the same inscription, they insert on the lateral tergal wall. It should be noted that in this complex muscle system only this small auxiliary muscle is wholly inserted directly on to the skeleton.

The principal functional attachments of the main flexor musculature are thus: *a*, the strong ventral inscriptions, which are firmly attached to a thickened region of the ventral surface membrane just posterior to each sternal bar (*St.*); *b*, the lateral attachments of the transverse membranes to the dorso-lateral tergal walls (*Tr. mb. l.*).

When this musculature is examined in the fully flexed position (see Fig. 2), certain interesting points emerge which help to clarify the function of the indirect muscles. First, the loop formed by the central muscle remains ventral to the hinge point even in full flexion. While this criterion is satisfied, contraction of the central muscle can be expected to produce a flexor force by its action around the "pulley-wheel" provided by the contracting muscles of the anterior oblique series (see Fig. 3). Second, the medial part of the transverse membrane is seen to have shifted dorsally relative to the position of the inter-segmental hinge. This initially surprising result of flexor action can be more readily understood when it is realized that the raising of the transverse membrane would be the sole function of the rather powerful dorso-lateral and transverse muscles if these alone were stimulated. The importance of this action in terms of the tail flick mechanism appears to lie in its effect on the changes in length of the direct muscles. Calculations based on measurements obtained from camera lucida drawings suggest that the observed dorsalward movement of the transverse membrane effectively halves the change in length of these muscles required to produce flexion. Because the mechanical situation is not greatly altered, a virtual doubling of the tail flick velocity might be expected.

It should be emphasized that this investigation has been based on the steady-state positions of full flexion and extension. The dynamic properties of this system will depend on the details of the timing of impulse arrival and on the rates of rise of tension in different muscles; the overall properties of the single functional unit

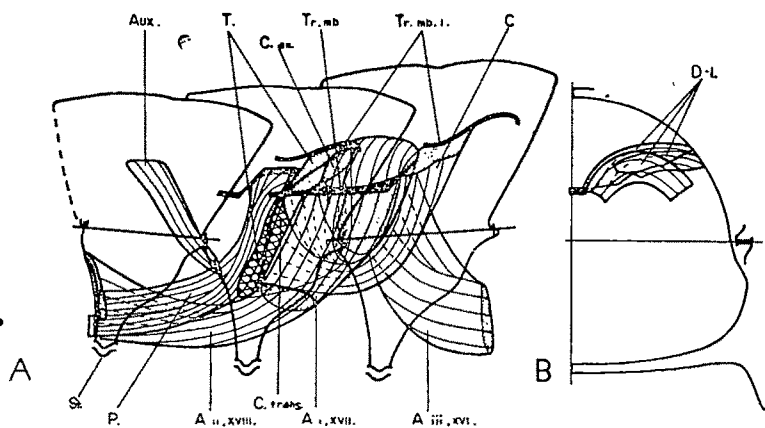


Fig. 1. Internal view of the crayfish abdominal flexor musculature in the extended position. A diagrammatic reconstruction prepared from camera lucida drawings. A, Medial view of the second, third, and fourth abdominal segments. B, Anterior view of the third abdominal segment (only the medial part of the transverse membrane is shown in this figure). See text for explanation of abbreviations. The lines drawn across the second and fourth segments are drawn through the intersegmental hinges, the positions of the hinges being marked by the short vertical bars. The parts of the transverse membrane and all inscriptions are shown stippled, with the exception that the mid-line inscription between the lateral halves of the transverse-running muscles is cross-hatched.



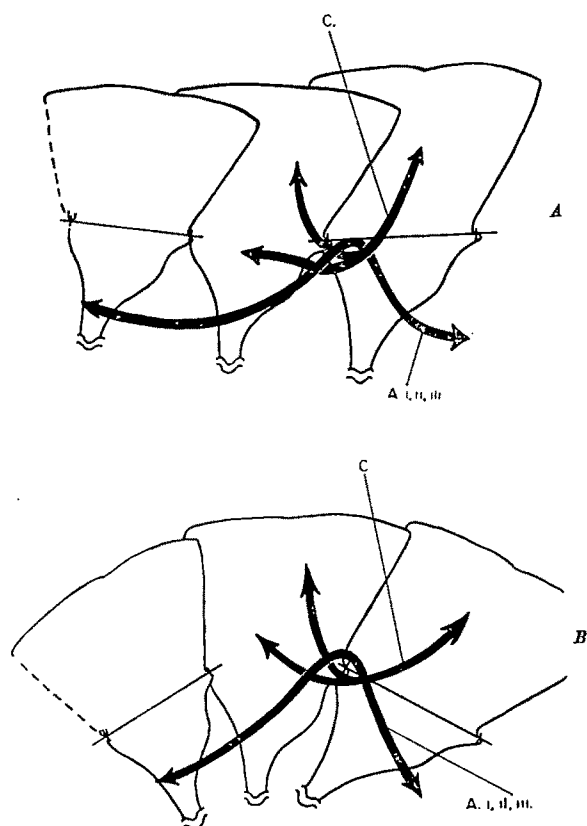


Fig. 3. Diagrammatic representation of the "pulley-action" occurring in the flexor musculature of the crayfish abdomen. Dimensions used were derived from camera lucida drawings. *A*, Second, third and fourth abdominal segments in the extended position. *B*, The same segments in the fully flexed position.

formed by the *A i* and *A ii* muscles connected in series are still an interesting problem, particularly as these muscles are known to be innervated from different segments (unpublished work).

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### Calcium Action at an Inhibitory Synapse

THE transmitter substance of the crossed olivo-cochlear fibres has not been identified<sup>1</sup>. The previously known actions of these inhibitory fibres are blocked by strychnine<sup>2</sup> and it may also be of significance that the fibres and their endings apparently contain acetylcholinesterase<sup>3</sup>.

The release of acetylcholine at the motor nerve terminals in the frog and the rat depends directly on the presence of calcium<sup>4</sup> and it has been reported recently that perfusion of the scala tympani in the cochlea of the guinea-pig with solutions which contained calcium in increased concentrations caused increased changes of sound evoked potentials. Thus cochlear microphonics and negative summing potentials were increased while the whole nerve action potential was decreased<sup>5</sup>.

The present investigation is concerned with the action of calcium and strychnine on the slow negative potential that

is recorded from the endolymph of the cochlea when the crossed olivo-cochlear fibres are stimulated repetitively. Inhibition of activity in the cochlear nerve by the crossed olivo-cochlear fibres is considered to be caused by the direct action of these fibres on outer hair cells, and the slow potential of the present investigation is considered to represent the post-synaptic, inhibitory activity of outer hair cells, generated by efferent endings<sup>6</sup>.

We used ten cats which were anaesthetized with pentobarbitone sodium and paralysed with gallamine triethiodide. The crossed olivo-cochlear fibres were stimulated electrically in the floor of the fourth ventricle. Cochlear potentials evoked by such stimulation, and by sound presented to the ear, were recorded from the scala media, with microelectrodes of resistance 1-5 MΩ filled with 3 molar potassium chloride solution. The membrane of the round window was removed. Perilymph was sucked away from the scala tympani to expose the basilar membrane so that the microelectrode could be pushed through the cochlear partition into the endolymphatic space under relatively good visual control. In control experiments, cochlear potentials were recorded to ensure that slight variations of the level of the perilymph in the scala tympani, or substitution of part of the perilymph with artificial perilymph, did not influence the results. The following solutions were used as artificial perilymph. Solution *A*: sodium chloride 125 mmoles, sodium bicarbonate 25 mmoles, potassium chloride 3.5 mmoles, calcium chloride 1.3 mmoles, magnesium chloride 1.14 mmoles, sodium dihydrogen orthophosphate 0.51 mmole, urea 130 mg/l., glucose 610 mg/l.<sup>7</sup>. Solution *B*: sodium chloride 140 mmoles, sodium bicarbonate 12.5 mmoles, sodium dihydrogen orthophosphate nil and the other components as in Solution *A*. Strychnine hydrochloride was added to Solution *A* or Solution *B* to a concentration of 0.012 mmoles, that is, strychnine hydrochloride  $5 \times 10^{-6}$ . The calcium concentration was increased by addition of calcium chloride, with or without deletion of an equivalent amount of sodium chloride. The solutions were heated to 38°-40° and equilibrated with 'Carbogen' (5 per cent CO<sub>2</sub>, 95 per cent O<sub>2</sub>) immediately before their application to the scala tympani with a thin glass tube.

In five animals strychnine was applied to the scala tympani. In one of these, strychnine was used twice before the application of a solution with an increased content of calcium, in one animal once before and once after such an application, and in three animals after calcium only. The amplitude of the slow potential evoked by efferent stimulation decreased by at least 80 per cent after the application of only strychnine and by at least 65 per cent when strychnine followed on calcium with an interval of 30 min or more. Strychnine also lengthened the latency of the slow potential. These effects of strychnine began to appear within 20 sec in two experiments and were always fully developed within 2-3 min. In two experiments, in which artificial perilymph was repeatedly applied to the scala tympani after strychnine, the effects of strychnine began to reverse in 21-24 min and in one of these experiments the slow potential had regained 90 per cent of its original amplitude in 40 min. Thus, because strychnine in low concentrations in the scala tympani partly blocks the generation of the slow potential under investigation, this potential is caused by efferent synaptic activity in the organ of Corti, by the same order of probability as are the cochlear actions previously ascribed to such activity<sup>2</sup>.

Results from a typical experiment with calcium are illustrated in Fig. 1. The potentials under investigation were reasonably stable (Fig. 1, *A*, *B* and *D*) and were not altered by the substitution of perilymph in the scala tympani by Solution *A* in which 20 mmoles of sodium chloride had been replaced by 40 mmoles of sucrose (Fig. 1, *B*, *C*). When 20 mmoles of sodium chloride had been replaced by 10 mmoles of calcium chloride, however, the slow potential evoked by efferent stimulation was

changed (Fig. 1, *D, E*). Both the latency and the time of rise were shortened, and the falling phase prolonged. The corresponding changes produced in another animal when 5 mmoles of calcium chloride had been added to Solution *B* in exchange for perilymph are illustrated in Fig. 2. The effects of calcium began to appear in one experiment within 10 sec and were always fully developed within 1–4 min. The effects began to reverse spontaneously in 17 to 25 min in some experiments; in others the effects were still fully maintained 25–30 min after the application of the solution which contained high calcium.

In all animals, application to the scala tympani of Solution *A*, or *B*, with an increased content of calcium, produced shortening of the latency, shortening of the rise time required to reach a maximum, and a prolongation of the falling phase of the slow potential from crossed efferent stimulation. Because such changes have not occurred spontaneously in any of the animals in which potentials evoked by efferent stimulation have been investigated<sup>4</sup>, they are most likely the consequence of an increase of calcium ion concentration within the organ of Corti.

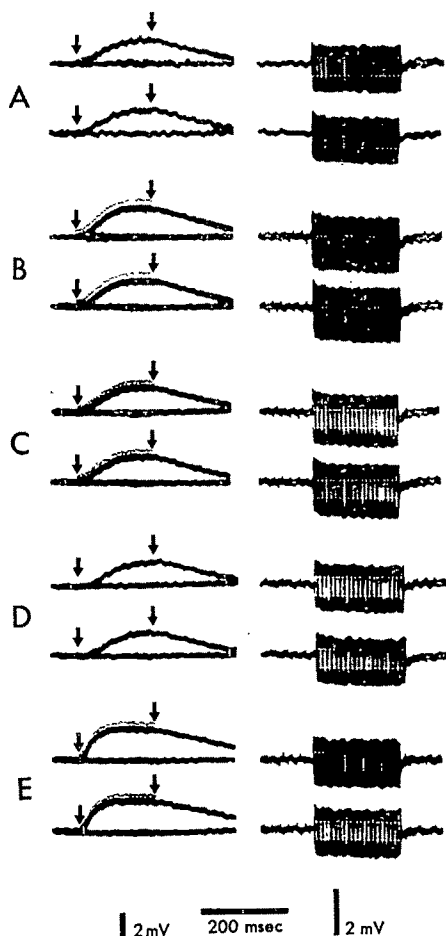


Fig. 1. Column 1 shows the slow negative potentials evoked by repetitive electrical stimulation of the crossed olivo-cochlear fibres in the brain stem (duration of stimulation is indicated by arrows). Column 2 shows the cochlear microphonic (CM) and the summating potential (SP) evoked by sound stimulation. The SP discussed here corresponds to the initial asymmetry of the full size CM relative to the baseline. All the records were taken with the microelectrode in one position in the endolymph of the cochlea. *A*, Control, perilymph in scala tympani. *B*, Control, 60 min after *A*. *C*, Control, 10 min after *B*. The perilymph of the scala tympani had been partly replaced by artificial perilymph (Solution *A*, minus sodium chloride 20 mmoles, plus sucrose 40 mmoles). Pictures were taken 3 min after the replacement. Note that the responses to both stimuli were unchanged. *D*, Control, 15 min after *C*. *E*, 15 min after *D*. The perilymph had been partly replaced by a test solution with an increased content of calcium chloride (Solution *A*, minus sodium chloride 20 mmoles, plus calcium chloride 10 mmoles). Pictures were taken 3 min after the replacement. The time calibration is common to all the records. Each voltage calibration is common to all records within each of the columns. The time constant of the recording system was 100 msec. Negativity is upwards.

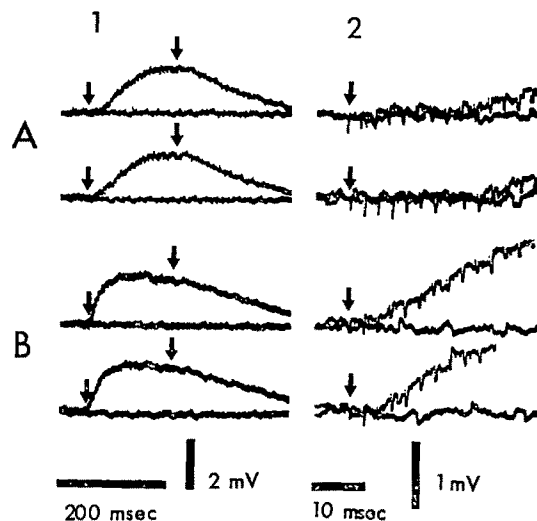


Fig. 2. Column 1 shows the full time course of slow negative potentials from efferent stimulation (duration of stimulation is indicated by arrows). Column 2 shows the initial course of such potentials, displayed with a fast sweep (start of stimulation indicated by arrow). All the records were taken with the microelectrode in one position in the endolymph of the cochlea. *A*, Control, perilymph in scala tympani. *B*, 7–8 min later than *A*. The perilymph had been partly replaced by a test solution with an increased content of calcium chloride (Solution *B* plus calcium chloride 5 mmoles). Pictures taken 2.5 min (*B2*) and 3 min (*B1*) after the replacement. See text for results. Note that time calibration and voltage calibration are different for the two columns. The time constant of the recording system was 100 msec. Negativity is upward.

There was no consistent change of the cochlear microphonics with increase of the calcium content in the scala tympani. The summing potentials, however, showed a small change towards the negative side in seven out of eight cats in which the summing potential was observed: these changes were generally about twice the size of that in Fig. 1, *D, E*. Such changes were as a rule not found when artificial perilymph was applied to the scala tympani, and were probably also caused by the increased content of calcium. If so, calcium may have acted directly on the hair cells generating the summing potential, or indirectly through efferent endings impinging on these hair cells.

These new findings lead to the conclusion that calcium participates in the synaptic mechanism of the crossed olivo-cochlear inhibitory efferents and strongly suggest that release of transmitter substance at this synapse, as at the neuro-muscular junction<sup>4</sup>, is dependent upon calcium.

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### Induction of Tumours in Denervated Skin

It has been shown that the number of fine axons in close relationship with the epidermis of human skin increases *pari passu* with a rise in the mitotic index in the germinal layer<sup>1,2</sup>. These observations prompted us to compare the rate of tumour induction by chemical carcinogenic agents in comparable zones of healthy as well as denervated

skin in the same animal, because it has been shown that in completely denervated skin the mitotic index falls<sup>3</sup>.

The right ears of ten Dutch male rabbits between 5 and 6 months old were completely denervated by methods suggested previously<sup>4-7</sup>, under 'Nembutal' and ether anaesthesia. In four animals both the inner and outer surfaces of both ears were evenly painted with 2 per cent methylcholanthrene dissolved in benzene (2) and liquid paraffin which covered comparable areas of skin previously outlined by indian ink. In four more animals the agent used for painting was 2 per cent 9,10-dimethyl-1,2-benzanthracene (DMBA) dissolved in benzene. In each case a standard volume of solution was placed in the centre of the area outlined, using a Pasteur pipette, with which it was evenly spread and allowed to dry. This was done twice daily for 2 weeks and twice weekly for the next 4 or 5 months. In two animals in each group the hairs within the treated area were removed by shaving and in the others the hairs were plucked out with forceps.

The results are set out in Tables 1, 2 and 3.

Table 1. TIME IN DAYS FROM FIRST APPLICATION OF CARCINOGEN TO DEVELOPMENT OF TUMOUR JUST VISIBLE TO NAKED EYE

Rabbit No.	Hairs	Right (treated) ear		Left (untreated) ear	
		Outer surface	Inner surface	Outer surface	Inner surface
Methylcholanthrene					
1	Shaved	Nil	119	150	Nil
2	Pulled out	150	Nil	Nil	Nil
3	Pulled out	115	129	Nil	122
4	Shaved	129	Nil	143	Nil
	Average	131	124	146	122
DMBA (see text)					
5	Shaved	57	57	97	90
6	Pulled out	76	66	82	66
7	Pulled out	46	24	85	73
8	Shaved	18	24	24	24
	Average	49	43	72	63
	Average whole ear	46		67	

Table 2. TIME IN DAYS BETWEEN THE FIRST APPEARANCE OF A TUMOUR AND ITS GROWTH TO A DIAMETER OF 0.5 MM

Rabbit No.	Right (treated) ear		Left (untreated) ear	
	Outer surface	Inner surface	Outer surface	Inner surface
Methylcholanthrene				
1	Nil	10	Nil	Nil
2	Nil	Nil	Nil	Nil
3	7	10	Nil	31
4	14	7	Nil	31
DMBA (see text)				
5	14	10	34	41
6	17	10	34	34
7	21	7	10	28
8	7	10	14	17

Table 3. TIME IN DAYS FROM APPLICATION OF CARCINOGEN TO ULCERATION OF TUMOUR

Rabbit No.	Right (treated) ear		Left (untreated) ear	
	Outer surface	Inner surface	Outer surface	Inner surface
Methylcholanthrene				
1	Nil	7	Nil	Nil
2	Nil	Nil	Nil	Nil
3	14	Nil	Nil	Nil
4	Nil	Nil	Nil	Nil
DMBA (see text)				
5	Nil	14	Nil	Nil
6	10	37	Nil	Nil
7	31	Nil	31	Nil
8	10	7	37	Nil

From Table 1 it can be seen that (a) 2 per cent DMBA is a more effective and consistent tumour inducing agent than 2 per cent methylcholanthrene in experiments of this kind, a fact which has already been established; (b) both carcinogenic agents induced tumours more readily in the denervated than in the control ears, and in any given animal the tumours appeared earlier on the denervated side; (c) on the control side tumours appeared more quickly on the inner than on the outer aspects of the ears; (d) there seems to be no obvious relationship between the rate of tumour formation and hair growth induced by plucking in this short series of animals.

Table 2 shows that (a) tumour growth was faster on the right (denervated) ear than on the left ear after the use of DMBA. The same was true in the case of methylcholanthrene, and although the number of tumours grown was few the trend was in the same direction; (b) tumour growth was slower on skin in the inner surface of the ear, and this was the case in both control and denervated ears.

Table 3 shows that ulceration occurred more often in denervated ears. At the start, the tumours which were induced looked like keratoacanthomas; later some of them ulcerated (Fig. 1), but histologically they could not be differentiated from squamous cell carcinomas either before or after ulceration (Fig. 2).

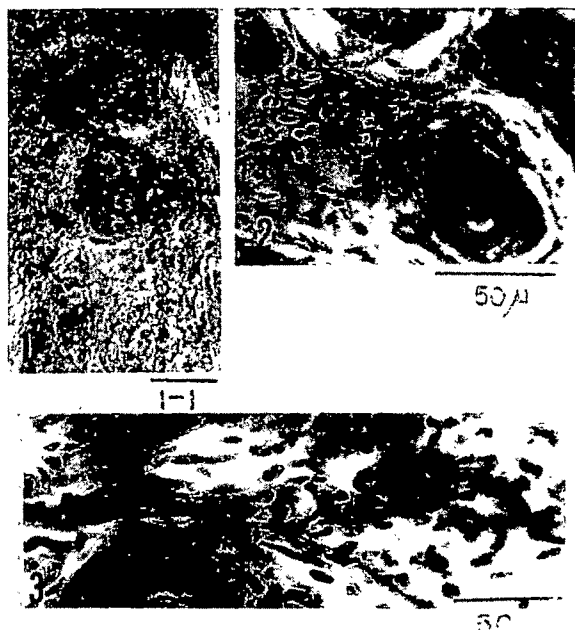


Fig. 1. Ulcerated tumour on inner surface of denervated rabbit ear induced by applying DMBA for 5 weeks.

Fig. 2. Section (stained with haematoxylin and eosin) which shows non-mature 'Pearl' and atypical cells showing individual keratinization.

Fig. 3. Section (stained with silver) which shows a nerve bundle between masses of tumour cells. Many axons are degenerating and towards the right neural elements have almost disappeared.

In sections stained with silver to display the neural elements<sup>8</sup>, only a few very fine axons were seen in some of the sections from the denervated side, but on the control side numerous fine axons and Schwann cells surrounded the invading cells. Within the tumour nerves were present only in the early stages of its development; later degenerating remnants were seen (Fig. 3) and later still no neural elements at all.

Despite the small number of animals so far examined in detail, some interesting facts have emerged. Tumours are induced more rapidly in completely denervated rabbit ears as compared with their opposite ears which served as controls. Tumours are also induced more rapidly in skin of the inner aspects of control ears. This is also interesting, for skin on the inner surface of the ear is far less densely innervated than skin on the outer surface. Complete denervations of the rabbit ear led to alterations in the blood supply of the skin on both of its surfaces, yet tumours grew faster in the skin of the inner aspects of the denervated ears, which in any case have a poorer blood supply than the outer surfaces. On the control side, however, the opposite was the case. Tumours grew more slowly in the skin of the inner aspects, so that the relevant factor concerned with tumour growth here must be connected

with an interference with the nerve supply and not with the blood supply.

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## Iron Absorption and Retention in Man

Conrad and Crosby<sup>1</sup> have shown that, in normal rats, a proportion of the iron which has been absorbed from the intestinal lumen is retained in the epithelial cells of the intestine, and lost with the faeces in the course of the normal desquamation of these cells. In rats with iron deficiency, scarcely any iron is retained in the intestinal epithelial cells.

Conrad and Crosby believe that a similar mechanism acts in man; they reached this conclusion on the basis of a study in which normal subjects and patients with iron deficiency received an oral test dose of radioactive iron and with carmine red. They found that the excretion of radioactivity in the faeces of normal subjects persisted considerably longer than the excretion of carmine red. In patients with iron deficiency, however, the faecal excretion of radioactivity and that of carmine red lasted about the same length of time. These findings suggested that the conventional clinical methods of measuring iron

absorption from the intestine after a given interval—usually 10–15 days<sup>2</sup>—do not determine iron absorption but iron retention, that is, the quantity of iron absorbed minus the quantity of iron which is excreted in the faeces after absorption.

Our objective was to study the absorption and ultimate retention of iron in man. For this purpose we used a tracer dose of radioactive iron and barium. The barium was added because, in the form of barium sulphate, it is believed not to be absorbed from the intestinal tract and can consequently serve as an inert indicator. Oral administration of barium-131 sulphate and iron-59 sulphate might therefore make it possible to assess the absorption of iron from the intestinal lumen by measuring the proportions of iron-59 and barium-131 in the test dose and the faeces<sup>3</sup>. By determining the total quantity of iron-59 excreted in the faeces it should be possible to determine the ultimate iron retention. The experiments were carried out in three normal healthy subjects with normal haemograms, normal serum iron concentrations and serum iron saturation percentages; two patients with sideropenic anaemia and two with iron overload (patient 6 had familial red cell aplasia with secondary haemochromatosis after some 150 blood transfusions, normochromic macrocytic anaemia, a serum iron concentration of 274 µg/100 ml. and a serum iron saturation percentage of 88; patient 7 had hereditary hypochromic sideroachrestic anaemia with haemosiderosis, a serum iron concentration of 185 µg/100 ml. and a serum iron saturation percentage of 86).

The test dose administered was 15 µc. barium-131 and 170 mg barium sulphate, 5 µc. iron-59 and 1 mg iron in the form of iron sulphate, 10 mg ascorbic acid and 2 g carmine red. The technical details will be published elsewhere<sup>4</sup>. The test results in the aforementioned patients are shown in Tables 1, 2 and 3. The results suggest that

Table 1. FAECAL IRON-59 AND BARIUM-131 AFTER ORAL TEST DOSE OF IRON-59 SULPHATE AND BARIUM-131 SULPHATE IN THREE NORMAL MEN

Days after administration of test dose*	<sup>59</sup> Fe† (%)	1. Normal Control <sup>131</sup> Ba‡ (%)	<sup>59</sup> Fe§ (%)	Absorption (%)¶	<sup>59</sup> Fe† (%)	2. Normal control <sup>131</sup> Ba‡ (%)	<sup>59</sup> Fe§ (%)	Absorption (%)¶	<sup>59</sup> Fe† (%)	3. Normal control <sup>131</sup> Ba‡ (%)	<sup>59</sup> Fe§ (%)	Absorption (%)¶
1	27.3	51.4	0.53	47	32.0	42.6	0.75	25	10.8	13.5	0.80	20
2	24.3	43.5	0.56	—	—	—	—	—	42.8	52.9	0.81	—
3	5.8	6.0	0.97	—	47.5	54.0	0.88	—	22.5	27.0	0.83	—
4	4.5	2.8	1.61	—	—	—	—	—	—	—	—	—
5	4.3	0.4	10.75	—	1.7	0.3	5.67	—	7.1	4.8	1.48	—
6	—	—	—	—	1.0	0	—	—	—	—	—	—
7	1.8	0	—	—	2.8	0	—	—	2.5	0.5	5.0	—
8	—	—	—	—	—	—	—	—	—	—	—	—
9	2.3	0	—	—	—	—	—	—	1.5	0.1	—	—
10	—	—	—	—	1.5	0	—	—	—	—	—	—
11	—	—	—	—	2.6	0	—	—	—	—	—	—
12	2.2	0	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	0.9	0	—	—	0.4	0.1	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—
Total percentage of test dose in faeces	72.5	104.1	—	—	90.9	96.9	—	—	87.6	98.9	—	—

\* For composition of the test dose, see text. † Faecal iron-59 expressed as percentage of test dose. ‡ Faecal barium-131 expressed as percentage of test dose. § Ratio between the percentage of iron-59 and the percentage of barium-131 in the faeces. ¶ Percentage absorption of iron-59 calculated from the <sup>59</sup>Fe : <sup>131</sup>Ba ratio in the test dose and in the first 24 h faeces showing carmine red. || —, No defaecation.

Table 2. FAECAL IRON-59 AND BARIUM-131 IN TWO PATIENTS WITH SIDEROOPENIC ANAEMIA AND TWO PATIENTS WITH IRON OVERLOAD, AFTER ADMINISTRATION OF AN ORAL TEST DOSE OF IRON-59 SULPHATE AND BARIUM-131 SULPHATE

Days after administration of test dose*	4. Iron deficient patient <sup>59</sup> Fe† (%)	<sup>131</sup> Ba‡ (%)	<sup>59</sup> Fe§ (%)	Absorption (%)¶	5. Iron deficient patient <sup>59</sup> Fe† (%)	<sup>131</sup> Ba‡ (%)	<sup>59</sup> Fe§ (%)	Absorption (%)¶	6. Iron overload patient <sup>59</sup> Fe† (%)	<sup>131</sup> Ba‡ (%)	<sup>59</sup> Fe§ (%)	Absorption (%)¶	7. Iron overload patient <sup>59</sup> Fe† (%)	<sup>131</sup> Ba‡ (%)	<sup>59</sup> Fe§ (%)	Absorption (%)¶
1	0	0	—	—	0	0	0	—	21.1	29.8	0.71	29	22.7	29.5	0.77	23
2	12.2	70.6	0.17	83	—	—	—	—	24.1	32.0	0.75	—	49.6	63.4	0.78	—
3	1.7	9.4	0.18	—	—	—	—	—	30.5	39.2	0.78	—	3.1	3.0	1.03	—
4	4.4	22.0	0.20	—	—	—	—	—	—	—	—	—	1.8	0.3	6.0	—
5	1.1	2.1	0.52	—	16.4	34.7	0.47	53	4.0	0.6	6.6	—	4.6	0.2	23.0	—
6	2.0	2.4	0.83	—	5.2	10.9	0.48	—	3.2	0	—	—	—	—	—	—
7	1.1	0	—	—	14.8	30.8	0.48	—	4.8	0	—	—	1.7	0.1	—	—
8	0	0	—	—	9.3	18.7	0.50	—	—	—	—	—	1.3	0	—	—
9	—	—	—	—	2.3	3.8	0.61	—	2.4	0	—	—	1.4	0	—	—
10	0	0	—	—	0	0	—	—	1.2	0	—	—	1.0	0	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	0	0	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total percentage of test dose in faeces	22.5	106.5	—	—	48.0	98.9	—	—	91.3	101.6	—	—	87.9	96.5	—	—

\* For composition of test dose, see text. † Faecal iron-59 expressed as percentage of test dose <sup>59</sup>Fe. ‡ Faecal <sup>131</sup>Ba expressed as percentage of test dose <sup>131</sup>Ba. § Ratio between the percentage of iron-59 and the percentage of barium-131 in the faeces. ¶ Percentage absorption of iron-59 calculated from the <sup>59</sup>Fe : <sup>131</sup>Ba ratio in the test dose and in the first 24 h faeces showing carmine red. || —, No defaecation.

Table 3. AMOUNT OF AN ORAL IRON DOSE ABSORBED BY THE GASTROINTESTINAL MUCOSA, THE PROPORTION OF THIS ABSORBED IRON LOST IN THE FAECES WITHIN 10-15 DAYS, AND THE PERCENTAGE OF THE IRON DOSE REMAINING IN THE ORGANISM AT THE END OF THIS PERIOD

Normal control persons and patients	Total iron absorbed Percentage of oral dose	Absorbed iron lost in the faeces		Iron retention after 10 to 15 days Percentage of oral dose
		Percentage of oral dose*	Percentage of total absorbed iron	
1. Normal	47	20	43	27
2. Normal	25	16	64	9
3. Normal	20	8	40	12
4. Iron deficient	83	6	7	77
5. Iron deficient	53	1	2	52
6. Iron overload	29	20	69	9
7. Iron overload	23	11	48	12

\* Total iron absorbed minus iron retention after 10-15 days.

barium sulphate is not absorbed; this was later confirmed with a whole-body counter\*. In the patients with iron deficiency (Table 2: Nos. 4 and 5), the iron-59 disappeared from the faeces only slightly later than did barium-131; this indicates that iron-59 sulphate and barium-131 sulphate have about the same rate of intestinal passage.

Absorption of iron from a test dose occurs for the most part within the first 2 h; the remaining absorption is believed to occur up to 20 h after administration of a test dose<sup>6</sup>. The proportionate radioactivity of iron-59 and barium-131 in the faecal samples shows that this coefficient is always lowest in the first sample to show carmine red; in samples taken on subsequent days this coefficient increases, and this may indicate that epithelial cells labelled with iron-59 enter the intestinal lumen soon after the absorption of iron-59 by the intestinal mucosa. This is plausible if we assume that in man both young and old epithelial cells of the intestinal villi absorb iron. This is known to be the case in rats. The oldest epithelial cells are likely to be lost in the intestinal lumen soon after absorption of iron; if they contain iron-59, they are bound to influence the ratio iron-59:barium-131. We have therefore calculated the percentage of absorption from the first faeces stained by carmine red. The ratio of iron-59 to barium-131 in subsequent samples of the faeces increased least in the patients with sideropenic anaemia (Table 2); in these patients, apparently, almost all iron-59 absorbed is directly passed by the epithelial cells of the intestinal mucosa and released into the blood.

In normal test subjects and patients with iron overload, radioactive iron is still present in the faeces long after barium-131 has ceased to be demonstrable. The rate at which the faeces pass through the intestinal tract varies, but faecal excretion of barium-131 is usually completed within 6 days. As mentioned before, the absorption of iron from the test dose is believed to occur within the first 24 h<sup>5</sup>. If it is true that the life span of epithelial cells of the human intestinal mucosa is 3-6 days<sup>6,7</sup>, then iron-59 found in the faeces 13 days or more after oral administration of iron cannot originate from the epithelial cells which first absorbed the iron-59. Possibly, this iron-59 enters the faeces so late because, after absorption, when it starts to circulate in the organism, it is re-distributed and in part taken up by newly formed cells of the intestinal mucosa<sup>8</sup>. These cells are desquamated into the intestinal lumen after a few days. Another possibility is that a proportion of the iron-59 in desquamated intestinal epithelial cells is again absorbed lower in the intestinal tract. Part of this re-absorbed iron-59 could then re-enter the faeces with desquamated epithelial cells. A proportion of the iron-59 in the faeces also probably originates from macrophages loaded with iron, which play a part in the absorption and re-distribution of iron in the organism<sup>9</sup>. Tables 1-3 show that absorption was greatest in the two patients with iron deficiency, in which nearly all iron absorbed was also retained (Table 3). Absorption in the normal test subjects varied from 20 to 47 per cent of the dose of iron-59 administered, and retention varied from

9 to 27 per cent. The difference in absorption and retention was about as great as that in the two patients with iron overload, that is, up to 20 per cent of the dose of iron-59 administered. One of the normal test subjects absorbed nearly as much (47 per cent) as one of the patients with sideropenic anaemia (52 per cent). The ultimate retention percentage (Table 3) shows, however, that in the patients with iron deficiency nearly all the iron absorbed was retained in the organism. In the normal test subjects, a large amount of the iron absorbed was not passed by the intestinal mucosa but entered the faeces.

The results of the tests described indicate that, in man, more iron is normally absorbed than is ultimately retained in the organism. Only in patients with sideropenic anaemia is the retention of orally administered iron nearly as great as the absorption. These tests confirm the assumption made by Conrad and Crosby on the basis of the results of their tests with radioactive iron in rats and with radioactive iron and carmine red in man.

With the aid of an oral test dose of iron-59 sulphate and barium-131 sulphate, the iron absorption percentage can be rapidly and easily calculated without the need for quantitative collection of faeces. In order to determine the ultimate retention of iron-59 in the organism after an oral test dose of iron-59, however, it is necessary either to make quantitative determinations of faecal iron-59 or to establish with the aid of a whole-body counter how much iron-59 remains in the organism 2 weeks or more after administration of a test dose of radioactive iron.

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### Meaning of Impedance Plethysmography

IMPEDANCE plethysmography is a method of measuring the resistive impedance of a limb segment. Nyboer<sup>1</sup> pointed out that the resistive impedance variation  $\Delta R$  during the heart cycle is proportional to the blood volume  $\Delta V$  transiently stored in the segment

$$\Delta V = -\rho \frac{l^3}{R_0^2} \Delta R$$

where  $\rho$  is the blood resistivity,  $l$  the length of the segment and  $R_0$  the mean steady value of the segment resistance. Nyboer<sup>1</sup> proposed a method of calculating the blood flow by extrapolating the post-systolic slope of the plethysmogram. This procedure is subject to some criticisms and may lead to wrong results.

We propose a quite different interpretation of the impedance plethysmogram.

The problem of pulsatile flow in arteries has been analysed from a biophysical standpoint by many authors. Womersley<sup>2</sup> pointed out that the radial expansion  $\xi$  of an artery during the pulse is not directly related to the pressure but to the average blood velocity  $\bar{v}$  (instantaneous mean value of the velocity in the section of the artery), to the resting radius  $r$  and the pulse-wave velocity  $c$  through the simple relation

\* Whole body counting was performed by the Radiological Workgroup, T.N.O., Arnhem.



$$\frac{\xi}{r} = \frac{\bar{v}}{c}$$

McDonald<sup>3</sup> showed that this relation can be clearly justified from the physical point of view, but it leads to an interpretation of the impedance plethysmogram quite different from that of Nyboer.

If we assume that the radial expansion  $\xi$  is very small in comparison with the radius of the artery<sup>4</sup>, we may put

$$\Delta V = 2\pi r \xi$$

so that

$$\xi = -\frac{\rho l}{2\pi r R_0^2} \Delta R$$

and the mean instantaneous velocity of the blood

$$\bar{v} = -\frac{\rho l c}{2\pi r^2 R_0^2} \Delta R$$

The instantaneous blood flow is given by

$$Q = \bar{v} \pi r^2 = -\frac{\rho l c}{2R_0^2} \Delta R$$

The resistive impedance variation  $\Delta R$  is thus proportional to the pulsatile flow, but the value of the constant of proportionality is a function of the velocity of the pulse wave. This velocity depends on the elastic behaviour of the artery, so that it is not possible to evaluate  $Q$  from the impedance plethysmogram without an independent measurement of  $c$ . The information that we may have

from the plethysmogram is only a value of the ratio  $\frac{Q}{c}$ .

As an experimental test, we have measured the mean flow in the segment of a limb for different values of  $c$ . The variation of the pulse wave velocity has been obtained by changing the transmural pressure of the arteries. As is well known, a reduction in the transmural pressure reduces the mean tension of the arterial wall. Arterial walls have elastomeric properties and the Young's modulus  $E$  largely varies with the tension<sup>5</sup>.

Because the pulse wave velocity is proportional to  $E^{1/2}$  (ref. 6), a reduction of the transmural pressure produces a reduction of  $c$ . We have measured the pulse-wave velocity in the segment by using the  $R$  pulse of the electrocardiogram as the external trigger of a double beam cathode-ray oscilloscope.

The  $y$  axes of the two beams are driven by the resistive impedance variations in two equal segments of the legs. In a segment of one of the thighs we have applied an external pressure with a pneumatic cuff. As the pressure is raised, the corresponding pulse is delayed and the amount of this delay is a measure of the velocity of the pulse wave (Fig. 1).

We have now measured the mean blood flow from the impedance plethysmogram in two segments, one in the thigh and the other in the leg on the same side for different

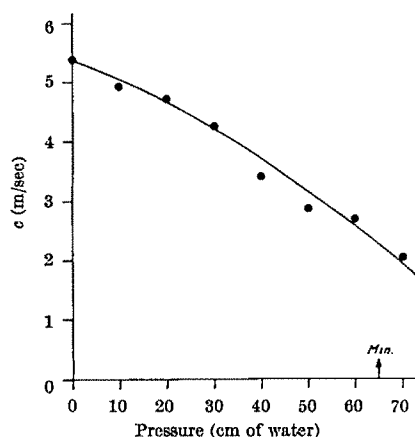


Fig. 1. Pulse-wave velocity as a function of external pressure in a segment of the thigh of a 14 year old boy.

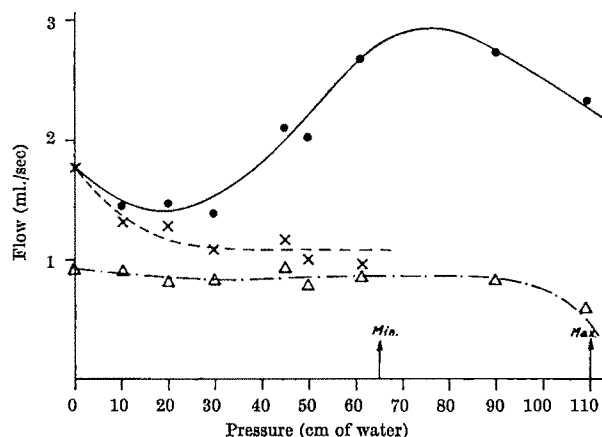


Fig. 2. Blood flow in the thigh and in the leg of a 14 year old boy as a function of an external pressure applied to the thigh. ●, Flow calculated assuming a constant wave velocity of 5.40 m/sec; ×, flow calculated with the measured value of the velocity; △, flow in the leg.

values of the pressure. A cuff is applied between the electrodes of the plethysmograph on the thigh and the flow measured. The cuff has been found to produce a variation of  $R_0$  and  $l$  of less than 1 per cent in the range of pressures from 0 to 100 cm of water and we therefore think that the flow measurement is independent of passive effects due to the cuff. If the pulse wave has a constant velocity, the increased flow through the thigh segment with the applied pressure is quite paradoxical. On the other hand, the values of the pulse wave velocity measured directly give a constant flow up to the maximum value of the arterial pressure as might be expected.

The decrease in the calculated flow for external pressures up to 20 mm of mercury is due to the contribution of the venous pulse in the external venous system.

These results may be of some interest to clinicians, because impedance plethysmography is an excellent tool in the diagnosis of peripheral vascular disease.

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## BIOLOGY

### Autoregulation of Release of Melanocyte Stimulating Hormone from the Rat Pituitary

MELANOCYTE stimulating hormone (MSH) enables amphibians such as the frog to change skin colour and thereby adapt to the environment. The function of this pituitary hormone in mammals such as the albino rat has not been established.

The release of MSH from the pituitary of the frog is controlled primarily by an area of the central nervous system—the hypothalamus. If the hypothalamus is destroyed, the frogs turn black<sup>1</sup>. These darkly pigmented frogs have increased amounts of MSH activity in their body fluids and decreased amounts of MSH activity in their pituitaries<sup>2</sup>.

There is evidence that the albino rat has a similar mechanism for the control of MSH release. The MSH content of a rat pituitary increases after injection of hypothalamic extracts<sup>3,4</sup>; it decreases selectively after

removal from direct hypothalamic influence<sup>6</sup> and after treatment with some of the same tranquilizers that cause frogs to darken<sup>6,7</sup>. Thus, in the rat as well as in the frog, it appears that increased pituitary content of MSH may be associated with inhibition of MSH release, and decreased pituitary MSH may mean an increased release of the hormone.

Melatonin, a serotonin derivative produced by the pineal gland, is the most potent agent known to lighten isolated frog skin<sup>8</sup> as well as frogs darkened by removal of the hypothalamic area<sup>9</sup>. In addition to exerting opposite effects on skin pigmentation in the frog, melatonin and MSH may also have opposite effects on the rat thyroid<sup>10,11</sup>.

The present investigations suggest a regulatory mechanism for the control of the release of pituitary MSH in the albino rat. Intravenous injection of exogenous MSH caused an elevation of the pituitary content of MSH, while injection of melatonin caused a depletion of the pituitary content of MSH. On the basis of the other studies already mentioned, it seems possible that release of MSH from rat pituitaries may be inhibited by raised concentrations of circulating MSH and perhaps stimulated by an increase in the concentrations of circulating melatonin.

In these experiments, 200 g albino Sprague-Dawley rats were anaesthetized with ether and 0.5 ml. of the test material diluted in 0.01 molar acetic acid-0.9 per cent sodium chloride was injected into the jugular vein. Twenty minutes later the rats were decapitated and their pituitaries removed. After being weighed, the pituitaries of four rats from each group were pooled, homogenized in acidified saline, and assayed for MSH activity in hypophysectomized frogs (*Rana pipiens*), as described previously<sup>12</sup>. Melatonin was initially dissolved in ethanol, the other compounds in 0.1 molar acetic acid.

The injection of highly purified natural and synthetic preparations of MSH caused a significant increase in the MSH content of the pituitaries of recipient rats as compared with the MSH levels of pituitaries of rats injected with only diluent (Tables 1-3). These increased pituitary MSH levels were related to the dose of MSH injected (Tables 1 and 2).

Possible explanations for the increased MSH content of rat pituitaries after injection of exogenous MSH include: *a*, accumulation of the exogenous MSH by the pituitary; *b*, direct feedback effect on the pituitary itself; *c*, inhibition of release of a possible hypothalamic factor stimulating MSH secretion<sup>3,13</sup>; and *d*, stimulation of release of the hypothalamic factor inhibiting MSH secretion<sup>3,4,14</sup>.

Table 1. EFFECT OF HIGHLY PURIFIED NATURAL MSH ON PITUITARY MSH CONTENT

Material	Dose/rat (mg)	Pituitary MSH (units/mg)	95 per cent confidence limits	Increment in pituitary MSH from control (units/mg)
$\alpha$ -MSH (Schally)	0.02	1,847	1,667-2,027	937
$\alpha$ -MSH (Schally)	0.005	1,536	1,418-1,654	626
$\beta$ -MSH (Schally: glu-2- $\beta$ -MSH)	0.02	1,524	1,379-1,669	614
Control (acidified saline)	—	910	881-939	—
$\alpha$ -MSH (Lerner, Lande and Upton)	0.02	1,717	1,568-1,866	786
$\alpha$ -MSH (Lerner, Lande and Upton)	0.005	1,134	1,066-1,202	203
Control (acidified saline)	—	931	864-998	—

Table 2. EFFECT OF SYNTHETIC MSH ON PITUITARY MSH CONTENT

Material	Dose/rat (mg)	Pituitary MSH (units/mg)	95 per cent confidence limits	Increment in pituitary MSH from control (units/mg)
$\alpha$ -MSH (Ciba)	0.02	1,242	1,167-1,316	940*†
$\alpha$ -MSH (Ciba)	0.001	315	289-341	13
$\beta$ -MSH (Ciba: ser-2- $\beta$ -MSH)	0.04	625	580-690	323
Control (acidified saline)	—	302	279-325	—‡

\* Kidney, adrenal, and muscle contained less than 1 unit of MSH/mg tissue.

† Liver contained 35 units of MSH/mg tissue.

‡ Liver contained 3 units of MSH/mg tissue.

Table 3. EFFECT OF VARIOUS SUBSTANCES ON PITUITARY MSH CONTENT

Material	Dose/rat (mg)	Pituitary MSH (units/mg)	95 per cent confidence limits	Change in pituitary MSH from control (units/mg)
Corticotropin A (W. F. White)	0.046	2,064	1,736-2,392	+963
Lysine vasopressin (Schally)	0.0004	1,080	932-1,198	-21
Control (acidified saline)	—	1,101	938-1,263	—
$\beta$ -MSH (Lerner, Lande and Upton)	0.02	1,741	1,557-1,925	+640
Melatonin	0.225	507	465-540	-594
Dexamethasone	0.8	1,614	1,424-1,804	+154
$\alpha$ -MSH (Lerner, Lande and Upton)	0.02	2,928	2,381-3,475	+1,408
Control (acidified saline)	—	1,460	1,358-1,562	—
Control (acidified saline containing 12 per cent ethanol)	—	1,435	1,237-1,633	—
Melatonin	0.112	604	525-683	-831

The small amounts of exogenous MSH accumulated by kidney, adrenal, muscle, and liver (Table 2) make it unlikely that the increased MSH content in the pituitaries was due to a simple deposition of the injected hormone. The possibility remains, however, that the pituitary has an avidity for concentrating MSH similar to that exhibited by the thyroid for trapping iodide. MSH apparently does not exert its effect directly on the pituitary, because the pituitaries of frogs the hypothalami of which have been destroyed contain a decreased amount of MSH in spite of elevated circulating levels of MSH<sup>2</sup>.

The most probable explanation is that administration of exogenous MSH caused an increase in the levels of this hormone in the plasma which, in turn, led to inhibition of the release of pituitary MSH. The reciprocal relationship between pituitary secretion of MSH and the plasma levels of this hormone may operate by a negative feedback mechanism similar to that existing for other hormones<sup>15</sup>. The hypothalamus, the probable target site of this feedback, may then regulate pituitary secretion by elaborating a factor controlling the release of MSH. Most evidence suggests that this hypothalamic factor primarily inhibits pituitary MSH release<sup>1-5,14</sup>.

Corticotropin A also increased the MSH activity of pituitaries from recipient rats (Table 3). The intrinsic MSH activity of the corticotropin was insufficient to explain this action. Because of the similarity of certain amino-acid sequences in both MSH and corticotropin, it is possible that corticotropin affected the hypothalamic receptors for the factor controlling MSH release. The failure of dexamethasone to increase pituitary MSH content significantly (Table 3) suggests that corticotropin did not exert its effect by stimulating the adrenals. The intrinsic MSH activity of corticotropin was also insufficient to account for more than 1 per cent of the rat pituitary MSH activity<sup>2,3</sup>. Larger doses of lysine vasopressin had no significant effect on pituitary MSH content (Table 3).

Injection of melatonin caused an effect on pituitary MSH content opposite to that obtained with MSH. Table 3 shows the significantly decreased MSH content found in the rat pituitary after injection of melatonin. The possibility of accumulation of melatonin by the pituitary was not completely excluded.

The data demonstrate that injection of exogenous MSH into the circulation of the rat causes an increase in pituitary MSH content. Conversely, injection of melatonin results in a decrease in pituitary MSH content. These findings indicate that MSH and possibly melatonin may be concerned with the regulation of MSH release in a class of vertebrates in which MSH has no clearly established function. In addition, this demonstration of regulatory mechanisms of pituitary MSH release in albino rats supports the concept that MSH may have an extra-pituitary function in mammals.

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### Distribution of Cytochrome Oxidase, Monoamine Oxidase and Carbonic Anhydrase in the Carotid Body of the Rabbit

Joels and Neil<sup>1</sup> showed that a high concentration of carbon monoxide in the fluid perfusing the carotid body of a cat produced an increase in discharge rate in the carotid sinus nerve. Carbon monoxide forms an unstable complex with the iron atom in cytochrome oxidase and this is broken down by strong light; illumination of the carotid body perfused with a large concentration of carbon monoxide reduces the intensity of the discharge induced by carbon monoxide. This was interpreted by Joels and Neil<sup>1</sup> to indicate the presence of cytochrome oxidase in the carotid body. Lee and Mattenheimer<sup>2</sup>, however, found only very low cytochrome oxidase activity in the carotid body glomus tissue of slaughterhouse-killed bullocks. The distribution of monoamine oxidase is of interest in connexion with the large concentration of a catecholamine, which appears to be dopamine, in the chemoreceptor or type I glomus cells<sup>3</sup>, and the possibility of a catecholamine acting as a transmitter in this tissue as suggested by Lever and Lewis<sup>4</sup>. Carbonic anhydrase activity was found to be high in the glomus tissue by Lee and Mattenheimer<sup>2</sup>, who used a histochemical method, but they did not examine its distribution within the glomus tissue. Black, McCloskey and Torrance<sup>5</sup> showed that inhibiting carbonic anhydrase with sodium acetazolamide slowed the carbon dioxide response of the cat carotid body.

The distribution of cytochrome oxidase, monoamine oxidase and carbonic anhydrase has now been investigated in the course of other work on the rabbit's carotid body. The animals were killed by a blow on the head. The carotid bodies were removed immediately and frozen on to a microtome chuck with a carbon dioxide freezer. Sections 20 $\mu$  thick were cut in a cryostat and floated on to the incubation media for the three enzymes. In addition, six carotid bodies were perfused through the carotid artery with 2.5 per cent glutaraldehyde in 0.1 molar phosphate buffer, pH 7.4, and fixed for a total of 30 min. After 2 h of washing in buffer they were frozen on to a microtome chuck and cut in the cryostat.

Cytochrome oxidase was demonstrated on sections of fresh tissue by the method of Burstone<sup>6</sup> with *p*-aminodiphenylamine and 1-hydroxy-2-naphthoic acid or 8-amino-1,2,3,4-tetrahydroquinoline as the coupling agent. The

method of Glenner, Burtner and Brown<sup>7</sup> was used to show monoamine oxidase in unfixed tissue sections and that of Morris and Swayne<sup>8</sup> for carbonic anhydrase on sections of both fresh and fixed tissue.

Cytochrome oxidase, with 1-hydroxy-2-naphthoic acid as the coupling agent, gave a red-brown colour which faded within 1 month. The precipitate with 8-amino-1,2,3,4-tetrahydroquinoline was dark blue and did not fade noticeably over 2 months. With both agents the colour was confined to the type I glomus cells. The precipitate was granular and the granules were of the same size as the mitochondria seen in electron micrographs of type I cells. It was absent from nerve fibres and connective tissue and only faint in the smooth muscle of blood vessels. The sustentacular or type II glomus cells could not be distinguished on the surface of the type I cells.

Monoamine oxidase activity is shown by a dark blue formazan precipitate in the method used. It was found in the cytoplasm of elongated or irregular cells surrounding the groups of type I cells in a single layer. These cells with nuclei much smaller than those of the type I cells are probably type II cells. Connective tissue did not stain, but some myelinated nerve fibres showed a granular deposit.

The final reaction product of the carbonic anhydrase method is black cobalt sulphide. The groups of glomus cells were strongly stained; in fresh tissue sections the precipitate was granular and largely confined to the cytoplasm of type I cells. In the fixed material the reaction was stronger but less well localized, although it was still heaviest in the type I cells. Any red cells present stained, and a few nerve bundles as well. Connective tissues did not stain. All staining was prevented by including 5 mmolar sodium acetazolamide in the incubation medium. The enzyme appears to be localized in type I cells though results obtained with the usual methods for this enzyme have been criticized by Pearse<sup>9</sup> and by Arvy<sup>10</sup>.

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### Nature of the Variation in Flower Colour in *Vicia*

From earlier work it was concluded that the principal factor concerned in promoting blueness in anthocyanin-pigmented flowers of *Lathyrus* species was the presence of flavonol co-pigments<sup>1,2</sup>. It was of interest to extend the observations into the related genus *Vicia* to determine whether a similar conclusion might be reached. In the latter genus the range of flower colour is similar to that found in *Lathyrus*. Species were selected for investigation which gave some representation of the colour range. Hydrolysed extracts of standard and wing petals were prepared and then examined by filter paper chromatography as described earlier<sup>1</sup>.

As in wild material of *Lathyrus*, the anthocyanins are glycosides of delphinidin and its related methylated derivatives petunidin and malvidin. The same flavonols, quercetin and kaempferol, are also present in the two genera. These occur as glycosides before hydrolysis.

Table 1. CONSTITUENTS OF HYDROLYSED EXTRACTS AND pH OF EXPRESSED SAP IN RELATION TO PETAL COLOUR

Species	Colour	pH	Anthocyanidins			Flavonols	
			D	P	M	Q	K
<i>V. atropurpurea</i> Desf.	S red	6.9	+	+	+	—	—
	W red	7.1	+	+	+	—	—
<i>V. dumetorum</i> L.	S bluish-red	6.7	+	+	—	+	+
	W bluish-red	6.8	+	+	—	+	+
<i>V. sepium</i> L.	S bluish-red	6.6	+	+	+	±	±
	W bluish-red	6.9	+	+	+	±	±
<i>V. sativa</i> L.	S bluish-red	6.1	—	+	+	+	+
	W red	6.0	—	+	+	—	—
<i>V. narbonensis</i> L.	S blue	6.1	—	+	+	—	+
	W blue	6.6	—	—	+	—	+
<i>V. cracca</i> L.	S blue	6.3	+	+	+	±	+
	W blue	6.4	+	+	+	±	+

D, delphinidin; P, petunidin; M, malvidin; Q, quercetin; K, kaempferol; S, standard; and W, wing petals: +, present; ±, trace; —, not detected on chromatograms at loading employed. Where more than one anthocyanidin was found, the predominant substance is indicated by +\*.

Delphinidin is the bluest of the anthocyanidins and malvidin the reddest. There is no evidence that flower colour is determined by the nature of the predominant anthocyanidin (Table 1), a conclusion also reached for *Lathyrus*<sup>1</sup>. The flavonols were found in those petals which were blue or bluish-red in colour. The situation in *V. sativa* is of particular interest because this has bicoloured flowers in which the standard petal is bluer than the wings; in those *Lathyrus* species with red/blue bicoloured flowers it is generally the wing petals which are bluer. In both *V. sativa* and the bicoloured *Lathyrus* species the flavonol content of the bluer petals is substantially greater than that of the redder petals<sup>1-3</sup>. The data reported here for *Vicia* are all consistent with the view put forward for *Lathyrus* that the flavonol glycosides act as co-pigments which modify the colour towards blue.

Determinations of the pH of cell sap expressed from petal tissue were made with a micro-electrode as described earlier<sup>1</sup>. The pH values varied according to species from 6.0 to 7.1. The data provide no evidence that blueness within the genus is promoted by higher pH, because the bluer petals tend to have lower pH's than the redder ones (Table 1).

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### Taxonomic Analysis of Herbarium Material by Gas Chromatography

In the recent investigation into the taxonomy of the spicate mints, cytogenetic and biometric investigations suggested that a redefinition of the species limits is necessary (R. M. Harley, in the press). One of these species, *Mentha spicata* L., the spearmint, is of commercial importance, as are many of its hybrids. The above work indicated that this species is a segmental allopolyploid derived from the diploid species *Mentha suaveolens* Ehrh (= *M. rotundifolia* auct.) and *M. longifolia* (L.) Huds. Because of the long history of cultivation mainly by vegetative propagation, and the ease with which hybrids are formed, the origins of the many cultivated forms are obscure, and because of the great morphological variability of *M. spicata* and its hybrids, such characters are not always sufficient by themselves for correct identification.

In an attempt to throw further light on the origins of *M. spicata* and its related hybrids, we have recently begun a taxonomic survey of mint oils by gas chromatography. One of us (R. M. H.) has brought together a large collection of live mints from all parts of the world, and this, which is kept in the University Botanic Garden, forms the basis of this survey.

The apparatus used is a 'Microtech GC 2000 R' gas liquid chromatograph fitted with a hydrogen flame

ionization detector and with glass columns 3/16 in. diameter, 6 ft. long. The columns are packed with 'Chromosorb G' with 2 per cent polypropylene sebacate liquid phase. The carrier gas is nitrogen with a flow rate of 35 ml./min and the column temperature is 70° C for 1 min, which is then programmed at 2° C/min up to 170° C.

A small segment of fresh or dried leaf from the middle of the main stem is inserted into an oven which leads directly to the column. In this way, only small quantities of material are needed, and no preliminary extraction is necessary.

Initial investigations indicate that the composition and relative amounts of the different terpenes in the essential oils of each species are highly characteristic of that species. Minor differences can also be used to distinguish infra-specific taxa, and also to some extent individuals within a population. In this respect the chromatographic trace can be compared with a fingerprint. Environmental factors, at least within the limits normally encountered, seem to cause very little disturbance in relative composition of the essential oils. In order to evaluate their importance, however, we are conducting a series of experiments on clonal material.

It was soon found that the terpene content of dried material differs very little from that of fresh material taken from the same plant. To see to what extent ageing of dried material occurred, we examined a range of herbarium material of two taxa, namely *M. alopecuroides* Hull and *M. citrata* Ehrh. These were chosen because it

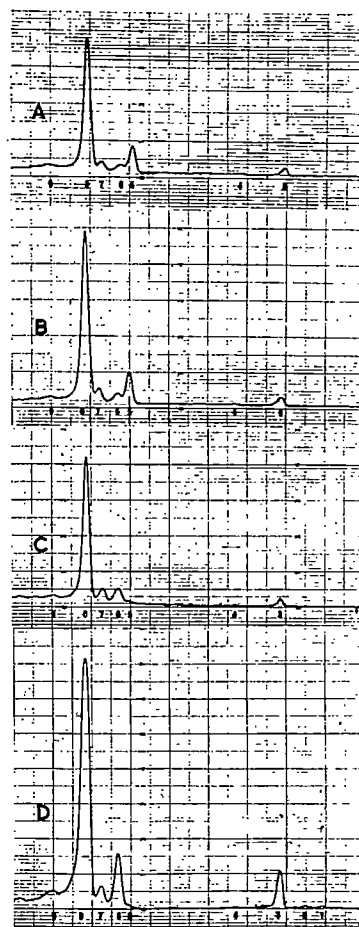


Fig. 1. Gas chromatographic traces of essential oils in herbarium specimens of *M. alopecuroides*. Each division of the chart paper represents two minutes. The peaks are numbered in order of appearance, from the right. Source of material: A, Aristotle Lane, Oxford, Leg. R. M. Harley, 1960; B, Upavon, Wiltshire, Leg. R. M. Harley, 1961; C, Essex, Leg. J. W. White, 1893; D, unlocalized, Leg. W. Sole, circa 1798.

was believed, on morphological and other grounds, that each represented a single sterile clone of hybrid origin. If this supposition were correct, any major differences in terpene content between herbarium material could be put down to ageing effects rather than genetic heterogeneity.

Both *M. alopecuroides* and *M. citrata* are widely cultivated plants which are occasionally found as garden escapes; and both are known to have been in existence for at least two hundred years.

Typical traces from four different specimens of *M. alopecuroides* are illustrated. The samples were run under identical conditions, although increased amplification was necessary in some cases, because of loss of essential oil with age. The similarity of the four traces is at once obvious. Specimens *A* and *B* (for further details see the figure) are both of recent origin and do not differ in terpene content from fresh material. They were collected in different years from different localities, and yet their traces are almost identical. The similarity is closer than that among the progeny from selfing *M. spicata*. Specimen *C* was collected in 1893 and specimen *D* in about 1798 by William Sole, although the specimen is undated. The Department of Botany at the University of Bristol is very fortunate in possessing a set of Sole's exsiccata of mints which follows the arrangement and nomenclature of his monograph *Menthae Britannicae* published in 1798.

These older specimens nevertheless have traces which are at once closely comparable with the more modern ones. Until the individual peaks have been identified, a detailed comparison is perhaps not justified, but attention is drawn to two points.

Peaks 1 and 2 are only visible on the oldest trace because of the much greater amplification. On increasing the amplification of *A*, *B* and *C*, peaks 1 and 2 at once appear. Peaks 5, 6 and 7 are clearly the least stable components on ageing, but their changes do not unduly affect the diagnostic value of the trace.

From these results, it seems very likely that *M. alopecuroides*, at least as represented by the plants so far examined, is a single clone. A similar conclusion was reached in the case of *M. citrata*, which also produced a series of almost identical traces, though much more material remains to be examined.

In the case of *M. spicata* itself we have evidence for a similar recurrence of certain clones in the material we have examined. Not only does the present technique offer considerable scope in experimental taxonomic and population investigations, but it also seems to be of value in the correct identification of old or fragmentary herbarium material, particularly where this affects typification, and where the material cannot be identified by more orthodox means. A case in point is Linnaeus' type specimen of *M. aquatica*, which may well be of hybrid origin. Gas chromatographic analysis should be able to solve this and similar problems.

One of us (M. G. B.) acknowledges the tenure of a Unilever research assistantship held during the course of this work.

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### Effect of Ectopic Pituitary Grafts on the Olfactory Block to Pregnancy in Mice

THE neuro-endocrine cause of the alien male-induced pregnancy block in newly mated female mice<sup>1,2</sup> is the failure of prolactin (luteotrophic) secretion by the adenohypophysis with consequent failure of the development of the corpora lutea and the return of the females to oestrus as if mating had not occurred. Administration of exogenous prolactin<sup>1</sup> or the increased secretion of prolactin

induced by suckling in lactating females<sup>3</sup> would prevent the block in newly mated females kept near to alien males. It is now clearly established that the pheromones which cause the pregnancy block are excreted in the urine of male mice<sup>4</sup>. Hypophyseal grafts to an ectopic site are known to secrete prolactin continuously<sup>5,6</sup> and so we investigated the effects of such grafts in newly mated female mice exposed to urine from alien males.

All the females and the stud males were outbred albinos of the Parkes stock. Females were about 10 weeks old at the start of the experiment. Each intact female received a graft of the adenohypophysis from a donor female of the same age. The graft was placed in the sub-capsular space of the right kidney. The external indication of a functioning ectopic pituitary graft in the intact female mouse is the appearance of a series of pseudopregnancy-like cycles<sup>6</sup>. After two or three consecutive pseudopregnancy-like cycles in each female, shown by daily examination of a vaginal smear, they were paired with the stud male. When the vaginal plug was found (day 0), the female was separated from the stud male and housed singly. About 5–7 weeks elapsed between the grafting of the pituitary and mating of the female. Twenty-four hours after mating (day 1 of pregnancy), the female was exposed to fresh urine from twelve inbred *CBA* males, as described elsewhere<sup>4</sup>, for 3 days. A control group bearing no ectopic pituitary grafts, that is, unoperated, was mated and similarly exposed to urine from males for 3 days. Normal females were used as controls because mock operation of the females or the placing of a piece of the brain tissue in their kidney capsules in no way modified the oestrous cycles. Another group of females was mated and left undisturbed after separation from the stud male in order to assess the rate of spontaneous failure of pregnancy. Daily vaginal smears were examined from all females up to day 7 *post coitum* and a return of oestrus during this period was considered to be the external manifestation of pregnancy failure<sup>2</sup>. The results are summarized in Table 1.

Table 1. INHIBITION OF PREGNANCY BLOCK IN NEWLY MATED MICE BY ECTOPIC PITUITARY GRAFTS

Treatment	Proportion and percentage of females returning to oestrus	Proportion and percentage of females remaining pregnant or pseudo-pregnant
Urine of <i>CBA</i> males and ectopic pituitary graft	11/64 (17%)*	53/64 (83%)
Urine of <i>CBA</i> males alone	45/52 (87%)*	7/52 (13%)
Undisturbed	2/20 (10%)	18/20 (90%)

\*  $P < 0.001$

It is evident that the ectopic pituitary graft removed from neural control and hence from the olfactory influence could prevent the male-induced pregnancy block from taking place in a large proportion of newly mated females. Obviously the prolactin secreted by the pituitary graft could sustain the corpora lutea over the period when the luteotrophic activity of the female's own pituitary was temporarily suspended. Thus, the inhibition of the pregnancy block in females exposed to urine from males by ectopic pituitary graft provides additional evidence in support of the view<sup>1</sup> that the block is caused by the failure of the luteotrophic function of the anterior pituitary.

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### Stridulation by a Cockroach during Courtship Behaviour

WE have found that the male of *Nauphoeta cinerea* stridulates during courtship. The male raises his wings and tegmina when courting the female, and exposes his tergum<sup>1</sup>. The female responds to a pheromone (seducin), produced in the male's abdomen, and mounts and palpates or "feeds" on his tergum<sup>2</sup>. The male initially courts without stridulating, and if the female is receptive, mating will occur quickly. If the female does not respond by mounting and feeding, however, or if she does mount and the male's attempts to grasp her genitalia are unsuccessful and she dismounts, he lowers his tegmina and wings, touches her with his antennae or legs, or stands within about 2 cm from her and stridulates. The male stridulates when the female remains quiescent; if she begins to move about he stops stridulating and usually turns facing away from her and raises his tegmina and wings.

Both adult sexes of *N. cinerea* (Olivier) have a stridulating apparatus on the pronotum (Fig. 1) and tegmina. In the male there are about forty parallel striae, each approximately  $4\mu$  apart, on the ventral surface of the latero-posterior edges of the pronotum (Fig. 2) and a row of about 400 striations, also about  $4\mu$  apart, arranged transversely down the dorso-proximal regions of the costal veins of the tegmina (Fig. 3).

Just before stridulating, the male rhythmically pumps and extends the abdomen with its tip arching upwards; as part of this co-ordinated activity the tegmina are raised and rotated slightly so that the anterior margins of the costal veins engage the ventral latero-posterior surfaces of the pronotum. The animal then begins to tremble and produces the courting sounds.

The sounds were picked up by a 1 in. Brüel and Kjaer microphone 'Model 4131', fed into and amplified by a '502A Tektronix' oscilloscope which was calibrated to register intensity. Records were stored on magnetic tape using an 'Ampex' tape deck and selected portions of the records were later filmed (Figs. 4-6) using a 'Grass C4H Kymograph' camera.

The phrase (Fig. 4) emitted by courting males begins with two to six (usually three) complex pulse trains each

having a 500 to 750 msec duration, followed by a series of disyllabic chirps, each of 60 to 80 msec duration. The terminology, with minor modification, is that of Broughton<sup>3</sup>. The phrases usually last 5-10 sec and are linked to form sentences lasting for as long as 3 min. Observations employing a dissecting microscope and stroboscope light source indicate that each pulse train is produced by a posterior, anterior, and side-to-side displacement of the pronotum over the costal veins. Chirps are produced by rapid, minute movements of the pronotum, first in a posterior, then in an anterior direction only.

We interpret the pulse train component of the courting phrase, shown in Fig. 5, to be composed of repeating units similar to the disyllabic chirps (Fig. 6). These repeating units, which precede and then follow the somewhat uniform central portion of the pulse train, probably result from lateral pivoting of the pronotal striations over the costal veins, whereas the midpulse region is the large slow posterior thrust of the pronotum observed midway in the pulse train cycle. The first syllable of a chirp appears to be made by a short, rapid, posteriorly directed displacement of the pronotum, while the second syllable results from an anterior movement. The first syllable is sharply damped.

The average sound level at the microphone placed 1 cm above the cockroach (in dB above a reference level where 0 dB =  $0.0002$  dynes/cm<sup>2</sup>) ranged from 60 to 65 dB for the pulse train peaks and from 55 to 60 dB for the chirp peaks. In a typical phrase, the pulse trains build in intensity from 55 to 65 dB, and the chirps which follow gradually decrease from 60 to about 55 dB before the next phrase begins.

An analysis of the sounds produced during courtship stridulation indicates that there is a wide frequency spectrum present in the components of the pulse train and the chirps. The audio spectrum consists of little more than a band of noise up to about 15 kc/s. This is not surprising when one considers the structure of the sound producing apparatus. The sound may be compared to that made by rubbing two finely ridged files together.

It is probable that pulse modulation of the mixed frequencies, or the rhythm of alternating pulse trains and chirps, or the rhythm of the chirps alone, provides the principal information unit, assuming the sound has meaning to the female. We have been unable to demon-

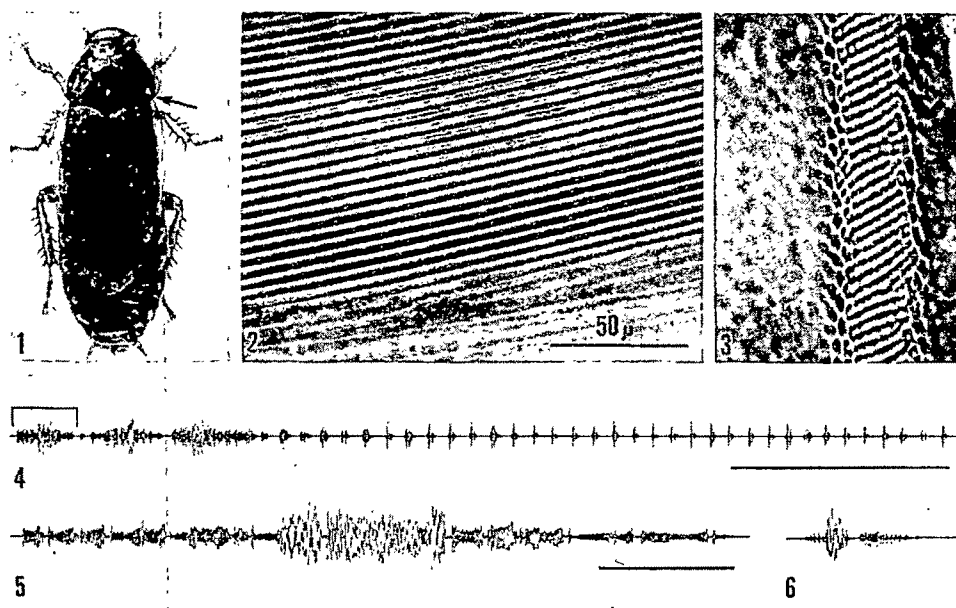


Fig. 1. *Nauphoeta cinerea* (male). Arrow points to site of the stridulating apparatus on the pronotum. Fig. 2. Part of the striae on the pronotum. Fig. 3. Part of the striae on the costal vein (magnification as in Fig. 2). Fig. 4. Oscilloscope record of a typical phrase. Time mark, 2 sec. Fig. 5. Bracketed section of Fig. 4 filmed at high speed to show details of the complex pulse train. Time mark, 100 msec. Fig. 6. A typical disyllabic chirp from the phrase in Fig. 4. Time scale as in Fig. 5.

strate that the female hears or responds to the males' stridulation. Dr. R. Alexander, who has listened to our tape recordings, considers this to be a "primitive" or "simple" sound, when compared with other stridulating Orthoptera, and there is little to distinguish the cockroach sounds from those made by the plum curculio, mutillid wasp or milkweed beetle. In his view, this is a "noisy" signal, in the information sense.

A stridulating apparatus similar to that found in *N. cinerea* has been described in *Leucophaea maderae* (Fabricius), and Vosseler<sup>4</sup> claimed that this species stridulated only when captured or frightened. Like *Leucophaea*, both sexes of *N. cinerea* stridulate when held, a response called protest or reflex-cry<sup>5</sup>, or alarm or danger calls<sup>6</sup>. Recently, Dumortier suggested that sound production by the female may play a part in the maternal behaviour of certain species of ovoviparous cockroaches<sup>5</sup>.

We have found pronotal-tegmenal stridulating apparatuses in five other species of Blaberidae: *Jagrehnia gestroi* (Saussure), *Oxyhaloa buprestoides* (Saussure), *Oxyhaloa* sp. (probably *ferreti* Reiche and Fairmaire), *Henschoudeutenia flexivitta* (Walker) and *Panchlora nivea* (Linnaeus), but not in eighteen other species belonging to sub-families of Blaberidae. Species of *Gromphadorhina* produce loud hissing sounds by expelling air through the second abdominal spiracles. The males hiss during courtship or in defence of territory<sup>7,8</sup>, and the females hiss if they are disturbed when they have young under or near them<sup>9</sup>. *Panchlora* is a member of the Panchlorinae, and *Nauphoeta*, *Leucophaea*, *Oxyhaloa*, *Jagrehnia*, *Henschoudeutenia*, and *Gromphadorhina* belong to the Oxyhaloinae<sup>10</sup>.

In the Blaberoidea, specialized sound producing devices have so far been found only in the highly evolved ovoviparous Blaberidae, and two different mechanisms for sound production (stridulation and expulsion of air through the spiracles) have evolved in some members of the Oxyhaloinae. The present finding is the first report of stridulation by a cockroach during courtship.

We thank Dr. K. Roeder for his help in determining the sound frequencies, Dr. R. Alexander for audiospectrograms, and Messrs. B. Crist and D. Loomis for technical assistance.

The stridulating structures were found in museum specimens of *Jagrehnia*, *Oxyhaloa*, and *Henschoudeutenia*. We thank Dr. A. Gurney, U.S. National Museum, and Dr. K. Princis, Lund University, for the loan of, and permission to dissect, specimens of these and other genera.

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poppy grows in abundance to a height of 2.5–3.5 ft. in sunny places, and blooms in June. Its flowers are 5–7 in. across and are scarlet in colour, black blotched at the base of petals and surrounded by conspicuous bracts (Fig. 1).

In July when the petals fall and the seed capsules are still immature, a juice is exuded from them by incision; it hardens when exposed in the air.

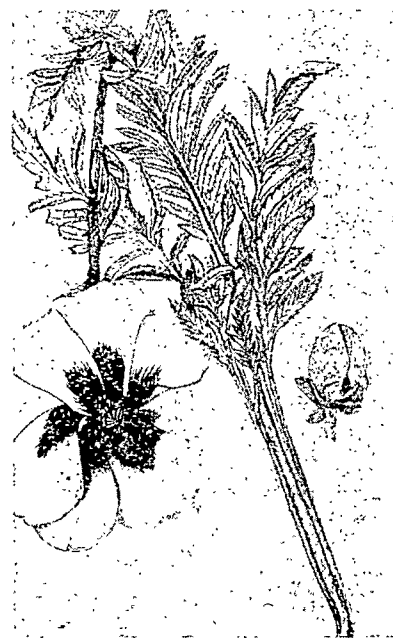


Fig. 1. *Papaver bracteatum* Lindl.

Some of this latex was dried and made into powder. To 10 g of this powder was added 30 ml. of water and the mixture was shaken gently overnight at 40° C. After filtration, the residue was twice shaken with 30 ml. of water at room temperature for 5 h and filtered. The collected filtrates were evaporated on a steam bath to a viscous state and, while still warm, an excess of anhydrous sodium carbonate was added. The contents turned into a solid which was powdered and extracted several times with a total volume of 100 ml. of warm benzene.

This was charcoaled and after evaporation of the solvent the residue was crystallized several times in absolute alcohol.

Through this method we obtained 2.6 g of pure thebaine (26 per cent of dry latex) which was identified by infra-red absorption and proton magnetic resonance spectra. Its melting point was 193° and it was not depressed by admixture with a sample of pure thebaine; its  $[\alpha]_D^{25}$  was  $-219^\circ$  ( $p=2$  in alcohol). It is noteworthy that no morphine was found in the latex of *P. bracteatum*.

In opium approximately 0.5 per cent of thebaine is present, and in the roots of *P. orientale* only a minimum amount of this alkaloid has been reported. Twenty-six per cent of thebaine in the dry latex of *P. bracteatum* Lindl. is therefore an unusually high figure.

We thank H. Golgolab and A. Zargari for their help in determining this poppy.

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### ***Papaver bracteatum* Lindl., a Highly Rich Source of Thebaine**

In the Alborz mountains in the north of Iran, on the slopes facing the Caspian Sea, at altitudes ranging from 5,500 to 7,500 ft. from the sea level we have found vast areas covered with *Papaver bracteatum* Lindl. This wild annual

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## GENETICS

## Blood Groups of the Chinese in Calcutta

THE purpose of this investigation was to record the distribution of A<sub>1</sub>A<sub>2</sub>BO, MN, Rh, Kell and Duffy blood groups and of ABH secretor factor and haemoglobin variants, if any, of the Chinese in Calcutta, most of whom are reported to have migrated from Canton about two or three generations ago. They live in groups and do not seem to intermingle with Indians.

Table 1. DISTRIBUTION OF ABO, A<sub>1</sub>A<sub>2</sub>BO, MN, Rh, DUFFY AND KELL BLOOD GROUPS AND SECRETOR FACTOR AMONG THE CHINESE IN CALCUTTA

ABO system								A <sub>1</sub> A <sub>2</sub> BO system*							
Phenotypes (number and per cent)					<i>p</i>	Genes (per cent)			Phenotypes (number and per cent)						
O	A	B	AB	Total		<i>q</i>	<i>r</i>		O	A <sub>1</sub>	A <sub>2</sub>	B	A <sub>1</sub> B	A <sub>2</sub> B	Total
252 44.52 (44.44)	170 30.04 (30.13)	113 19.96 (20.06)	31 5.48 (5.37)	566 100.00 (100.00)	19.69 ± 1.25	13.65 ± 1.06	66.66 ± 1.49		252 46.41 (46.33)	142 26.15 (25.66)	12 2.21 (2.21)	113 20.81 (20.79)	20 3.68 (4.57)	4 0.74 (0.44)	543 100.00 (100.00)
A <sub>1</sub> A <sub>2</sub> BO system*					MN system				Rh system (tested with anti-D only)						
Genes (per cent)				<i>M</i>	Phenotypes (number and per cent)			Total	Genes (per cent)	Phenotypes (number and per cent)			Total	Genes (per cent)	
<i>p</i> <sub>1</sub>	<i>p</i> <sub>2</sub>	<i>q</i>	<i>r</i>		MN	N				Rh(D)	Rh(d)			<i>D</i>	<i>d</i>
16.47	1.60	13.86	68.07	68 32.54 (28.98)	89 42.58 (49.71)	52 24.88 (21.32)	209 100.00 (100.00)	53.83	46.17	473 99.79	1 0.21	474 100.00	95.42	4.58	
Rh system (Tested with anti-C, -c, -D, -E and -e only)															
Phenotypes (number and per cent)										Chromosomes (per cent)					
CDe	cDE	CcDe	cDEe	CcDEe	cDe	CDEe	cde	CcDE	Total	<i>CDE</i>	<i>CDe</i>	<i>cDE</i>	<i>cDe</i>	<i>cde</i>	
82 50.93 (49.07)	10 6.21 (4.95)	12 7.45 (10.19)	4 2.49 (3.24)	48 29.82 (31.23)	3 1.86 (0.40)	1 0.62 (0.60)	1 0.62 (0.13)	0 0.00 (0.19)	161 100.00 (100.00)	0.43	70.05	22.25	3.63	3.64	
Duffy system					Kell system					ABH secretion					
Phenotypes (number and per cent)				Genes (per cent)	Phenotypes (number and per cent)				Genes (per cent)	Phenotypes (number and per cent)				Genes (per cent)	
Fy(a+)	Fy(a-)	Total	<i>Fy</i> <sup>a</sup>		<i>Fy</i> <sup>b</sup>	Kell +	Kell -	Total		<i>K</i>	<i>k</i>	Secretor	secretor		Total
122 96.06	5 3.94	127 100.00	80.16	19.84	1 0.76	131 99.24	132 100.00	0.38	99.62	406 73.15	149 26.85	555 100.00	48.19	51.81	

Figures in parentheses represent expected percentage.

\* Obtained from ABO data.

During June–December, 1965, blood specimens were collected mainly from students and teachers of three Chinese schools in Calcutta and were tested for blood group antigens like A, A<sub>1</sub>, A<sub>2</sub>, B, M, N, C, c, D, E, e, Kell (K) and Duffy (Fy<sup>a</sup>), but because of non-availability of antisera all the specimens could not be tested for every antigen. All the antisera with the exception of anti-A and anti-B were received from 'DADE' in U.S.A. Kell and Duffy antigens were determined by Coombs test, while other antigens were determined by standard methods. The specimens of saliva were examined by the method of Race and Sanger<sup>1</sup> for ABH secretor factor. Paper electrophoresis technique was used for the detection of haemoglobin variants. All the results have been summarized in Table 1.

In a sample of 566 individuals, 44.5 per cent belong to group O, 30.0 per cent to group A, 20.0 per cent to group B and 5.5 per cent to group AB. The maximum likelihood estimates of *p*, *q* and *r* for three allelomorphous genes *A*, *B* and *O* are 19.7, 13.6 and 66.7 per cent respectively. The incidence of *B* gene in China is less than 20 per cent and that of *A* gene is more than 20 per cent, in contrast to India and South Eastern Asia where *B* is generally in excess of *A* (ref. 2). The incidence of *B* in China, however, is greater than is found in Europe. It has been noted<sup>3-7</sup> that the Chinese are characterized by complete absence of A<sub>2</sub> gene, but as in the present investigation, occurrence of A<sub>2</sub> gene at a low frequency has also been reported<sup>8,9</sup>.

Among the Chinese the incidence of *M* gene is greater than that of *N* and it varies from 50.5 per cent to 63.0 per cent against 60 per cent to 70 per cent in India. The frequency of *M* gene obtained in the present investigation is 53.8 per cent while its mean value calculated by pooling the results of all workers<sup>3-6,8-11</sup> including ours is 56.9 per cent.

The gene frequencies for *D* and *d* among 474 Chinese in Calcutta are 95.4 per cent and 4.6 per cent respectively, giving 99.8 per cent Rh-positive and 0.2 per cent Rh-negative individuals. In the detailed analysis of Rh groups, the chromosome frequencies are in general agreement with those previously reported<sup>2,4-6</sup>. The Chinese differ from the Indians in having a higher frequency of *CDe* and *cDE* and a lower frequency of *cde* chromosomes.

The Duffy positive is almost universal in Chinese as observed by Miller *et al.*<sup>5</sup> and Layrisse and Arends<sup>9</sup>, while 96 per cent among 127 individuals in the present survey are

found to be Duffy positive giving approximately 80 per cent of Fy<sup>a</sup> genes. One of 132 individuals examined was found to be Kell positive. Earlier Miller *et al.*<sup>5</sup> and Sussman<sup>6</sup> reported complete absence of Kell antigen in the blood of the Chinese.

In the present survey of 555 individuals, 73 per cent were secretors and 27 per cent non-secretors; the gene frequencies for secretors and non-secretors were 48.2 per cent and 51.8 per cent respectively. As far as we know, this is the first report of the distribution of secretor factor among the Chinese. No abnormal haemoglobin was detected.

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## IMMUNOLOGY

### Inhibitory Effect of Isoantibody on *in vivo* Sensitization and on the *in vitro* Cytotoxic Action of Immune Lymphocytes

KALISS was able to show that the enhancement of tumour grafts in allogeneic recipients is mediated by antibody<sup>1</sup>. Three alternative mechanisms by which antibodies might exert their effect have been postulated by Billingham *et al.*<sup>2</sup>: (1) an afferent inhibition in which antibody combined with allogeneic cells prevents antigenic determinants from reaching immunologically competent cells; (2) a central inhibition in which antibody, by direct action on lymphoid cells, prevents their sensitization; (3) an efferent inhibition in which antibody reacting with target cells protects against the cytotoxic action of immune lymphoid cells which are competing for the same antigenic receptors. Both the afferent and the central mechanisms would affect sensitization of lymphoid cells, thought to be the most important mediators of homograft rejection. Brent and Medawar<sup>3</sup> demonstrated that extracts of antigenic tissue injected into recipients treated with antiserum did not provide immunization against a subsequent skin graft. Snell *et al.*<sup>4</sup> showed that lymphoid cells from tumour allograft recipients which had been treated with antiserum were less efficient in inhibiting tumour growth than lymphoid cells from untreated recipients. Passive immunization is also known, however, to inhibit the synthesis of antibodies<sup>4-6</sup>, and experiments involving transfer of immunologically competent cells cannot critically distinguish between cellular and humoral immunity. We have therefore analysed the effect of passive immunization on "cell bound immunity" using *in vitro* methods.

Recipient C57BL mice were immunized both actively with DBA/2 tumour cell allografts, and passively with isoantiserum. The cytotoxic action of the spleen cells of the recipient mice was evaluated by counting under a microscope and/or by an *in vitro* assay of the potential of the target cell population to form clones<sup>7</sup>. Hyperimmune anti-DBA/2 isoantiserum was prepared by injecting mice of the lymphoid cell donor strain (C57BL) between six and eight times with DBA/2 mastocytoma cells. A strongly reacting serum was obtained, with an agglutination titre against DBA/2 erythrocytes of 1:64,000 and a cytotoxic titre against DBA/2 mastocytoma cells of 1:256-1:512. Indirect immune fluorescence tests using living mastocytoma cells showed the serum to be strongly active at dilutions up to 1:128.

In four experiments, 0.2 ml. volumes of the isoantiserum were injected intravenously into C57BL mice 1 h after the immunizing dose of  $30 \times 10^6$  DBA/2 mastocytoma cells. A second group of mice received the same number of mastocytoma cells only. After 8 days, the spleens of the recipient and of untreated C57BL control mice were collected. An enhancing effect on the growth of tumour cells in most of the passively immunized mice was suggested by the formation of increased amounts of ascitic fluid containing large numbers of living mastocytoma cells.

For the *in vitro* assay, an equal volume of  $2 \times 10^7$ /ml. spleen cells was mixed with  $2 \times 10^5$ /ml. target mastocytoma cells maintained as *in vitro* cultures. Separate reaction mixtures were prepared with spleen cells from the two groups of recipient mice and from untreated control mice. One millilitre of the suspension was placed in each of two Leighton tubes and cloning assays were performed after incubation at 37° C for 24 h.

As can be seen from Table 1, the *in vitro* assay revealed that the ability of lymphoid cells of passively immunized recipients to inhibit the potential of target cells to form clones was greatly reduced or abolished. That is, the development of cell bound immunity was inhibited by the passive immunization.

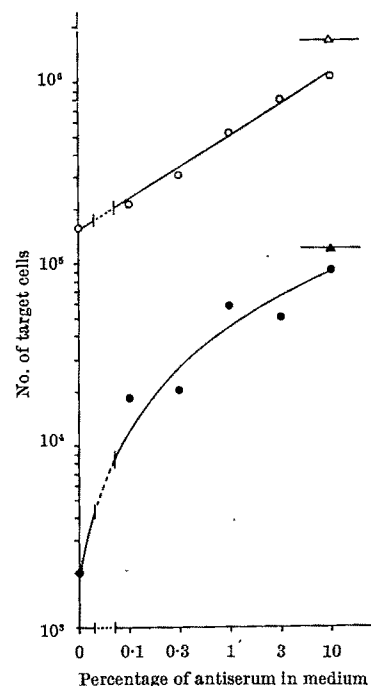


Fig. 1. *In vitro* inhibition of the immune lymphoid cell reaction by antiserum.  $\Delta$ , Microscopic counts of target cells after 48 h of contact with normal lymphoid cells in presence of 10 per cent isoantiserum.  $\circ$ , microscopic counts of target cells after 48 h of contact with immune lymphoid cells in presence of increasing concentrations of isoantiserum.  $\blacktriangle$ , clones formed by target cells after 24 h of contact with normal lymphoid cells in presence of 10 per cent isoantiserum;  $\bullet$ , clones formed by target cells after 24 h of contact with immune lymphoid cells in presence of increasing concentrations of isoantiserum.

The inhibiting effect of isoantibody on lymphoid sensitization was complete in experiment 30. Incomplete but still very marked inhibition was observed in the other three experiments. An inverse relationship seems to exist between the level of inhibition and the efficacy of sensitization. In the three experiments in which the control mice showed stronger sensitization of the lymphoid cells the inhibitory effect of antibody was incomplete.

The third possible mechanism of tumour enhancement concerns reactions at the target cell level. Antibodies covering antigenic sites are thought to protect target cells against the cytotoxic action of immune lymphoid cells. Möller<sup>8</sup> was able to demonstrate the progressive growth of a tumour coated *in vitro* with antitumour antibodies even in recipients which had been immunized with normal allogeneic tissue. *In vitro* experiments by Erna M. confirmed the *in vivo* results, which suggested that antibody mediated protection against the immune lymphoid cells of the host. Brondz<sup>10</sup>, however, was unable to confirm this in a similar system.

To evaluate a possible protective effect of a passive antiserum, various concentrations of the same antiserum that had been used for passive immunization (inactivated at 56° C for 30 min) were added to reaction mixtures containing  $1 \times 10^6$ /ml. target mastocytoma cells,  $1 \times 10^7$ /ml. immune lymphoid cells of recipient C57BL mice which had received a sensitizing injection of  $30 \times 10^6$  DBA/2 mastocytoma cells 8 days previously. In control suspension, spleen cells of normal C57BL mice

Table 1. EFFECT OF ACTIVE-PASSIVE IMMUNIZATION ON *in vitro* LYMPHOID SENSITIZATION: CLONES FORMED BY TARGET CELLS AFTER 24 H OF CONTACT WITH DIFFERENT LYMPHOID CELL PREPARATIONS

Exp. No.	No. of clone forming target cells after incubation for 24 h in the presence of		
	Normal cells	Immune cells	"Active-passive" immune cells
29	146,000	3,200	88,000
30	141,000	19,000	168,000
31	800,000	2,000	72,000
32	810,000	580	280,000

added to target cells either in the presence of the highest concentration (10 per cent) of antiserum used in the test or without serum. The mixtures were placed in Leighton tubes, and cloning assays and microscopic counts were performed after incubation for 24 and 48 h.

The results shown in Fig. 1 clearly demonstrate the protective effect of isoantibody on the immune lymphocyte reaction. The effect increased with the amount of antibody added, and led to an almost total inhibition of the immune lymphoid cell reaction at the highest concentration used. The presence of 10 per cent antiserum added to control suspensions containing normal spleen cells did not affect the number of target cells which could form clones.

The experiments described here have allowed us to differentiate between two distinct mechanisms of immunological enhancement. Homotransplantation and adoptive transfer experiments indirectly demonstrated (1) an afferent or central inhibitory effect of passive immunization on lymphoid cell sensitization, and (2) an efferent protective effect of antibody against the action of immune cells.

Our *in vitro* studies, allowing a critical distinction between cellular and humoral immunity, support the possible simultaneous functioning of both mechanisms by demonstrating directly that (a) passive immunization inhibits the induction of cell-bound immunity, and (b) antibody protects against the cytotoxic action of immune lymphoid cells *in vitro*.

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### Comparison of Different Isoprecipitin Sera

A SERUM has been described from a polytransfused patient (C. de B.) which gave precipitate formation in gels with some but not all normal sera<sup>1,2</sup>. The isoprecipitin was subsequently shown to react with serum low density ( $\beta$ ) lipoprotein<sup>3,4</sup>. It was recognized that C. de B. antiserum exhibited more than one kind of reaction<sup>1</sup> or had more

than one specificity<sup>1,5</sup>. Further work on serum C. de B. showed that this antiserum contains at least three different antibodies (anti-a, anti-x and anti-z)<sup>7,8</sup>. Another isoprecipitin serum (L. L.) was found which contained one of the antibodies (anti-x) present also in serum C. de B.<sup>9</sup>. The present report deals with the comparison of the following human isoprecipitin sera: C. de B.<sup>1</sup>, L. L.<sup>9</sup>, E. S., Gi<sup>10</sup>, B. B.<sup>11</sup>, E. Z.<sup>12</sup>, 700 (S. L.), 928 (J. R.), 1121 (H. K.)<sup>8</sup>, 352 (J. A.), 931 (G. R.).

To compare these sera, a panel of twenty-seven normal human sera from regular blood donors and staff at the State Institute for Blood Group Serology in Stockholm was collected and kept at  $-20^{\circ}\text{C}$  until used (within 5 months of collection). Absorptions were carried out in test-tubes by mixing equal amounts of selected panel sera with the isoprecipitin sera to be absorbed and incubating the mixture overnight at  $+4^{\circ}\text{C}$ . A gel diffusion technique on microscope slides was used as described elsewhere<sup>7</sup>.

The panel sera were characterized by testing against serum C. de B. unabsorbed as well as absorbed with each of fifteen of the twenty-seven panel sera. In this way the panel sera could be divided into six groups (Table 1). The reaction patterns of the different unabsorbed isoprecipitin sera against this panel are given in Table 2.

Additional tests on other normal sera from between ten and two hundred unrelated individuals living in the Stockholm area did not reveal any difference between the reaction spectra for sera E. S., Gi, B. B., E. Z., 700 and 928 as compared with serum L. L. Absorption tests or presence of interference reactions in agar gel gave no evidence for more than one single kind of antibody molecules in these isoprecipitin sera. The above-mentioned sera are consequently assumed to be all of the specificity anti-x. Sera 1121, 352 and 931 gave the same reaction spectrum as serum C. de B. when tested unabsorbed (Table 2). After absorption of these sera with selected panel sera, differences were, however, revealed (Table 3).

The absorption data for serum 1121 can be accounted for by assuming the presence of anti- $a_1$ , +anti-x but absence of anti-z in this serum. Similarly, the absorption data for 352 can be accounted for according to the present model by assuming that 352 does have the "complex" antibody anti-xz in addition to the "simple" antibodies anti- $a_1$  and anti- $z_2$  (ref. 13). Consequently, addition to the antiserum of samples which possess the antigenic determinant z will inhibit the activity not only of the simple antibody anti-z but also (according to definitions<sup>13</sup>) of the complex antibody anti-xz whereby the antiserum will no longer react with samples having only the x and/or the z antigen.

In the same way, the absorption data for serum 931 are accounted for according to the present complex-complex model of classification by assuming the existence of the complex antibody anti-a<sub>1</sub>x in addition to the simple antibody anti-x.

Table 1. CHARACTERIZATION OF AND NOTATION FOR PANEL SERA AS DEFINED BY SERUM C. DE B. UNABSORBED AS WELL AS VARIOUSLY ABSORBED

Group	Sera No.	Notation	C. de B. Anti-a <sub>1</sub> Anti-x Anti-z	C. de B. +I — — —	C. de B. +II — — Anti-z	C. de B. +III — Anti-x —	C. de B. +IV — Anti-x Anti-z	C. de B. +V Anti-a <sub>1</sub> — Anti-z	C. de B. +VI Anti-a <sub>1</sub> Anti-x Anti-z
I	1-2	(a <sub>1</sub> + x + z +)	+	—	+	+	+	+	+
II	3-11	(a <sub>1</sub> + x + z -)	+	—	—	+	+	+	+
III	12-19	(a <sub>1</sub> + x - z +)	+	—	+	—	+	+	+
IV	20	(a <sub>1</sub> + x - z -)	+	—	—	—	+	+	+
V	21	(a <sub>1</sub> - x + z -)	+	—	—	+	+	—	—
VI	22-27	(a <sub>1</sub> - x - z -)	—	—	—	—	—	—	—

Table 2. REACTIONS OF DIFFERENT UNABSORBED ISOPRECIPITIN SERA AGAINST PANRL SERA

[illegible]



Table 3. ABSORPTION TESTS ON SERA 1121, 352 AND 931 AND SUGGESTED ANTIBODIES IN THESE SERA

Group	Notation	1121	Antibodies: anti-a <sub>1</sub> , anti-x					
			1121 +I	1121 +II	1121 +III	1121 +IV	1121 +V	1121 +VI
I	(a <sub>1</sub> +x+z+)	+	-	-	+	+	+	+
II	(a <sub>1</sub> +x+z-)	+	-	-	+	+	+	+
III	(a <sub>1</sub> +x-z+)	+	-	-	-	-	+	+
IV	(a <sub>1</sub> +x-z-)	+	-	-	-	-	+	+
V	(a <sub>1</sub> -x+z-)	+	-	-	+	+	-	+
VI	(a <sub>1</sub> -x-z-)	-	-	-	-	-	-	-

Group	Notation	352	Antibodies: anti-a <sub>1</sub> , anti-xz, anti-z					
			352 +I	352 +II	352 +III	352 +IV	352 +V	352 +VI
I	(a <sub>1</sub> +x+z+)	+	-	+	-	+	+	+
II	(a <sub>1</sub> +x+z-)	+	-	-	-	+	+	+
III	(a <sub>1</sub> +x-z+)	+	-	+	-	+	+	+
IV	(a <sub>1</sub> +x-z-)	+	-	-	-	-	+	+
V	(a <sub>1</sub> -x+z-)	+	-	-	-	+	-	+
VI	(a <sub>1</sub> -x-z-)	-	-	-	-	-	-	-

Group	Notation	931	Antibodies: anti-a <sub>1</sub> x, anti-x					
			931 +I	931 +II	931 +III	931 +IV	931 +V	931 +VI
I	(a <sub>1</sub> +x+z+)	+	-	-	+	+	-	+
II	(a <sub>1</sub> +x+z-)	+	-	-	+	+	-	+
III	(a <sub>1</sub> +x-z+)	+	-	-	-	-	-	+
IV	(a <sub>1</sub> +x-z-)	+	-	-	-	-	-	+
V	(a <sub>1</sub> -x+z-)	+	-	-	+	+	-	+
VI	(a <sub>1</sub> -x-z-)	-	-	-	-	-	-	-

Absence of (a<sub>1</sub>-x+z+) and (a<sub>1</sub>-x-z+) samples means that the possibility that additional complex antibodies with, for example, the antibody specificity anti-z are present in the antisera which are regarded as having the antibody specificity anti-a<sub>1</sub> cannot be dismissed. Thus, serum 931 may either have the additional complex antibody anti-a<sub>1</sub>xz or have this antibody instead of anti-a<sub>1</sub>x. The present model does thus only represent an attempt to account for the data in a qualitative way according to the most economic model with regard to symbols introduced and under the basic assumption that one antigenic determinant reacts with and absorbs only those antibodies which have the corresponding antibody specificity. Other models for the Ag-system based on "simple-complex", "complex-simple" or "complex-complex" notations<sup>13</sup> are thus possible and will be discussed elsewhere.

Several other isoprecipitin sera have and most probably will be discovered, and so it would be of great interest if the definition of their specificities could be co-ordinated with the present work. It is therefore suggested that small samples (about 2 ml.) should be submitted to one or the other of us for definition and comparison of their specificities.

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## Development of Immunological Capacities in Normal and Thymectomized Mice

THE presence of thymus has been shown to be essential for the normal development of immunological capacities by a number of investigators. Antibody response and homograft immunity are the usual criteria of the immunological response. The capacity to produce antibody plaque-forming cells and thus to produce serum antibody in response to sheep erythrocyte antigen has been reported to be impaired in adult mice by neonatal thymectomy<sup>1-3</sup>. In this report the development of capacities for the production of haemolysin and homograft immunity in thymectomized mice as compared with those of sham-thymectomized control mice will be presented.

Inbred mice of strain *SL* were thymectomized or sham-thymectomized 24-36 h after birth according to the method of Sjodin *et al.*<sup>4</sup>. On days 5, 8, 13, 20, 30, 45, 60 and 120 of life, both groups of mice received a single intraperitoneal injection of 50 per cent (v/v) washed sheep erythrocyte suspension. Mice younger than 20 days of age received 0.025-0.50 ml. of the antigen suspension according to their body weights, but 0.1 ml. was injected into all the mice older than 30 days of age. More than six mice for each group were killed by bleeding after cutting the femoral vessel 4 days after injection of the antigen. Sera were stored at -25° C and were titrated together at the completion of the experiment. After inactivation, serial twofold dilutions of sera with an initial dilution of 1:40 in 0.2 ml. of magnesium saline solution were mixed with 0.2 ml. of 2 per cent sheep erythrocyte suspension. The mixtures were incubated at 37° C for 30 min after addition of guinea-pig complement. A limit dilution giving complete haemolysis was considered the titre of the serum. The method of Jerne *et al.*<sup>5</sup> was used with minor modifications<sup>1</sup> to count antibody-forming cells in the spleen. Male mice of strain *C57BL*, which differs from *SL* at the *H<sub>2</sub>* histocompatibility locus, were used as skin donors. Recipient mice of strain *SL* received skin grafts on days 15, 30 and 60 of life. The skin homografts were full-thickness circular specimens, 6 mm in diameter. They were grafted on the dorsal surface of the recipients and a pressure dressing was applied. The grafts were examined daily after day 8.

The results of haemolysin assay are shown in Fig. 1. In the thymectomized group most of the mice immunized younger than 45 days old showed the haemolysin titre less than 1:80. On the other hand, the sham-thymectomized control group immunized at 13 days old developed 1:200 of mean haemolysin titre which increased gradually with the age up to 1:2,720 at 120 days old. It is interesting that the gradual increase of antibody response was also observed in the thymectomized group. In this group the mean haemolysin titre at 120 days old was about 1:430, which approximately corresponds to the titre at 30 days old in the control group.

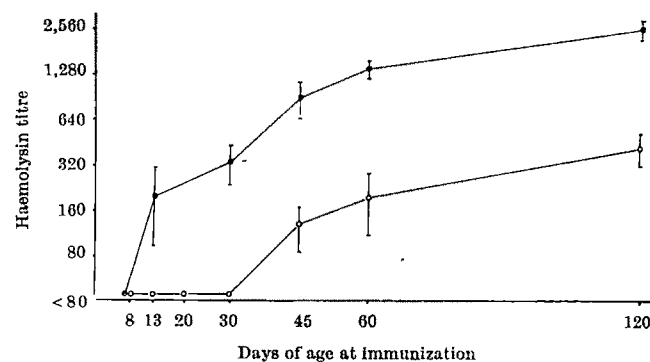


Fig. 1. Haemolysin titres in *SL* mice 4 days after immunization at various ages. Bars represent standard errors. O, Thymectomized mice; ●, sham-thymectomized mice.

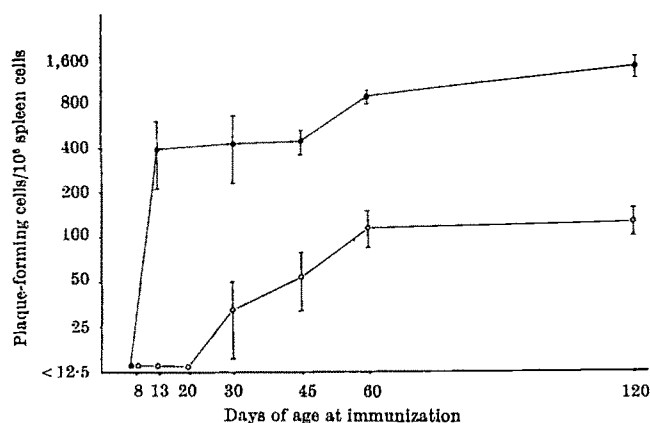


Fig. 2. Number of antibody-forming cells in spleens 4 days after immunization at various ages. Bars represent standard errors. O, Thymectomized mice; ●, sham-thymectomized mice.

The mean numbers of antibody plaque-forming cells in both groups of mice at various ages are shown in Fig. 2. They are roughly parallel to the results of haemolysin production. In the control group, immunization at 5 days failed to elicit a detectable response, while in mice immunized at 8 days old an increase in the number of plaque-forming cells was found. Thereafter, the cell number increased rapidly at 13 days of age and then gradually up to 120 days of age. On the other hand, in the thymectomized group no appreciable plaque-forming cells could be found in mice immunized until 30 days of age, when only less than one tenth of the number of cells as compared with that of the control group were detected. After 30 days the number gradually increased up to 120 days of age.

The skin homograft survival times are shown in Table 1. In the control group, skin grafts on the mice older than 15 days of age were rejected regularly after 10–13 days of survival. In the thymectomized group mice grafted at 15 days old showed a marked prolongation of rejection time and most of them accepted the skin graft permanently. On the other hand, a considerable degree of homograft immunity was observed in mice grafted at 60 days of age; these rejected the grafts mostly within 30 days.

Table 1. SURVIVAL TIMES OF C57BL SKIN GRAFTS TRANSPLANTED TO NEONATALLY THYMECTOMIZED AND SHAM-THYMECTOMIZED SL MICE OF VARIOUS AGES

Age of recipients (days)	Survival times of skin homografts (days)	
	Sham-thymectomized	Thymectomized
15	10, 11, 12	> 60, > 60, > 60, 42
30	10, 11, 11, 12, 13, 13	> 60, > 60, > 60, 41*, 33*, 27*, 16
60	11, 11, 11, 12	58*, 27*, 22, 19, 16, 16, 11

\* Died with intact skin graft.

The present results demonstrate that in normal mice the capacity to produce haemolysin plaque-forming cells and serum haemolysin develops rapidly between 8 and 13 days after birth and then increases gradually. The full homograft immunity was already recognized 15 days after birth in normal mice. The results of the plaque-forming cell response of normal mice of various ages coincide well with those reported in the rat<sup>6</sup>. It has recently been reported that newborn mice of different strains start to respond to immunization with sheep erythrocyte at different ages between 5 and 10 days<sup>7</sup>. According to the present results the SL mice appear to begin to respond at about 8 days of age.

In neonatally thymectomized mice, it was suggested that a low rate of production of competent cells for serum antibody and homograft immunity still continues even after thymectomy, because the number of haemolysin plaque-forming cells and serum haemolysin titre increases,

and homograft immunity is restored to some extent by the age of the thymectomized mice. These results are supported by recent findings of Rogister<sup>8</sup>, which were obtained by comparison of haemagglutinin production and homograft immunity between young and adult groups of neonatally thymectomized A.S.W. mice. It will be of interest to see whether this low rate of production of competent cells is caused by the influence which the thymus had exerted before thymectomy or by the function(s) of as yet unidentified system(s).

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### Cholera Toxin Neutralization and Some Cellular Sites of Immune Globulin Formation in *Cercopithecus aethiops*

As a rule, *Vibrio cholerae* does not enter the intestinal tissues and the blood stream. Cholera is a disease due to the toxins of the causative vibrio which are liberated in the lumen of the gut. The toxins have not yet been completely defined but the most important of them seem to be those of group 2 of Burrows<sup>1</sup>. These produce a cholera-like syndrome when injected into the upper part of the small intestine of infant rabbits or into the ligated intestinal loop of adult rabbits<sup>1,2</sup>. The serological responses of vervet monkeys (*Cercopithecus aethiops*) to crude cholera toxin prepared according to Oza and Dutta<sup>3</sup> and Burrows *et al.*<sup>2</sup>, phenolized cholera vaccine, and *V. cholerae* lipopolysaccharide have been investigated by our group<sup>4,5</sup>. It was found that the serum immune globulin G is the most important carrier of the *V. cholerae* toxicity neutralizing factor(s) in the circulating blood. The purpose of these experiments was to establish the antibody producing capability of mesenteric lymph gland and spleen cells, as well as to investigate the relationship of serum Ig-bound antibodies to the toxin neutralizing capability of Ig in the secretions and excretions of the intestinal tract.

Six adult vervets weighing 2 to 2.5 kg, of both sexes and of approximately the same age, were used. Two of them were immunized with the Watanabe type<sup>6,7</sup> *V. cholerae* lipopolysaccharide (LPS) prepared from El Tor strain B 17. Two injections of 1 mg each were given in six month intervals, and four animals were not immunized. Two of the latter group were challenged, together with those that received LPS, with the intracellular vibrio toxin (IC) of Burrows<sup>1,2</sup> administered through a duodenal sonde 2 weeks after completed immunization. The two unimmunized vervets served as controls. The amount of the IC used per monkey was derived from 5 to 5.4 × 10<sup>11</sup> of strain B 17 vibrios. Blood was drawn from all vervets before this operation, and all animals were bled 4 days later. Saliva was collected with the aid of plastic sponges<sup>8</sup> inserted for 30 min between the cheek and the gums of the vervets under anaesthesia. Laparotomy was performed on the same day on all six animals, under general anaesthesia. The intestines of the vervets that received IC appeared distended, engorged and contained much

Table 1. IMMUNE GLOBULINS IN BODY FLUIDS OF VERVETS AFTER INGESTION OF GROUP 2 CHOLERA TOXIN

Fluid	Ig	Collected	Vaccinated, challenged		Not vaccinated, challenged		Not vaccinated, not challenged	
			263*	620	83	116	610	621
Serum	G	b†	1,009‡	987	1,103	1,027	947	1,113
		a	1,237	1,118	1,205	1,107	969	1,116
	A	b	427	382	401	405	394	412
		a	477	425	414	439	399	409
Saliva	M	b	132	121	112	104	100	107
		a	151	168	129	122	98	104
	G	a	5	<5	5	7	<5	<5
		a	12	18	18	15	10	14
Stomach contents	M	a	<5	<5	5	6	<5	<5
		a	<5	5	6	8	<5	<5
	A	a	32	36	27	30	26	31
		a	<5	<5	<5	<5	<5	<5
Jejunal contents	G	a	162	154	109	128	62	70
		a	70	65	56	49	18	14
	M	a	7	6	5	<5	<5	<5
		a						

\* Serial number of vervet monkey.

† b, Before challenge; a, 4 days after challenge.

‡ In mg per cent.

liquid. Splenic biopsies were performed, and one mesenteric lymph gland near to the ileo-caecal valve was collected from each monkey. The contents of the upper jejunum were drawn into a syringe after punch enterotomy of an isolated 6 cm long loop. Gastric contents were aspirated by similar entry with a syringe into 0.07 M phosphate buffer, pH 7.8. The bleeding from the spleen was controlled by oxidized cellulose sponge application. The incisions of the jejunal and of the stomach wall were closed using inverting sutures with a continuous overlay to assure complete closure. The post-operative course was uneventful and complete in 7 days.

IgG, IgA and IgM from the sera and excreta were separated according to the method of Vaerman *et al.*<sup>8</sup>. The polysaccharide from *V. cholerae* biotype El Tor strain E 17 was absorbed on sheep red blood cells and the number of antibody producing cells in the splenic tissue and in the mesenteric lymph gland was determined by the procedure of Halliday and Webb<sup>9</sup>. IgA and IgM producing cells were suppressed by anti-A and anti-M sera to allow the enumeration of haemolysing IgG cells. On other plates, IgG and IgM were suppressed to allow the IgA forming cells to be seen. In the third experiment IgM was distinguished after addition of anti-G and anti-A sera. The cholera toxigenic activity of IC was titrated with the aid of the method of Read<sup>4,10</sup>. All tests were carried out in triplicate.

Table 1 demonstrates that all serum immune globulins increased after the challenge, while saliva contained principally IgA. This is in accordance with the observations of Tomasi *et al.*<sup>11</sup>, who found that this immune globulin predominates in the saliva. The gastric contents also revealed a preponderance of IgA. Fisher *et al.*<sup>12</sup> and Hurlimann<sup>13</sup> pointed out, however, that proteins in the gastric contents may be decomposed in spite of the neutralizing effect of the collecting fluid. More IgG was found in the upper jejunum, but the IgG : IgA ratio was lower than in the serum, a phenomenon discussed by Fisher *et al.*<sup>12</sup> for gastric disorders. Ig levels were not increased significantly in the saliva or in the gastric contents after cholera toxin administration when compared with those in unvaccinated and unchallenged animals. Higher IgG and IgA titres were found, however, in the intestinal contents of those vervets which received IC than in the control animals.

Table 2. NUMBER OF IMMUNE GLOBULIN PRODUCING CELLS IN VERVETS AFTER INGESTION OF GROUP 2 CHOLERA TOXIN

Tissue	Ig	Vaccinated, challenged		Not vaccinated, challenged		Not vaccinated, not challenged	
		263*	620	83	116	610	621
Spleen ‡	G	210†	230	106	132	<5	<5
	A	68	82	37	51		
	M	22	20	15	17		
Mesenteric gland	G	272	281	166	181	<5	<5
	A	97	102	61	74		
	M	15	15	10	12		

\* Serial number of vervet monkey.

† Per 10<sup>6</sup> cells, geometric mean of 3 determinations.

‡ Collected 4 days after challenge.

Table 2 shows that the proportion of cells producing immune globulins was elevated in unvaccinated but challenged vervets, and the number of such cells was even larger in vaccinated and challenged animals. It seems that the mesenteric lymph glands contained proportionally more Ig forming cells than the spleen. Chordirker and Tomasi<sup>14</sup> and Tomasi *et al.*<sup>11</sup> showed in their experiments with other antigens that adjacent lymph nodes produce more antibody than distant accumulations of lymphoid-plasma cells, and this rule may also apply to the present experiments in which the toxic substance was administered into the area drained by the examined mesenteric lymph glands.

The neutralizing capacity of the immune globulins could not be determined in all samples because of insufficient material. Table 3 shows that elevated titres were found in the blood sera of immunized vervets, and these levels increased further after challenge. Higher values were found in challenged and unimmunized monkeys than in the controls. The toxin neutralizing capability of IgA isolated from blood serum, saliva and stomach contents was rather poor, but this capacity was marked in IgA from the jejunal contents. The cholera toxin neutralizing capability of IgG from the jejunal contents per mg globulin was greater than that of the serum IgG, supporting the theory of Chordirker and Tomasi<sup>14</sup> and Tomasi *et al.*<sup>11</sup> about the production on mucosal surfaces of different subtypes of immune globulins. In our experiments, the sera against vervet IgG, IgA and IgM were prepared against globulins separated according to Vaerman *et al.*<sup>8</sup>. While these antisera gave one single narrow precipitation band in agar gel diffusion tests, it is quite possible that some or all immune globulins consist

Table 3. GROUP 2 CHOLERA TOXIN NEUTRALIZING CAPABILITY OF IMMUNE GLOBULINS IN VERVETS

Source of immune globulin	Ig	Collected	Vaccinated, challenged		Not vaccinated, challenged		Not vaccinated, not challenged	
			263*	620	83	116	610	621
Serum	G	b†	1.8 ‡	2.1	<0.5	<0.5	<0.5	<0.5
		a	2.9	3.1	1.1	0.9		
	A	b	<0.5	<0.5	<0.5	<0.5		
		a	<0.5	<0.5	<0.5	<0.5		
	M	b	<0.5	<0.5	<0.5	<0.5		
		a	<0.5	<0.5	<0.5	<0.5		
Saliva	A	a	0.8	0.5	<0.5	<0.5	<0.5	<0.5
Stomach contents	A	a	<0.5	0.6	<0.5	<0.5		
Jejunal contents	A	a	8.2	8.9	8.2	2.3		
	G	a	16.2	18.2	8.1	8.9		

\* Serial number of vervet monkey.

† b, Before challenge; a, 4 days after challenge.

‡ Units per 5 mg immune globulin.

of 2 or more fractions with distinct immunological behaviour, but there is at present no means of separating them. The experiments reported here do not, however, exclude the possibility that some circulating immune globulins are excreted selectively into the intestinal lumen, and it is quite possible that immune globulins reacting with cholera toxins are both excreted into the lumen of the intestines and also produced locally.

It should be noted that circulating toxin could not be demonstrated in the serum on the fourth day after its administration, nor did any of the excreta and secreta investigated by us reduce the neutralizing capability of specific immune serum against cholera vibrio IC when filtrates were tested at pH 7.6 to 8.2. It must be assumed, therefore, that cholera toxin did not interfere with these investigations. Finally, there may be errors inherent in the enumeration of cells producing Ig as well as errors introduced by the need to work with relatively small amounts of immune globulins in these experiments. We hope that further refinements of the techniques and the use of larger groups of animals will allow a more thorough investigation of the phenomena observed during these preliminary investigations.

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## HAEMATOLOGY

### Inhibition of Platelet Aggregation by a Sulphonic Polysaccharide

THE anticoagulant property of heparin is well known, but some of its other effects—such as lipolysis<sup>1</sup> and inhibition of platelet aggregation<sup>2-6</sup>—are less definite. Some compounds of similar structure may possess more lipolytic and anti-aggregating activity and less anticoagulant effect than heparin, and these compounds could conceivably have clinical uses. We have investigated a sulphonic polysaccharide similar to heparin (Fig. 1) in structure, which has a marked fibrinolytic action<sup>7,8</sup> and little anticoagulant effect.

The present communication is a preliminary account of the inhibition of platelet aggregation by this compound, which could be considered as an antithrombotic drug.

Platelet aggregation was measured by Born's method<sup>9</sup> with the results given as absolute and percentage values. Citrated bovine blood was used (30 ml. of 19 per cent sodium citrate added to each 1,000 ml. of blood). The citrated blood was then centrifuged at 1,500 r.p.m. for 20 min, giving a supernatant containing  $1.5 \times 10^5$ – $3 \times 10^5$  platelets/mm<sup>3</sup> and a few erythrocytes and leucocytes. Heparin (2 U/ml.) was added to the platelet rich plasma

(PRP) to prevent clotting. At this dosage platelet aggregation is not impaired<sup>10</sup>.

The PRP was incubated with the polysaccharide for 1 h at room temperature (about 20° C). The platelet aggregation was then measured by the variations of the optical density of the PRP in a spectrophotometer. The PRP was then placed in OS 1,001 tubes, with an equal amount of 0.013 molar calcium chloride and shaken with an electric agitator at 500 r.p.m. Readings were taken at 1 min intervals for 10 min.

The results are expressed in terms of absolute variations in optical density and by the index of platelet aggregation, that is, the ratio between the initial and final values of each curve obtained over 10 min, taking the initial value as 100 per cent of the optical density. This method was applied to 100 different plasmas<sup>10</sup>. The mean index of platelet aggregation was 46, with a standard deviation of 9.0, the normal limits being between 25 and 64.

Twenty-five different plasmas were investigated, each incubated with different amounts of the polysaccharide. The average of twenty-five curves of each amount was then obtained.

Table 1. MEAN AGGREGATION CURVE FROM TWENTY-FIVE PLASMAS WITH DIFFERENT DOSES OF THE SULPHONIC POLYSACCHARIDE

Time (min)	Optical density				
0	34.2	34.0	34.2	33.4	34.7
1	24.6	24.0	25.8	26.6	30.2
2	20.6	21.6	22.7	25.7	28.0
3	18.8	20.6	22.7	25.7	28.0
4	19.2	20.2	21.4	24.8	28.2
5	19.4	20.4	21.0	24.9	27.6
6	19.7	21.0	22.7	25.7	28.6
7	20.7	23.0	23.9	26.7	29.4
8	21.8	23.8	25.6	27.0	30.4
9	22.0	24.6	25.4	27.9	30.7
10	23.2	25.2	26.0	29.6	31.2
Aggregation index	54.9	59.4	62.5	74.2	79.5
Concentration of compound (mg/ml.)	0.00	0.006	0.012	0.120	0.30

Table 1 shows that as the dose of the polysaccharide increased, the ability of platelets to aggregate decreased, that is, the optical density diminished less, and the index of platelet aggregation increased. These increases correlate closely with the dose of polysaccharide used. It can be seen that with 0.12 mg/ml. plasma the index is 74, that is, the index lies outside the limits already mentioned.

It seemed to us very interesting to find a drug which is able to inhibit platelet aggregation without impairing the haemocoagulative state. This could have more interest if the drug is also capable of fibrinolysis at the same time, because the former action can be used in prophylaxis and the latter in therapy.

The polysaccharide investigated clearly inhibits platelet aggregation. This activity is present even with a dose of

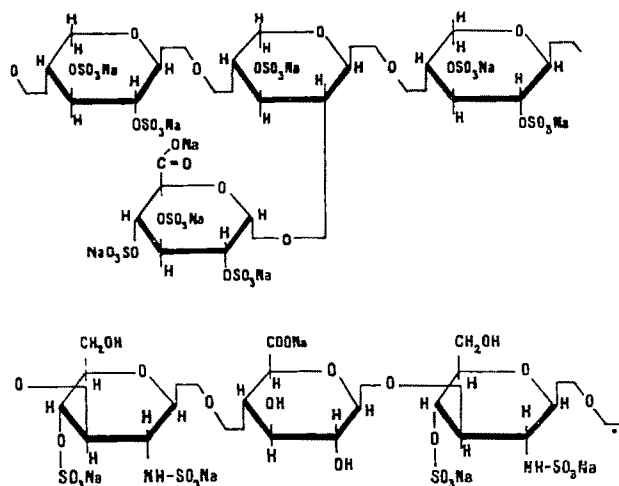


Fig. 1. Comparison between the structure of the sulphonic polysaccharide (top) and that of heparin (bottom).

0.012 mg/ml. of plasma, which is lower than that used in patients (about 0.06 mg/ml. of plasma). We therefore assume that this antithrombotic activity is evident *in vivo* as well as *in vitro*.

We believe that this substance may inhibit platelet aggregation by liberating peptides from fibrinogen, and that they might be adsorbed to the platelet membrane thus altering its ability to aggregate. This effect could also result from a lipolytic activity of the compound<sup>11</sup>, modifying the lipoproteins and phospholipids of the platelet membrane.

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## BIOCHEMISTRY

### Tissue Content of Citrate and Citrate-cleavage Enzyme Activity during Starvation and Refeeding

It is known that citrate, as well as being an intermediate of the tricarboxylic acid cycle, may also be involved in metabolic regulation because it increases the substrate affinity of acetyl-CoA carboxylase<sup>1</sup> and also acts as a direct inhibitor of phosphofructokinase<sup>2,3</sup>. In view of the contradictory reports concerning the concentrations of rat liver citrate in different physiological conditions it is of interest to present data on the citrate concentrations of different rat tissues during starvation and refeeding with carbohydrate. The rats used were normal adult females weighing 200–300 g which had been raised on a standard diet (Instytut Leków, Warszawa). During the refeeding period animals were maintained on a high-glucose diet<sup>4</sup>. Rats were always decapitated at 12 o'clock. Pieces of liver and whole kidneys, heart and brain were rapidly removed, in this order, and placed in a mixture of carbon dioxide and ethanol. The frozen tissue was weighed and ground in a mortar, which was precooled, with 5 per cent trichloroacetic acid, and made up to volume for citrate analysis. Citrate was estimated according to McArdle<sup>5</sup>. Before determining the concentration of citric acid, samples were heated in a boiling water bath for 10 min.

Table 1 shows the influence of starvation and refeeding with carbohydrates on the content of citrate in the brain, heart, liver and kidney of rats. It can be seen that after

Table 1. EFFECT OF FASTING AND REFEEDING WITH CARBOHYDRATE DIET ON THE CONCENTRATION OF CITRATE IN RAT TISSUES

Treatment	Liver Concentration (μmoles/100 g wet weight of tissue)	Kidney Concentration (μmoles/100 g wet weight of tissue)	Heart Concentration (μmoles/100 g wet weight of tissue)	Brain*
Normal fed	12 ± 3 (8)	19 ± 4 (12)	22 ± 8 (5)	23, 22
24-h fast	9 ± 1 (12)	27 ± 3 (11)	39 ± 10 (5)	20, 24
48-h fast	12 ± 3 (15)	41 ± 5 (10)	37 ± 11 (5)	21, 23
72-h fast	9 ± 3 (6)	37 ± 7 (6)	31 ± 8 (7)	23, 23
8-h refeed	18 ± 2 (5)			
16-h refeed	18 ± 3 (9)			
24-h refeed	18 ± 3 (8)	21 ± 5 (12)	20 ± 7 (7)	23, 21
48-h refeed	14 ± 4 (19)	23 ± 7 (12)	22 ± 5 (7)	24, 24
72-h refeed	10 ± 2 (4)	18 ± 7 (4)	18 ± 2 (4)	23, 22

The values are the mean ± S.D. The number of animals in each group is indicated in parentheses.

\* Two rats were used in each experiment.

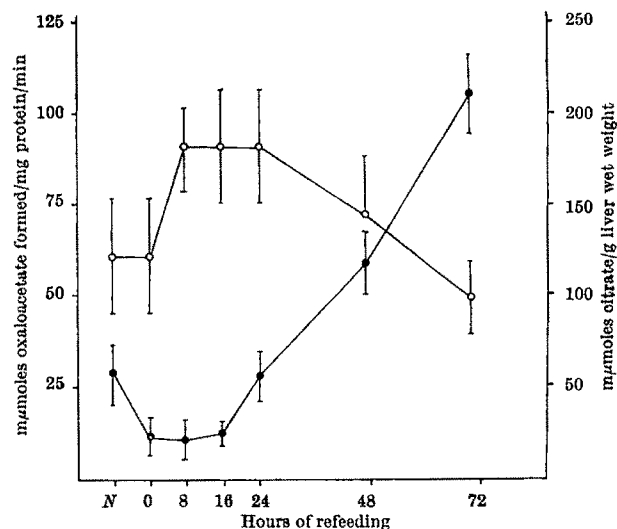


Fig. 1. The activity of citrate-cleavage enzyme and the concentration of citrate in liver of control rats, starved rats, and rats refeed with carbohydrate. The activity of citrate-cleavage enzyme was measured spectrophotometrically with the malate dehydrogenase method of Srere<sup>12</sup>. Rates of NADH oxidation were strictly proportional to protein concentration and linear with time. The high speed supernatant fraction (37,000g for 45 min) prepared in a medium containing 150 mmolar potassium chloride was used. The results are given as mumoles oxaloacetate formed/mg liver protein/min at 37° C (●—●), and mumoles of citrate/g of liver (○—○). Normal values (N) and values obtained 48 h after starvation (0) are shown for comparison. Mean values are also given. The vertical lines represent ± S.D. Each group contained between four and fifteen animals.

starvation for 24 h the concentration of citrate in the liver is about 30 per cent lower than normal. In contrast, the concentration of citrate in heart and kidney is greater by a factor of two than the control values. A similar pattern of change was observed after 48 h of starvation for heart and kidneys, whereas the concentration of citrate in liver returned to control values. The concentration in brain tissue did not change even after starvation for 72 h, or during the refeeding period. After 24 h of refeeding with carbohydrate, the concentration of citrate in the liver became about 50 per cent greater than the control values, while the concentrations in kidney and heart tissues decreased to normal. After refeeding for 48 h the concentration of citrate in the liver dropped to control values and on the next day of refeeding was even lower than normal. The results described here are compatible with the data reported by Parmeggiani and Bowman<sup>6</sup> for concentrations of citrate in heart both in the normal state and after fasting for 48 h. Our results are different from the unpublished data quoted by Tubbs and Garland<sup>7</sup> in respect of concentration of citrate in rat liver during starvation. These authors stated that "liver citrate concentrations are doubled or trebled by starvation". Quite opposite results were obtained by Lynen *et al.*<sup>8</sup> and they showed that "the citrate level in the rat liver after 24 h fasting has sunk to 50–60 per cent of the normal values". These latter results are similar to the data presented here. The significantly different results obtained by Frohman *et al.*<sup>9</sup> may be attributable, among other factors, to the different method used to determine the concentration of citrate and also to the different procedure used in sampling.

It is suggested that concentration in the soft tissue may be related to the difference between the activity of the citrate condensing enzyme and the activity of the citrate-cleavage enzyme. From the data presented by Srere<sup>10</sup> it is found that the ratio of the activity of the citrate condensing enzyme to that of the citrate-cleavage enzyme in rat liver is about 8, whereas analogous ratios for rat kidney and heart are 48 and 112, respectively. It is therefore plausible to assume that significant changes in the activity of citrate-cleavage enzyme, which depend on the degree of carbohydrate utilization<sup>11</sup>, may be correlated with the



concentration of citrate in the liver tissue. Fig. 1 illustrates the relation between the concentration of citrate and the activity of citrate-cleavage enzymes in rat liver tissue during starvation and refeeding with a carbohydrate diet. It can be seen that the activity of citrate-cleavage enzyme decreases in the liver of starving rats and remains at the same low level during the first 16 h after refeeding with a carbohydrate diet. During this last period the concentration of citrate in the liver is increased. This is followed by the low activity of citrate-cleavage enzyme up to 16 h of refeeding. The increase observed in the concentration of citrate in the liver may have resulted from either an increase in activity of the citrate condensing enzyme and reversal of the reaction of the extra-mitochondrial isocitrate dehydrogenase, or alternatively, from persistent low activity of the citrate-cleavage enzyme. Fig. 1 reveals a reverse relation between the concentration of tissue citrate and the activity of citrate cleavage enzyme after 48 h of refeeding. A marked increase in the activity of citrate-cleavage was associated with a gradual reduction in the concentration of citrate even to less than the normal range.

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### Turnover of Brain Messenger RNA

RECENT advances in molecular biology have indicated the importance of messenger RNAs which enable the nucleotide sequence of a specific DNA region to be translated into a specific protein. In mammalian brain, as in liver, such RNA templates can be operationally defined by the presence of RNA strands which help to bind ribosomes into large functional aggregates, polysomes, and by their ability to direct the incorporation of amino-acids into proteins (refs. 1 and 2, and Appel, S. H., Scott, S., and Davis, W., in preparation). The fact that environmental stimuli can alter neuronal RNA raises the question of whether brain template RNAs are sufficiently stable to persist as permanent records of environmental changes.

Recent investigations using actinomycin D give conflicting values for the apparent half-life of mammalian liver messenger RNA. Staehelin *et al.*<sup>3</sup> originally estimated the half-life to be several hours, using large doses<sup>3</sup> (5 mg/kg) and an acellular system. On the other hand, Revel and Hiatt<sup>4</sup> demonstrated that liver messenger RNAs were stable for at least 17 h. These authors contend that the results in the cell-free system are related to an indirect action of actinomycin D which reduces the number of polyribosomes present in disrupted tissue<sup>5</sup>. Furthermore, Acs *et al.*<sup>6</sup> demonstrated a direct degradative effect on cellular RNA with actinomycin D, and Honig and Rabinovitz<sup>7</sup> demonstrated that glucose could reverse the inhibition of protein synthesis which followed the inoculation of actinomycin D.

In the present investigations I used small doses of actinomycin D to inhibit the synthesis of brain messenger

RNA and, in turn, the synthesis of protein. There was no discrepancy between the results of the *in vivo* and *in vitro* methods of analysis. Outstanding was the fact that small doses (1–20 $\gamma$ ) were lethal for rats weighing 150 g in 36–72 h when the drug was inoculated intracerebrally. Intraperitoneal injections, on the other hand, had little effect on the metabolism of brain RNA presumably because of the failure to pass the blood-brain barrier. The toxicity of the drug in such small doses may well reflect compartmentalization within the brain, but it may also reflect either the sensitivity of brain tissue to the inhibition of RNA synthesis or other actions of the drug. Measurements were made of respiration on brain slices prepared from animals inoculated 12 h previously with 20 $\gamma$  of actinomycin D and compared with animals similarly inoculated with manitol (Dr. J. Lazlo kindly performed these determinations). The results were identical in the two groups. Measurements of anaerobic glycolysis, however, yielded a 30 per cent inhibition in slices from the animals treated with actinomycin D. The meaning of this observation is not clear because of the relative insignificance of this energy producing pathway in brain.

For the studies on the turnover of brain messenger RNA, Osborn-Mendel rats weighing 150 g were injected into the frontal lobes with 20 $\gamma$  (20  $\mu$ l.) of actinomycin D (solubilized with mannitol) and were compared with animals similarly injected with mannitol on its own. The animals were then killed at varying intervals after inoculation. As an index of RNA synthesis *in vivo*, animals were inoculated intracerebrally with radioactive uridine approximately 45 min before they were killed. It had been established in previous experiments that incorporation into RNA was linear for at least 60 min, and that under these conditions actinomycin D inhibited the incorporation of uridine into total brain RNA of 60, 72, 78, and 84 per cent in 1, 4, 8, and 24 h, respectively. Preliminary experiments indicated a decrease in amino-acid incorporation into protein of 70 per cent by 12 h after treatment with actinomycin D. *In vivo* studies with isotope tracer were complicated, however, by the fact that the availability of messenger RNA may not have been the rate-limiting step under the experimental conditions used. An action of actinomycin D, for example, on the availability of high energy substrate may well make diffusion of the isotope, isotope passage across cell barriers, charging of tRNA, or the assembly of amino-acyl-tRNA on ribosomes the rate-limiting step.

As a more direct evaluation two types of *in vitro* experiments were used. It was argued that following interruption of RNA synthesis the rate of protein synthesis *in vitro* would more accurately reflect the availability of messenger RNA. Brain slices were prepared from animals at varying intervals after inoculation of actinomycin D. They were incubated with Krebs-Ringer phosphate or Krebs-Ringer bicarbonate solutions and radioactive leucine and the incorporation into tissue proteins determined. As can be seen in Fig. 1, there is a decline in the ability to incorporate amino-acids which depends on the duration of *in vivo* exposure to actinomycin D, and which appears more rapidly in the subcortical regions. Furthermore, the inhibitory effect was entirely independent of the concentration of glucose present in the incubation media. As a confirmation, protein synthesis was measured in a cell-free system under conditions in which messenger RNA was limiting. Ribosomes were prepared from either the cortex or subcortex of animals killed at different periods after *in vivo* inoculation with actinomycin D. An equivalent concentration of ribosomal RNA or protein, presumably with different concentrations of messenger RNA present, was incubated with a supernatant or pH 5 fraction from control brain, and appropriate cofactors. Here, as in the brain slice experiments, a decrease in the rate of protein synthesis was noted (Fig. 2).

In both brain slice and ribosomal systems there is a more significant inhibition of protein synthesis in the subcortical

tissue suggesting a more rapid turnover for messenger RNA. This may have been related to the greater sensitivity of subcortical tissue or to the greater concentration of drug reaching the areas. Either of these possibilities would explain the striking morphological effect on the hippocampus and contiguous structures and 15 per cent greater inhibition of RNA synthesis in the subcortex than in the cortical areas after *in vivo* injections<sup>8</sup>. When actinomycin D is incubated directly with brain slices *in vitro*, however, the inhibition of RNA synthesis is only slightly increased; there is a consistent but small further increase in the inhibition of the synthesis protein in the subcortex compared with the cortex. This observation suggests the presence in subcortical tissue of a messenger RNA with a more rapid turnover than that in the cortical tissue; however, the most plausible explanation for our results is the higher concentration of drug reaching the subcortex. These data, however, are only tentative because of the low level of RNA synthesis in the brain slice preparations. The more complete inhibition of RNA synthesis in the subcortex permits a valid estimate in that region of the turnover of messenger RNA in brain as assayed by the decay in rate of protein synthesis. It is of interest that after the ability to incorporate initially declines to 25 per cent of the zero time control, no further decline is noted from 4 h to 24 h in both brain slice and cell-free systems. This would suggest that in terms of stability at least two populations of messenger RNA may exist in the subcortex: a labile fraction with a half-life of less than 4 h representing most of the template RNAs present, and another smaller population of molecules which are stable for greater than 20 h. The data also suggest a population of relatively stable molecules in the cortex with a turnover of less than 10–12 h. Because of the slower inhibition of RNA synthesis, however, presumably secondary to the lower concentration of drug, analyses of cortical messenger RNA turnover are necessarily imprecise. Furthermore, the investigations are not of sufficient duration to rule out a second more stable population of mRNAs in the cortex as seen in the subcortex.

Measurements of the turnover of RNA in the brain slices and cell-free material do not encounter the discrepancies noted in liver systems. On the other hand, these investigations do not exclude an indirect effect of

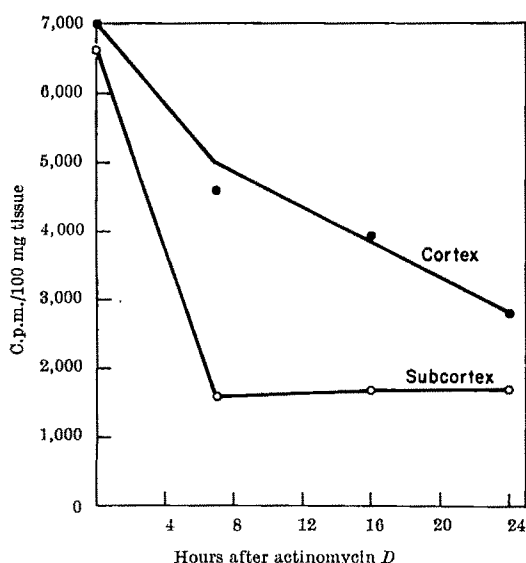


Fig. 1. At intervals after treatment with actinomycin D, rats were decapitated, their brains excised, the cortex separated from subcortex and sliced with a razor. 100 mg portions were blotted and incubated at 37° C in Krebs-Ringer phosphate buffer with dextrose and leucine-14 after gassing with oxygen. Incorporation of radioactivity into hot TCA insoluble protein was sampled at 60 min, during the linear portion of incorporation. Each point is an average of slices from three animals each performed in triplicate. There were no significant time-dependent differences in either medium or intracellular radioactive pools.

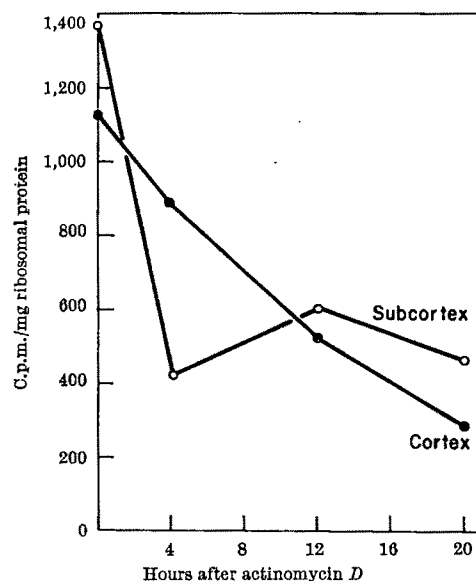


Fig. 2. Rats were decapitated at intervals after treatment with 20 $\mu$ g actinomycin D, the brains excised, the cortex separated from subcortex, suspended in 0.25 molar sucrose containing 0.005 molar magnesium chloride, 0.025 molar potassium chloride, 0.05 moles tris Cl pH 7.6 and homogenized in a loose glass homogenizer. After centrifugation at 10,000 g for 10 min and 20,000g for 20 min, the supernatant solution was made 0.9 per cent in sodium deoxycholate and spun through a 0.5 molar sucrose/2.0 molar sucrose gradient containing the above salts at 105,000g for 2 h. The clear pellet was used as the source of ribosomes. Other additions to the incubation consisted of a supernatant fraction prepared from uninjected animals, ATP, and ATP generating system, GTP, ammonium chloride and leucine-14C. The incorporation of radioactivity into protein insoluble in hot trichloroacetic acid was linear for at least 30 min. The points on the graph represent the averages of incorporation at 20 min with ribosomal preparations from four animals; each experiment was performed in triplicate. For the zero time point the animals were injected with actinomycin D and immediately killed.

actinomycin D on the breakdown of messenger RNA, independent of its inhibiting nucleic acid synthesis. In these circumstances our data may reflect faster rates for turnover than would obtain under most physiological situations.

The presence of both labile and stable brain messenger RNA moieties would confer significant adaptive value allowing shifts in certain aspects of the metabolic expression of the cell while other aspects remained stable. It is tempting to speculate that either the labile or stable components may affect intercellular communication and synaptic activity. In these circumstances stability of information may be determined by both quantitative and qualitative features of neuronal activation and may depend on the half-life of the mRNAs involved as well as on the configurational changes and half-lives of enzymes which catalyse the interneuronal metabolic processes.

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### Glucosamine Acetoacetate Condensate: a New Anti-Alloxan-Diabetes Factor

"It has been postulated that a gradual accumulation of ketone bodies in the system might be responsible for the onset of hyperglycemia and that the rise of blood sugar might be due not only to overproduction or nonutilization of glucose but, also, to a natural mechanism to combine

with some toxic metabolites in the system.<sup>11</sup> It has been proposed that the condensation product of glucose and acetoacetate (furoate condensate) prevents alloxan diabetes even when the product is in very low concentration, and that this condensation product is formed in the urine of normal people and not in those of diabetic patients<sup>2</sup>. A new route of glucose utilization and ketolysis is suggested, therefore<sup>3</sup>.

The condensation product of D-glucosamine and acetoacetate, ethyl 2-methyl-5-(D-arabinotetrahydroxybutyl)-3-pyrrolecarboxylate (pyrrole condensate)<sup>4</sup> could be involved in the biological phenomenon of antiketogenesis or ketolysis and the prevention of experimental diabetes, such as that produced by alloxan. The occurrence of the condensation product of glucosamine and acetoacetate *in vitro*, without the need of catalysis as needed in the formation of furoate condensate, suggested that perhaps the pyrrole condensates may be of importance as an antidiabetogenic factor.

Our results indicated that the 10 per cent ferric chloride test alone on urine as described by Nath and Sahu<sup>5</sup> is not sufficiently specific for use as a criterion for detecting the presence of furoate condensate. Of fifty-two diabetic urine samples tested, 27 per cent showed positive or any colour formation, and of thirteen normal urine samples 23 per cent showed any positive colour formation. As described by Nath, only normal urine gave a positive ferric chloride test indicating the presence of a furoate. Further, our paper electrophoretic investigations of normal and diabetic urine using Beckman model R at pH 7.4 of phosphate buffer followed by elution with 95 per cent ethyl alcohol and thin-layer chromatography (Eastman chromogram silica gel with butanol-acetic acid-water solvent, 4:1:5) showed that out of sixteen diabetic urines six showed pyrrole condensate and thirteen showed furoate condensates. Out of ten normal urine samples tested five showed the presence of furoate. The furoate condensate showed a characteristic dark blue fluorescence with an  $R_F$  value of 0.90 and ultra-violet wavelength 2840 Å and the pyrrole condensate shows a characteristic light blue fluorescence at the same wavelength and with an  $R_F$  value of 0.83.

Our data indicated the presence of furoate condensate both in normal and diabetic urine and the pyrrole condensate only in diabetic urine. This finding is further substantiated by our urine hydrolysis experiments<sup>6</sup> which show an increase, up to 100 per cent in five samples of diabetic urine, of glucosamine content after hydrochloric acid hydrolysis without any increase noted in the normal urine sample (Fig. 1).

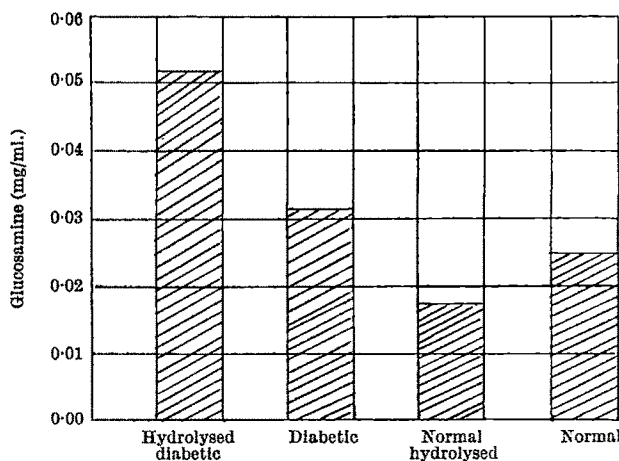


Fig. 1. Average value of experimental data versus concentration.

The role of pyrrole condensates in experimental diabetes was investigated using white rats weighing 250–300 g, which have received approximately 300 mg of alloxan/kg of body weight simultaneously with pyrrole or from 30 min to 1 h afterwards. The results are shown in Table 1.

We conclude that the glucosamine-acetoacetate condensate was capable of preventing alloxan diabetes symptoms in approximately 86 per cent of the rats challenged with alloxan. The manner by which alloxan diabetes is prevented is not demonstrated except that liver glycogen<sup>8</sup> depletion which occurs in rats treated with alloxan was completely inhibited, possibly indicating that the pyrrole condensate is involved in experimental diabetes as a glycogenic factor, and that its presence in human diabetic urine suggests some role as an antidiabetogenic metabolite.

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Table 1

Group I. Pyrrole condensate and alloxan injected simultaneously in the molar ratio of 1.5 to 1

No. of animals	Glucosuria*	Ketosuria*	Blood sugar concentrations†
5	—	—	Normal (72–114)†
2	—	—	Below normal (38–62)

\* Determined using 'Clinitest' and 'Ketostix' made by Ames Co.

† Normal values for blood sugar concentrations were considered 68–150 mg/100 ml.

Group II. Pyrrole condensate injected before alloxan in the molar ratio of 1.5 to 1

No. of animals	Glucosuria	Ketosuria	Blood sugar concentrations
8	—	—	Normal (70–132)
5	—	—	Above normal (202–300)
2	Positive	—	High (480–862)

Group III. Pyrrole condensate injected before alloxan in the molar ratio of 0.5 to 1.0

No. of animals	Glucosuria	Ketosuria	Blood sugar concentrations
19	—	—	Normal (68–150)
7	—	—	Below normal (22–60)
5	Positive	—	High (154–776)

Group IV. Injected with alloxan only (0.0004 moles)

No. of animals	Glucosuria	Ketosuria	Blood sugar concentrations
11	Positive	Positive	(800–1,108)
8	Positive	—	(320–520)

Group V. Injected with pyrrole condensate only (0.0006 moles)

No. of animals	Glucosuria	Ketosuria	Blood sugar levels
15	—	—	(54–130)

## Chondroitin Sulphate and Collagen in Inherited Skeletal Defects of Chickens

MUTANT chickens and chick embryos with well characterized hereditary defects of the skeleton<sup>1,2</sup> were obtained from the breeding flocks of the Agricultural Experimental Station of the University of Connecticut by kind permission of Drs. W. Landauer and L. Pierro.

Creepers, heterozygous for the dominant micromelia to which the breed owes its name, and normal siblings 12 weeks old were compared by methods described elsewhere<sup>3</sup> with respect to the composition of sternum cartilage and long bones in each sex. No differences in the content and type of chondroitin sulphate or in the content of collagen were found. Bones contained only chondroitin sulphate A, while the acid mucopolysaccharides of cartilage consisted of a mixture of 80 per cent chondroitin sulphate A and 20 per cent chondroitin sulphate C.

Three types of micromelic chick embryos, each homozygous for a recessive lethal mutation, were compared with normal siblings at the age of 17 days with reference to concentrations of acid mucopolysaccharides in sternum

cartilage and limb cartilage. Tissues from between thirty and sixty embryos of each type were pooled. No differences were found for the micromelia characteristic of the crooked-neck dwarf mutant or for that of Lamoreux's chondrodystrophy. In both instances, the acid mucopolysaccharides of cartilage consisted of about 60 per cent chondroitin sulphate A, 30 per cent chondroitin sulphate C and 10 per cent repeating periods of chondroitin sulphate lacking sulphate, and this was true for the mutant embryos as well as for their normal sibs. Cartilage of nanomelic embryos<sup>2</sup>, however, contained only about 10 per cent of the normal amount of chondroitin sulphate, a difference from normal easily demonstrated histologically by staining with toluidine blue. An investigation of this material was therefore undertaken in greater detail.

Various tissues were obtained from two groups of between thirty and forty nanomelic and normal embryos. Acid mucopolysaccharides were isolated and fractionated with cetyl pyridinium chloride and on 'Dowex 1x2' resin columns. The results in Table 1 indicate that nanomelic cartilage contains about a tenth of the chondroitin sulphate of normal cartilage, while a smaller reduction occurs in bone. Concentrations of collagen are comparable in cartilage but seem to be greater in nanomelic bone, possibly as a result of decreased mineralization. The purified chondroitin sulphate preparations from nanomelic and normal limb cartilage are similar in electrophoretic mobilities with near equimolar sulphate and galactosamine contents and have identical intrinsic viscosities of  $0.39 \pm 0.02$ . The principal residual amino-acid of chondroitin sulphate is serine in molar ratio to galactosamine of 0.020 and 0.026, respectively, for the nanomelic and the normal preparation.

Nanomelic skin has an essentially normal content of acid mucopolysaccharides. Similarly, nanomelic vitreous humour does not differ from normal in contents of total glucosamine and total galactosamine, which were 0.21 and 0.12  $\mu$ moles/ml., respectively.

Polymeric acid mucopolysaccharides could not be isolated from allantoic fluids of normal or nanomelic embryos. The normal and nanomelic fluids (about 2 ml. for each embryo) contained 1.44 mg/ml. and 1.58 mg/ml. of total hexosamine, respectively, divided almost equally between glucosamine and galactosamine; sulphate was less than 0.1 mole/mole hexosamine in both fluids. Because excretion of breakdown products in the allantoic fluid was not detected, it seems unlikely that the reduced content of chondroitin sulphate in nanomelic cartilage arises from the greater breakdown of the macromolecule.

We may therefore tentatively assume that a mechanism of synthesis is defective. The apparently normal rates of biosynthesis of glucosamine and galactosamine in tissues other than skeletal would presumably rule out a systemic deficiency of remote biosynthetic precursors such as glucose<sup>4</sup>. Furthermore, the local nutritional environment is unlikely to play an important part because the concentration of chondroitin sulphate is lower than normal at all skeletal sites. The simultaneous, almost normal concentrations of collagen (measured by hydroxyproline)

indicate the specificity of the effect on biosynthesis of the polysaccharide because normal, embryonic chick chondrocytes apparently produce chondroitin sulphate and collagen at the same time<sup>5-7</sup>.

It is necessary, however, to account for the apparently normal metabolism of chondroitin sulphate in nanomelic skin. In normal embryonic and adult chick cartilage, chondroitin sulphate chains are covalently linked as side chains to serine residues (unpublished observations) of an extended polypeptide backbone to yield a large macromolecule<sup>8,9</sup>. It is probable that chondroitin sulphate of embryonic chick skin is similarly linked to a polypeptide backbone. First, it can be assumed that the polypeptide backbones of chondroitin sulphate-polypeptide molecules of skin and cartilage, respectively, are different, and that mutation has affected a structural gene coding for the specific polypeptide in cartilage. Then, because synthesis of chondroitin sulphate depends on previous synthesis of a polypeptide<sup>10,11</sup>, production of a defective acceptor polypeptide results in restricted formation of polysaccharide chains.

Alternatively, if the molecular structure of the chondroitin sulphate-polypeptide is determined by identical structural genes in both tissues, then the inherited defect appearing only in chondrocytes may be in the genetic regulatory apparatus which controls the biosynthesis of the macromolecule in this type of cell. A defective enzyme for a reaction rate-limiting only in cartilage, however, is not excluded.

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## PATHOLOGY

### Effects of Tobacco Smoke on Lung Tissue as measured by Electron Spin Resonance

THE presence of free radicals in tobacco smoke has been reported already. During the course of a programme concerned with free radicals and alkylating agents in tobacco smoke it was observed that the concentration of free radicals in tobacco smoke condensate varied considerably as a function of methods used for collecting and subsequent treatment with solvents. To determine the biological effects of free radicals it became apparent that a method was needed whereby smoke could be directly applied to lung tissue without first collecting and processing the condensate. The method reported here enabled lung tissue to be directly exposed to cigarette smoke in a manner similar to normal smoking and resulted in the observation of distinct changes in the lung tissue as measured by electron spin resonance (ESR).

Table 1. COMPOSITION OF NANOMELIC AND NORMAL TISSUES\*

Type	Tissue (%)	Nitrogen (%)	Hydroxy-proline (%)	GluN (%)	GalN (%)	GalN of CS (%)
• Normal	Limb cart.	10.6	3.5	0.67	4.30	2.87
Nanomelic	Limb cart.	11.8	4.1	0.55	0.39	0.34
Normal	Bone	5.8	2.2	—	—	0.28
Nanomelic	Bone	6.5	3.2	—	—	0.08
Normal	Sternum cart.	—	—	—	—	2.16
Nanomelic	Sternum cart.	—	—	—	—	0.20
Normal	Skin	—	2.1	0.29†	0.29	0.070 ‡
• Nanomelic	Skin	—	1.8	0.25†	0.25	0.073 ‡

\* Dry-weight basis. GluN, glucosamine; GalN, galactosamine; CS, chondroitin sulphate.

† 0.090 per cent GluN of normal skin and 0.070 per cent GluN of nanomelic skin isolated as sodium hyaluronate.

‡ In both normal and nanomelic skins, about a third of the CS is chondroitin sulphate B; the remainder is a mixture of chondroitin sulphates A and C.

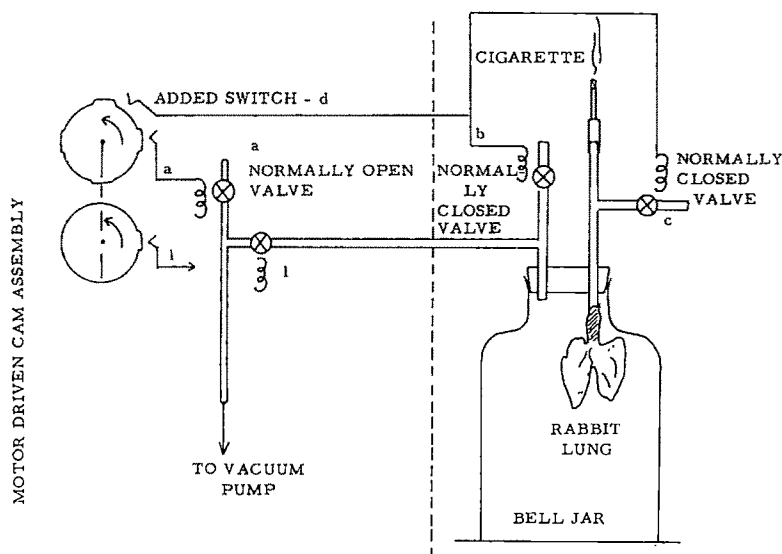


Fig. 1. Smoking apparatus.

Large adult rabbits of unknown strain and obtained locally were used. Each animal was killed by cervical dislocation and the lungs and trachea were excised intact as rapidly as possible. This was carried out within 10 min of death. The trachea was then attached to the apparatus shown in Fig. 1 using thin twine to hold it in place. The smoking apparatus (Fig. 1) consists of a bell jar, valving, and a Phipps and Bird smoking machine that was modified to exhaust the bell jar partially, hold it in the exhausted state, and then admit atmospheric air into the jar. The timing of the cycle is controlled by the cam and motor arrangement contained in the smoking machine. The smoking parameters used were: puff frequency, 30 sec; puff duration, 2 sec; inhalation time, 4 sec; butt length, 15 mm. The puff volume varied with the capacity of the lungs during each experiment. The volume was adjusted so that the lungs would fully inflate without rupturing. During the initial phases of this work several lungs did rupture, thereby allowing smoke to enter the bell jar. This was later prevented by first using the minimal puff volume that would inflate the lungs and step-wise increasing the volume until the lungs would inflate with a turgid appearance. King size, non-filtered cigarettes, manufactured in the United States, were used. The cigarettes were not preconditioned before use. They were inserted into a holder composed of rubber tubing and were

ignited by means of a lit cigarette of the same brand. A total of six cigarettes were smoked into the lungs for each experiment.

As soon as the smoking was completed the lungs were removed from the apparatus and homogenized at room temperature for 30 sec in an 'Omnimixer'. A cylindrical sample of the homogenate was then prepared and placed into a Varian liquid nitrogen dewar. Electron spin resonance spectra of the sample were then recorded at 77° K using a Varian '4500 X' band spectrometer using 100 kc/s modulation. A typical spectrum is shown in Fig. 2 together with the spectrum obtained from an unsmoked lung using the same instrument parameters and sample size. The observed spectrum was reasonably reproducible, although small changes in the relative magnitudes of the three line pattern superimposed on the broad resonance line were noted from sample to sample indicating that the observed pattern is composite. A considerable amount of additional data is required before we can

hope to arrive at any definite conclusions about the species giving rise to the signal. The similar pattern that Swartz observed in irradiated blood suggests, however, that the observed spectrum could be produced by the interaction of the free radicals in tobacco smoke with the red blood cells. This is being further investigated on lung experiments and on a series of model systems including metal porphyrin complexes. In Fig. 3 we have included the

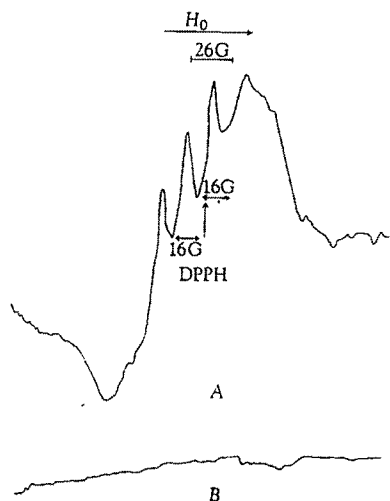


Fig. 2. A, ESR of lung which has smoked six cigarettes; B, ESR of control.

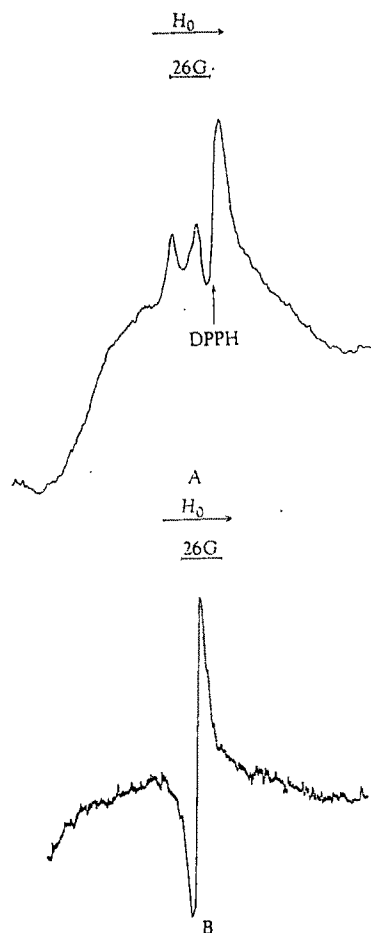


Fig. 3. A, ESR of vacuum dried smoked lung at 77° K; ESR of vacuum dried lung at room temperature.



spectrum obtained from freeze dried lung tissue exposed to smoke. Fig. 3A represents the spectrum obtained at 77° K and Fig. 3B the same sample run at room temperature. The room temperature signal consists of a single narrow line which is typical of the electron spin resonance signal obtained from tobacco smoke condensate. On refreezing to 77° K the electron spin resonance signal observed reverts to that shown in Fig. 3A.

It is apparent from this work that the smoking system that is reported has proved to be a reliable method for exposing intact lung tissue to fresh cigarette smoke comparable with the manner of human exposure. The results are not biased by changes in the smoke that occur as a result of trapping and processing. We cannot at this time be certain of the origin of our observed electron spin resonance signal. The similarity of it to that observed by Swartz on irradiation of red blood cells causes us to anticipate that both his and our results can be explained by the formation of a covalent hexacoordinated ferric haemoglobin complex formed because of the interaction of certain molecules found in tobacco smoke or molecules formed by irradiation damage, with the haemoglobin molecule in which the bonding is normally ionic in nature. Further work is being conducted to establish this point.

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### Carbon Tetrachloride Effect on Liver Autotransplants with Totally Reversed Blood Flow

LOCALIZATION of injury to specific zones in the liver lobule after the administration of many toxins is well recognized<sup>1</sup>. Carbon tetrachloride, an agent extensively examined in hepatotoxicology, characteristically produces centrilobular necrosis and lipid accumulation. Himsworth<sup>2</sup> and, more recently, Brody<sup>3</sup> suggested that the centrilobular location of injury after acute carbon tetrachloride intoxication resulted from centrilobular ischaemia secondary to the hydrocarbon's effect (directly or indirectly) on blood passing from the periphery to the centre of the lobule. This concept has been challenged on several grounds including the observation that the earliest signs of parenchymal cell injury precede vascular changes associated with ischaemia<sup>4</sup>. Several investigations have indicated that hepatic cells within different zones of the lobule have dissimilar function<sup>5</sup>. This has been ascribed to the relation of the hepatic cell to the blood flow through the lobule which causes these cells to adopt particular functions in accordance with available substrates or metabolites in the blood. Wilson describes certain zonal functions as representing habitual patterns of activity which become characteristic of hepatocytes in a particular location<sup>6</sup>. Zonal heterogeneity of function may explain the centrilobular location of acute carbon tetrachloride injury if it is assumed that hepatocytes in this location are susceptible to its effect.

Both the vascular and metabolic explanations of the zonal nature of the carbon tetrachloride lesion are related to the direction of the blood flow in the liver lobule, and so an experiment was designed to observe the effects on liver tissue of acute carbon tetrachloride poisoning in conditions of total blood flow reversal in the liver. A preliminary report of this work has been presented elsewhere<sup>7</sup>.

In mongrel dogs of both sexes (9.8–14.7 kg body weight),

the left lateral and left central lobes of the liver were removed. The left central lobe was utilized for baseline histological tissue sections. The left lateral lobe (about 25 per cent of the entire liver) was transplanted to the right side of the neck after ligation of the lobar bile duct and hepatic artery. The details of the grafting technique are given elsewhere<sup>7</sup>, and only a general description is given here. In "straight" blood flow liver autografts, transplantation was achieved by anastomosis of the lobar portal vein to the cephalic end of the carotid artery and the hepatic vein to the jugular vein. "Reverse" blood flow grafts were constructed by anastomosis of the carotid artery to the lobar hepatic vein and the portal vein to the jugular vein. Both anastomoses were end-to-end. The period of graft ischaemia was about 30 min. Arterial blood entered the cephalic segment of the carotid artery through collateral channels and blood flow and pressure (about 70 per cent of normal mean arterial pressure) were less than in the proximal segment. The hepatic artery of the graft was ligatured, and so the entire source of blood supply was thus arterialized blood at reduced pressure. "Straight" blood flow grafts served as controls for observations on the "reverse" blood flow transplants. One to twenty-three days after the grafts were established, each animal was injected intramuscularly with a single dose of carbon tetrachloride (0.5 ml. of carbon tetrachloride mixed with 0.5 ml. of sesame oil/kg body weight). Twenty-four hours after this injection, the animals were re-anaesthetized with pentobarbital and the grafts and non-transplanted livers were removed. There were twelve animals with "straight" and eleven with "reverse" blood flow grafts.

Tissue sections stained with haematoxylin and eosin and frozen sections stained with sudan IV obtained from grafted and non-transplanted liver were examined. The non-transplanted liver showed the typical changes reported at 24 h after acute carbon tetrachloride intoxication. Centrilobular necrosis was seen in all sections. The central hepatocytes adjacent to the zone of necrosis were filled with lipid droplets. "Straight" blood flow autografts exhibited the same general pattern of injury—central necrosis and lipid accumulation. Fig. 1 shows a portal tract in a "straight" flow graft surrounded by relatively normal looking hepatocytes. Lipid filled cells and necrotic areas are located beyond the periportal site. The amount of fatty deposition in these grafts appeared to be less than in the corresponding non-transplanted liver. Varying degrees of lobular atrophy, particularly centrilobular, with sinusoidal dilation were seen in these grafts. These changes resembled those seen in "straight" blood flow liver autografts without administration of carbon tetrachloride and consequently appeared to be the sequelae of the grafting procedure *per se*.

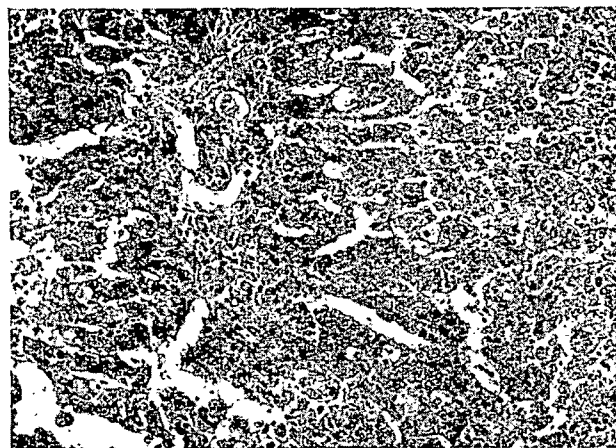
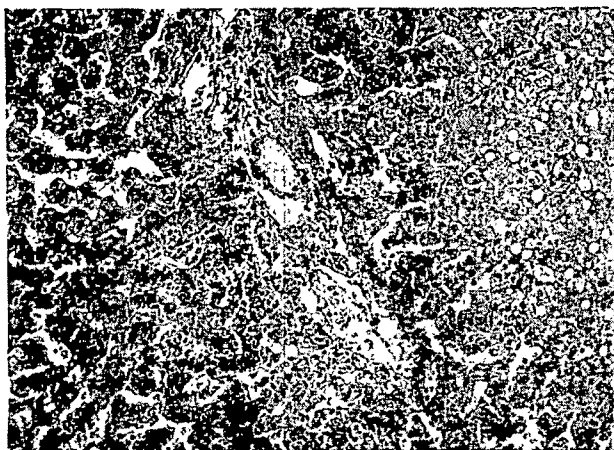


Fig. 1.  $\times 104$ .

Fig. 2.  $\times 104$ .

"Reverse" flow autografts showed the same pattern of central necrosis and lipid accumulation as the "straight" blood flow transplants and the untransplanted livers after administration of carbon tetrachloride. Fig. 2 shows a typical portal area in a "reverse" flow graft with the cells immediately adjacent appearing normal. Outside this zone, hepatocytes show marked lipid accumulation. As in the "straight" flow grafts, these changes were present together with alterations usually seen in "reverse" flow autografts. Parenchymal atrophy with centrilobular preponderance is more pronounced in "reverse" flow than in "straight" flow autografts. Where depletion and injury of parenchymal cells was extensive, collars or normal looking hepatocytes could usually be discerned in the periportal areas after treatment with carbon tetrachloride in both types of grafts. The atrophic changes attributable to the grafting procedure progressed with the age of the transplant. Consequently, the most clear-cut demonstration of persisting carbon tetrachloride effect was seen in the early grafts. In the grafts observed at 21–24 days, however, the general pattern of injury described still prevailed in both types of transplants.

This experiment suggests that carbon tetrachloride produces an effect in liver autotransplants which is comparable with that seen in otherwise normal liver. The amount of lipid in these grafts may be less than in non-transplanted liver. The amount of lipid, however, appears to be greater than that initially present in the graft. The same lobular zone lesion is produced in non-transplanted liver and in autografted liver with "straight" or totally "reversed" blood flow. It is noteworthy that R. W. Brauer commented at the NATO Symposium that, on the basis of unpublished work in his laboratory, the zone of necrosis from chlorinated hydrocarbons was the same in perfused *in vitro* rat liver preparation regardless of the direction of flow<sup>9</sup>.

Our investigation presents additional evidence against the contention that carbon tetrachloride effect on the liver is a result of ischaemia. In this experiment, centrilobular zones which received well oxygenated blood first still showed the typical response after administration of carbon tetrachloride. While the zonal pattern of acute carbon tetrachloride intoxication may result from the most susceptible cells being present in the area about the central vein, this zonal susceptibility tends to persist despite reversal of the direction of lobular blood flow in the time span investigated. Consequently, if lobular zone heterogeneity of function is the explanation for the specific site of the carbon tetrachloride lesion, and if this heterogeneity is a cellular adaptation to the direction of blood flow, then it must be assumed that cell function becomes relatively fixed despite later alteration of blood flow.

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### Effect of Bursectomy on the Rous Virus Tumour Induction in Chickens

NEONATAL thymectomy has a pronounced inhibiting or stimulating effect on the growth of tumours induced by various oncogenic viruses<sup>1–12</sup>. Our task was to investigate the role of thymus in the induction of Rous virus tumours. The interest of such experiments is that there are two organs in the chickens (thymus and bursa Fabricius), one of which has the functions of a mammalian thymus.

It is well known that the function of these organs is different<sup>10,13–16</sup>. This communication concerns the role of bursa Fabricius on the induction of Rous virus tumours and on tumour growth. For this purpose the chickens were bursectomized during the first 48 h of their life. As controls I used intact or sham-operated chickens which hatched on the same day as the experimental ones. The experimental and control birds were simultaneously inoculated with the Rous sarcoma virus (strain Carr) at the age of 14–76 days. As a source of the Rous virus I used a cell-free Rous sarcoma extract, which was stored in a frozen state (at  $-70^{\circ}\text{C}$ ) for 7 months. The virus titre, which was estimated by means of virus injection to 1-day-old chickens, was  $1 \times 10^7$  LD<sub>50</sub>/ml. All experiments were made with the same pool of tumour extract. The inoculated bursectomized and control birds were observed for a period of 1.5 month after virus injection. During this period we palpated the tumours every 2–3 days and autopsied every dead chicken. All birds which remained alive to the end of the experiment were killed and autopsied. The results of the experiments are given in Tables 1 and 2.

The results of these experiments showed that the incidence of tumours in the bursectomized chickens was somewhat lower as compared with the control birds. The observed difference, however, was not large (the difference

Table 1. INCIDENCE OF TUMOURS IN BURSECTOMIZED AND CONTROL BIRDS, 30 DAYS OLD, INOCULATED WITH ROUS SARCOMA VIRUS

Virus dilu- tions	No. in- oculated	Bursectomized No. of birds with tumours	Log LD <sub>50</sub>	No. in- oculated	Control No. of birds with tumours	Log LD <sub>50</sub>
$10^{-3}$	23	22 (95.6 per cent)		16	16 (100 per cent)	
$10^{-4}$	40	22 (55 per cent)		18	15 (83.3 per cent)	
$10^{-7}$	16	3 (18.7 per cent)	6.1	12	2 (16.7 per cent)	6.5
$10^{-8}$	9	0 (0 per cent)		9	0 (0 per cent)	

Table 2. INCIDENCE OF TUMOURS IN BURSECTOMIZED AND CONTROL BIRDS, INOCULATED WITH THE ROUS SARCOMA VIRUS ON THE SIXTIETH AND SEVENTY-SIXTH DAYS OF THEIR LIFE

Virus dilu- tions	No. in- oculated	Bursectomized No. of birds with tumours	Log LD <sub>50</sub>	No. in- oculated	Control No. of birds with tumours	Log LD <sub>50</sub>
$10^{-4}$	12	11 (91.7 per cent)		11	11 (100 per cent)	
$10^{-5}$	12	5 (41.7 per cent)	4.8	11	7 (63.6 per cent)	5.4
$10^{-6}$	12	0 (0 per cent)		11	3 (27.3 per cent)	
$10^{-7}$	12	0 (0 per cent)		11	0 (0 per cent)	

between log  $LD_{50}$  in both groups was  $< 1$ ). When the bursectomized and control chickens were inoculated with the virus at the age of 14 days, the difference in incidence of tumours was still lower. There was no difference in the duration of the latent period, tumour growth rate and incidence of metastasis in bursectomized and control birds inoculated with the Rous sarcoma virus at the age of 14–76 days. Thus, bursectomy had no significant effect on the Rous sarcoma virus induction and tumour growth rate. It was known that bursa Fabricius played a principal part in regulation of the humoral antibody production in birds. Therefore, our results gave further support to the conception that the humoral antibodies did not take any important part in the immune reaction against the new cell antigen, which was induced in the host cells transformed by the oncogenic viruses.

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### Adrenal Cortical Control of the Appearance of Rat Slow Alpha<sub>2</sub>-globulin

We have previously shown<sup>1</sup> that the appearance of slow alpha<sub>2</sub>-globulin among the blood serum proteins of the rat after the administration of the lipopolysaccharide (endotoxin) of *Salmonella abortus equi* is prevented by adrenalectomy. The slow alpha<sub>2</sub>-globulin response to this agent can be restored in the adrenalectomized rat by the administration of corticosterone. We wish to demonstrate that this adrenal cortical control is complete when certain other provoking agents or situations are used, but that the control is not complete when the provoking situation is immediately post-partum.

Three situations known to provoke the appearance of slow alpha<sub>2</sub>-globulin were used: partial hepatectomy<sup>2,3</sup>, growth of an implanted neoplasm<sup>4–7</sup>, and the immediate post-partum state of both mother and young<sup>4,8–9</sup>. In each situation, three experimental conditions were used: (a) the normal animal was placed in the desired situation; (b) the adrenalectomized animal was placed in the same situation; and (c) the adrenalectomized animal was placed in the situation but was given corticosterone as replacement therapy. The general procedures, including vertical starch gel electrophoresis, and the rats used have been described previously<sup>8</sup>.

Adrenalectomies were carried out as before<sup>1</sup> and all adrenalectomized rats were given 1 per cent sodium chloride instead of drinking water. Partial hepatectomies were performed by a modification<sup>2</sup> of the method of Higgins and Anderson<sup>10</sup>. If partial hepatectomy and adrenalectomy were to be combined, the former was performed 21 days after the latter. Replacement therapy consisted of the subcutaneous administration of 1 mg of corticosterone twice daily from the day before hepatec-

tomy until the second day after this operation; at this time the blood samples were taken.

Dorsal subcutaneous implantations of the Walker 256 carcinosarcoma were used to produce a growing neoplasm. In the appropriate animals, adrenalectomy was performed 2 days before the neoplasm was implanted. Blood samples were taken between 15 and 19 days after implantation of the carcinosarcoma, depending on the growth rate of the malignancy. Replacement therapy again consisted of 1 mg of corticosterone administered twice daily, beginning on the eleventh day after tumour implantation and lasting until the day when the blood sample was taken.

In the post-partum situation, blood samples were taken for analysis from both mother and newborn on the second day after birth. For each analysis, blood from at least three young of the same litter was pooled. Adrenalectomies were here performed on the second day of pregnancy (the day of the appearance of sperm in the vagina is counted as the first day). Preliminary experiments had shown that the administration of 1 mg of corticosterone twice a day caused many abortions or neonatal death, and so only 0.5 mg of corticosterone was given twice daily, beginning on the nineteenth day of pregnancy. The young of adrenalectomized mothers tended to be considerably smaller than those of untreated mothers.

The results obtained are summarized in Table 1. It will be seen that, when either partial hepatectomy or a growing neoplasm constitutes the stimulus used to provoke the appearance of slow alpha<sub>2</sub>-globulin, control is exercised by the adrenal cortex in the same manner as when a bacterial lipopolysaccharide was previously used. Further, the loss of response to these provoking agents in the adrenalectomized animal can again be reversed by the administration of corticosterone. A similar sequence of events has been reported when turpentine inflammation was used as the provoking agent<sup>11</sup>. The adrenal cortical control over the appearance of slow alpha<sub>2</sub>-globulin, therefore, seems to exist for a wide variety of provoking agents.

The data of Table 1 further show, however, that, in the case of the post-partum animals, the adrenal cortical control is not as strong as in the other situations, because slow alpha<sub>2</sub>-globulin will generally be present in the blood serum of a rat 2 days after the birth of a litter whether or not the animal is adrenalectomized. Slow alpha<sub>2</sub>-globulin is present in the young regardless of the adrenal status of the mother. The observation that the incidence of slow alpha<sub>2</sub>-globulins in the adrenalectomized mothers is only 57 per cent whereas in adrenalectomized and corticosterone treated animals it is 100 per cent can be regarded as evidence that adrenal cortical control is effective in the post-partum situation but incomplete, that is, factors other than the activity of the adrenal cortex substitute, at least in part, for the activity of the adrenal cortex. It can be suggested that the elevated concentrations of steroid hormone associated with pregnancy and lactation are involved here.

Table 1. ADRENAL CORTICAL CONTROL OF THE APPEARANCE OF SLOW ALPHA<sub>2</sub>-GLOBULIN

Test situation	Adrenal-ectomy	Replacement therapy (corticosterone)	No. of rats	Percentage of samples showing slow alpha <sub>2</sub> -globulin
Hepatectomy	No	No	8	100
Hepatectomy	Yes	No	9	0
Hepatectomy	Yes	Yes	8	75
Neoplasm	No	No	20	95
Neoplasm	Yes	No	13	0
Neoplasm	Yes	Yes	7	86
Post-partum, mother	No	No	20	80
Post-partum, young	No	No	22†	100
Post-partum, mother	Yes*	No	14	57
Post-partum, young	Yes*	No	8‡	100
Post-partum, mother	Yes*	Yes†	5	100
Post-partum, young	Yes*	Yes†	5‡	100

\* Mother only adrenalectomized.

† Mother only given replacement therapy.

‡ Number of pools of young.

The following conclusions can be drawn. (1) Adrenalectomy prevents the appearance of slow  $\alpha_2$ -globulin in a series of diverse situations in which this globulin normally appears. (2) The effect of adrenalectomy with respect to the appearance of slow  $\alpha_2$ -globulin can be reversed by the administration of corticosterone in all cases investigated. (3) The adrenal cortical control of the appearance of slow  $\alpha_2$ -globulin is incomplete in the 2 day post-partum maternal rat, because the globulin can be demonstrated in most adrenalectomized animals in this condition. (4) Adrenal cortical control over the appearance of slow  $\alpha_2$ -globulin is not simply absent in the post-partum maternal animal, because the percentage of adrenalectomized rats with demonstrable slow  $\alpha_2$ -globulin can be increased by the administration of corticosterone.

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### Enzymes in Cultivated Human Fibroblasts derived from Patients with Down's Syndrome (Mongolism)

FORTY-SEVEN chromosomes have been found in cells of children with Down's syndrome<sup>1</sup>. The extra chromosome, a small acrocentric, is believed to be either number 21 or 22<sup>1-3</sup>. The finding of larger concentrations of alkaline phosphatase in leucocytes of patients with Down's syndrome<sup>4-6</sup> suggested that the gene determining the enzyme is located on the chromosome associated with mongolism. Increased concentrations of whole blood and leucocyte galactose-1-phosphate uridyl transferase<sup>7-12</sup>, erythrocyte and leucocyte glucose-6-phosphate dehydrogenase<sup>9-12</sup>, leucocyte acid phosphatase<sup>9,12</sup>, blood galactokinase<sup>10,13</sup>, erythrocyte phosphohexokinase<sup>14</sup> and leucocyte 5-nucleotidase<sup>12</sup> have been reported. On the other hand, no increase of glucose-6-phosphate dehydrogenase or acid phosphatase could be detected in the platelets of patients with Down's syndrome<sup>15</sup>.

Tissue culture techniques did not show any difference in concentrations of acid phosphatase and  $\beta$ -glucuronidase between strains derived from patients with Down's syndrome, 18 trisomy syndrome and D trisomy syndrome<sup>16</sup>. No difference between two strains of fibroblasts derived from Down's syndrome patients and eight strains from controls was found<sup>17</sup>.

In an attempt to determine whether the changes in peripheral blood of patients with Down's syndrome were

caused by the consistent influence of the genetic material carried by the extra chromosome, cultured cells derived from skin biopsies of trisomic Down's syndrome patients were examined.

Biopsies were obtained from ten trisomic mongoloid children and ten age-matched controls. After cleaning an area of the forearm, a sterile 26 gauge needle was inserted intradermally and skin was elevated. A piece of skin several millimetres long was removed from around the needle using sterile curved scissors. The biopsies were cut into several pieces, immobilized under cover slips in 35 mm Petri dishes containing culture medium (nutrient mixture F10, G.I.B. Co., 15 per cent foetal calf serum, 50  $\mu$ /ml. of penicillin and 50  $\mu$ /ml. of streptomycin), and placed in a carbon dioxide incubator. After 3-4 weeks the strains were sub-cultured and chromosome analyses were carried out by the method of DeMars<sup>18</sup>. After further sub-culture replicate aliquots of the appropriate cell suspensions were introduced into plastic Petri dishes and sampled at intervals during growth for determination of enzyme activity and protein content.

Glucose-6-phosphate dehydrogenase activity was measured by the procedure described by Zinkham and his co-workers<sup>19</sup>. The cells were mechanically disrupted in  $2 \times 10^{-5}$  molar nicotinamide-adenine dinucleotide phosphate. Assay was carried out in a system containing 60  $\mu$ moles of *tris*, 10  $\mu$ moles of magnesium chloride, 1.33  $\mu$ moles of NADP, and 6.6  $\mu$ moles of glucose-6-phosphate in a final volume of 1.0 ml. at 37° C. The blank contained all of the reagents except the substrate. Activity was expressed as changes in density at 340 m $\mu$ .

Activity of alkaline phosphatase was determined by the method of F. Walker (unpublished results). The cells were air dried and overlaid with 0.7 ml. of a mixture containing equal parts of 0.4 molar glycine, pH 9.2, and 0.001 molar *p*-nitrophenyl phosphate. Incubation was carried out at 37° C for 90 min, the reaction stopped by the addition of 0.3 ml. of Cox buffer and read at 410 m $\mu$ .

Activity of acid phosphatase was determined by the method of DeMars<sup>16</sup>. The cells were air dried and overlaid with 1.0 ml. of reaction mixture containing  $3 \times 10^{-4}$  molar *p*-nitrophenyl phosphate (PNP) in 0.4 molar sodium citrate buffer, pH 5.0. Incubation was carried out at 37° C for 60 min, 4.0 ml. of 0.1 normal sodium hydride was added, and the optical density was read at 410 m $\mu$ .

Galactose-1-phosphate uridyl transferase was assayed in fibroblasts using  $1-2 \times 10^6$  cells. The suspension of cells was sedimented, the supernatant fluid decanted, and the cells were homogenized with 50  $\mu$ l. of a 0.2 per cent solution of saponin. The enzyme activity was determined by incubating with UL-<sup>14</sup>C galactose-1-phosphate and measuring the formation of uridine diphosphate galactose-<sup>14</sup>C according to the procedure of Anderson *et al.*<sup>20</sup> as modified by Inouye and Hsia (unpublished results). Protein was determined by the method of Oyama and Eagle<sup>21</sup>. Data were either analysed daily or pooled, and no significant differences between strains derived from patients with trisomic Down's syndrome and controls could be demonstrated as shown in Table 1.

Several explanations have been offered to explain the changes in peripheral blood seen in Down's syndrome. It has been suggested that chromosome "21" might contain the "structural" genes for certain specific enzymes<sup>3,6,14</sup>, the "regulator" genes for certain groups of enzymes<sup>9,12</sup>, or the genes that regulate isoenzymes<sup>10</sup>. There is no convincing evidence for any of the above hypotheses.

Table 1. POOLED DATA FROM ALL EXPERIMENTS PERFORMED WITH FIBROBLASTS GROWN IN CULTURE FROM NORMAL CONTROLS AND PATIENTS WITH DOWN'S SYNDROME  
(All values are expressed as mean  $\pm$  standard error)

Enzyme	Controls	Down's
Alkaline phosphatase		
$\mu$ g PNP utilized/h/ $\mu$ g total proteins $\times 10^{-3}$	6.66 $\pm$ 2.76	6.60 $\pm$ 3.38
$\mu$ g PNP utilized/h/cell $\times 10^{-4}$	4.48 $\pm$ 2.10	4.34 $\pm$ 2.10
Acid phosphatase		
$\mu$ g PNP utilized/h/ $\mu$ g total protein $\times 10^{-4}$	6.48 $\pm$ 3.02	6.04 $\pm$ 2.68
$\mu$ g PNP utilized/h/cell $\times 10^{-7}$	4.02 $\pm$ 2.06	4.80 $\pm$ 2.12
Glucose-6-phosphate dehydrogenase		
$\mu$ moles NADP utilized/h/ $\mu$ g total protein $\times 10^{-4}$	5.81 $\pm$ 3.75	6.13 $\pm$ 3.04
Galactose-1-phosphate uridyl transferase		
$\mu$ moles UDP galactose- <sup>14</sup> C formed/h/ $\mu$ g total protein	64.11 $\pm$ 12.40	64.93 $\pm$ 26.00

## MICROBIOLOGY

Enrichment of *Bacillus subtilis* Transformants by Zonal Centrifugation

A COMPETENT culture of *Bacillus subtilis* which has been exposed to DNA consists of at least two metabolically distinct cell populations—competent cells which have taken up DNA and are potentially transformable, and untransformable cells. The potential transformants are relatively resistant to penicillin, actinomycin D, and puromycin and appear to be non-dividing (refs. 1–3 and personal communication from H. O. Kammen, R. H. Beloff, and E. S. Canellakis). We suspected that these cells were lighter than the majority of actively dividing incompetent cells. Zonal centrifugation of a competent culture of *B. subtilis* in a sucrose gradient shows that these two kinds of cells can, in fact, be separated.

The media and transformation procedures were those of Anagnostopoulos and Spizizen<sup>4</sup>. *B. subtilis* strain 168 *ind*<sup>-</sup> (indole requiring) was used as the recipient and *B. subtilis* 168 prototroph as the donor of DNA. The DNA was extracted by the method of Marmur<sup>5</sup>. About 10 ml. of a linear sucrose gradient (4.5 per cent w/v to 18 per cent w/v sucrose in 0.1 molar sodium chloride and 0.05 molar sodium citrate at pH 7.4) was layered in a sterile 5/8 in. × 3 in. cellulose nitrate centrifuge tube by means of a gradient apparatus. The solutions were sterilized by filtration.

A competent culture of *B. subtilis ind*<sup>-</sup> was exposed to 5 µg/ml. of prototrophic DNA for 40–50 min at 37° C (ref. 4). The cells were centrifuged and resuspended in one tenth of the original volume of the medium and 0.2 ml. of this suspension was layered on the top of the sucrose gradient. The tubes were centrifuged for 30–40 min at 1,000–2,000 r.p.m. in the swing-out head of an 'MSE minor' clinical centrifuge at room temperature (~22° C). The cells move as a visible band, the position of which at any time can be determined by inspection. The tubes were connected to a drop collecting device<sup>6,7</sup> and punctured. Five drop fractions were collected serially. Alternate fractions were assayed for transformation to prototrophy and the viable count. The sucrose concentration along the gradient was measured by an 'Abbe-3L' refractometer.

The results of a typical experiment are given in Fig. 1. The majority of the transformed cells form a peak (A), which is quite distinct from the peak (B) formed by the

An alternative suggestion is that enzyme activity in the leucocyte and possibly also in the erythrocyte is dependent on cell age and that the shortened life span of the leucocyte in Down's syndrome results in the presence of younger white cells with higher enzyme concentrations<sup>22,24</sup>. Measurement of the survival of granulocytes in four adult patients with Down's syndrome, however, has demonstrated no significant difference from normal<sup>25</sup>.

Significant differences have been reported between trisomic and translocation Down's syndrome patients for the following enzymes: leucocyte, erythrocyte, and whole-blood galactose-1-phosphate uridyl transferase, leucocyte acid phosphatase, leucocyte alkaline phosphatase, erythrocyte glucose-6-phosphate dehydrogenase, and leucocyte 5-nucleotidase<sup>12</sup>. The translocated position of the chromosome could perhaps interfere with the normal gene action (position effect) or the deletion occurring in the process of translocation could be the site on the chromosome which determines the expression of the enzymes<sup>12</sup>.

The different responses of these enzymes between fibroblast cultures and leucocytes of trisomic Down's syndrome patients suggest that the increased activity of certain enzymes reported in trisomic individuals cannot be interpreted in terms of either gene locations or triple gene dose effect. The difference may be caused by one or more of the following: (a) specific properties of the white blood cell, including shortened life span; (b) tissue specific variation in enzyme regulation; (c) isoenzymes with different control mechanisms; (d) more complicated genetic interactions, and (e) effect of tissue culture.

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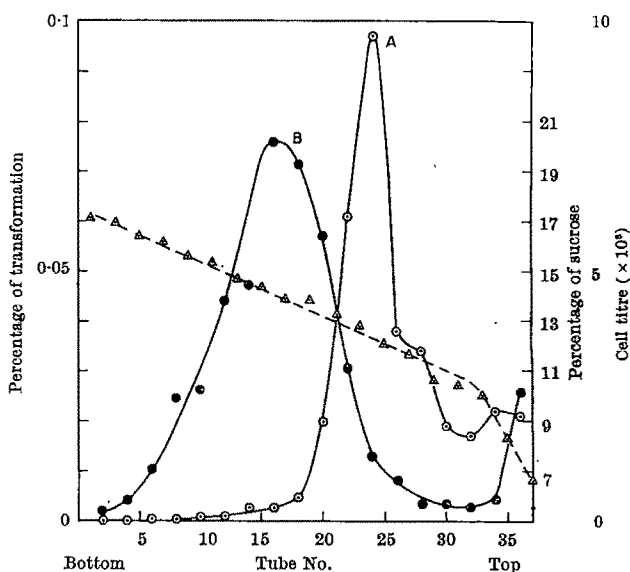


Fig. 1. Sedimentation of a competent culture of *B. subtilis* 168 *ind*<sup>-</sup> in a sucrose gradient. The percentage of transformation in the unfractionated culture was 0.015. O, Percentage of transformation; ●, cell titre; Δ, percentage of sucrose.



bulk of the cells. The frequency of transformants in the peak fraction is four to eight times greater than the average transformation frequency of the culture. By collecting smaller fractions enrichment greater than this can be easily obtained.

The centrifugal fractionation of a competent culture in addition to yielding higher transformation frequencies provides a convenient method for investigating the metabolic properties of the potential transformants.

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### Brief Consideration of the Meaning of the Lysogenic Conversion in *Salmonella anatum* Phage System

THE unusual properties of the  $\epsilon$ -group of *Salmonella anatum* phages to change the antigenic structure of the surface of their host bacterium first described by Uetake<sup>1,2</sup> are also interesting from the point of view of their biological meaning. The results given by Uetake seem to justify this approach. Uetake found that a non-lysogenic strain of *Salmonella anatum* (A) with antigenic structure 3,10 and sensitive to the phage  $\epsilon$ -15, when lysogenized by it, gives rise to a lysogenic strain A ( $\epsilon$ -15) with antigenic structure changed from 3,10 to 3,15 and sensitive to the phage  $\epsilon$ -34. When this new strain (with antigenic structure 3,15) is lysogenized by  $\epsilon$ -34, the resulting strain, containing both prophages A ( $\epsilon$ -15,  $\epsilon$ -34), has again a new antigenic structure (3) (15) 34. When the original strain A (with antigenic structure 3,10) is lysogenized in special conditions by  $\epsilon$ -34 only<sup>3</sup>, no antigenic change occurs. On the basis of a very detailed analysis the authors conclude that the information for the antigenic change was brought into the cell by the infecting phage and that for the formation of the antigen 34 a mechanism for the synthesis of antigen 15 is required, so that a "genetic co-operation" between the two phages occurs.

The results of these experiments as given by Uetake<sup>2</sup> are:

Strain	Plaque formation		Killing		Adsorption	
	$\epsilon$ -15	$\epsilon$ -34	$\epsilon$ -15	$\epsilon$ -34	$\epsilon$ -15	$\epsilon$ -34
	virulent	virulent	virulent	virulent	virulent	virulent
A	+	-	+	-	+	-
A( $\epsilon$ -15)	-	+	-	+	-	+
A( $\epsilon$ -34)	+	-	+	-	+	-
A( $\epsilon$ -15, $\epsilon$ -34)	-	-	-	-	-	-

Without regard to the chemical basis of this phenomenon<sup>4</sup> we find an interesting correlation; the bacteria lysogenic for  $\epsilon$ -15 do not adsorb  $\epsilon$ -15, the strain lysogenic for  $\epsilon$ -34 does not adsorb the phage  $\epsilon$ -34, and the strain lysogenic for both phages does not adsorb either of them. Thus, the changes caused by the lysogenization seem to serve as a tool depriving the bacterial host of the affinity to the phages related to the prophages present. Apparently, the phages carry information for the changes of their own receptors on the host cell. On the other hand, the  $\epsilon$ -34 phage introduced "by mistake"<sup>2</sup> to the strain A non-lysogenic for  $\epsilon$ -15 does not cause any detectable changes of the host's surface properties.

This view throws a new light on the meaning of the lysogenic conversion in this system. The logic of this

phenomenon, that is the tendency to prevent superinfection by the homologous phage, may be closely related to that of lysogenic immunity. This new barrier seems to be far more efficient, because it prevents superinfection by the relatively frequent virulent mutations which overcome the cytoplasmic immunity of the lysogenic cell. A host mutation, on the other hand, would have to be a virulent one to infect this lysogenic cell, a double mutant, which is unlikely to occur.

In view of this, it may be unnecessary to differentiate between strictly viral genes and those responsible for lysogenic conversion. The problem of the origin of the latter—so often discussed (see, for example, ref. 1, p. 504; and ref. 3, p. 209)—could merge with that of the phylogenetic origin of the phage itself. The existence of lysogenic conversion would seem to support the idea of Campbell<sup>5</sup> that phage genes advantageous to the host would be preferred and selected during evolution because they compensate for the handicap the phage imposes on the host and thus enable the host bacteria—and therefore its phage—to survive.

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### Linkage of Arginine-sensitive (*ars*) and Uracil-Arginine Requiring (*pyrA*) Loci of *Salmonella typhimurium*

ISHIDSU<sup>1</sup> has isolated a mutant (*ars-I*) of *Salmonella typhimurium* that grows normally on a minimal medium but is strongly inhibited on addition of arginine. This sensitivity to arginine can be overcome by the addition of uracil.

With the knowledge that genes controlling co-ordinated functions are often clustered together<sup>2</sup>, co-transduction experiments were performed to determine whether the *ars-I* locus is closely linked to a locus for a related biochemical step, such as biosynthesis of arginine and (or) uracil. As can be seen in Table 1, it was found that *ars-I* is closely linked to *pyrA*, single step mutants of which require both uracil and arginine<sup>3</sup> (see Sanderson and Demerec<sup>4</sup> for linkage map of *S. typhimurium*).

The procedure for joint testing for joint transduction was to grow donor phage P22 on *ars-I*. For each transductant plate, about  $2 \times 10^8$  phage particles were added to 0.1 ml. of an overnight culture of each of the *pyrA* recipients (about  $2 \times 10^8$  cells/ml.) and the mixture was spread on the surface of the plate containing minimal agar. After 2 days of incubation, transductant colonies formed; these represented transfer of donor *pyrA*<sup>+</sup> to *pyrA* recipient cells. The next step was to determine how many of the transductants also received the *ars-I* mutational site along with the *pyrA*<sup>+</sup>. This was scored in two different ways: (1) transductant colonies were replica plated on to plates containing minimal agar plus 100  $\mu$ g of arginine/ml. A large fraction (Table 1) of the transductant colonies

Table 1. JOINT TRANSDUCTION OF ARGININE-SENSITIVE (*ars-I*) AND URACIL-ARGININE (*pyrA*<sup>+</sup>) SITES IN *S. typhimurium*

Test No.	Recipient	No. of <i>pyrA</i> <sup>+</sup> transductants	No. containing <i>ars-I</i>	Percentage co-transduction
1	<i>pyrA</i> 47	53	31	58
2	<i>pyrA</i> 127	58	32	55
3	<i>pyrA</i> 129	106	32	30
4	<i>pyrA</i> 180	146	87	60
5	<i>pyrA</i> 180	92	41	45
6	<i>pyrA</i> 180	106	44	41
7	<i>pyrA</i> 180*	121*	0*	0*

\* Control, in which wild type *ars-I*<sup>+</sup> donor phage was substituted for *ars-I*

failed to grow, and on testing were found to contain the *ars* mutation; (2) it was found in later experiments that *pyrA*<sup>+</sup> *ars-1* colonies could be distinguished from *pyrA*<sup>+</sup> colonies without the need for replica plating. By incorporation of a small amount of arginine (2 µg/ml.) in the initial transductant plates, those recipient cells that had received the *ars-1* mutation as well as the *pyrA*<sup>+</sup> were smaller in size as a result of the arginine sensitivity. Testing of individual transductants showed that scoring by colony size was completely accurate.

In *S. typhimurium*, *pyrA* probably controls the biosynthesis of the enzyme glutamino-carbamoyl phosphate synthetase, as in *E. coli*<sup>5</sup>. It is interesting to note that a uracil-sensitive (*urs*) locus in *E. coli* is also linked to *pyrA* (ref. 6).

The arginine sensitivity of *ars-1* and its relationship to the enzymes involved in arginine-uracil biosynthesis is now being investigated by Ishidsu<sup>1</sup>.

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### Rapid Method for the Quantitative Estimation of Microbial Lipases

THE investigation of microbial lipases has been considerably impeded by a lack of knowledge about their natural substrates and their physiological role in the metabolism of the organisms. Any substrate chosen to detect microbial lipases must therefore be arbitrary and it is difficult to evaluate data of different workers because of the wide choice of substrates, assay conditions and methods employed. The underlying principle in the assay of microbial lipases has usually been the estimation of free fatty acids liberated from triglycerides after a suitable incubation time. We have found that direct titration methods are not suitable, because most cultures contain alkaline or acid degradation products. This can be overcome by extracting the fatty acids from the medium after incubation with the substrate<sup>1</sup> but the procedure is laborious and difficult to carry out quantitatively. A long incubation time is also open to the criticisms that the products of lipolysis may inhibit lipase activity<sup>2-4</sup> or be used in the reverse, synthetic reaction<sup>5</sup>. Short duration assays have been developed such as the indirect manometric method employed by Forster and co-workers<sup>6</sup> or the direct method of continuous automatic titration of the acid liberated from an appropriate substrate in a pH stat<sup>7</sup>, but the lipase activities of the micro-organisms that we have tested have been too low to be assayed by such techniques.

The fact however, that all of these micro-organisms produced zones of hydrolysis when grown on tributyrin agar, and some when grown on butterfat-Victoria blue agar media<sup>8</sup>, suggested that it should be possible to develop an agar diffusion method that would be more sensitive and versatile than existing assay techniques. Proteins diffuse through agar gel as through a plain solvent when the concentration of agar is below 1.5 per cent<sup>9,10</sup>. Cooper<sup>11</sup> has shown that the mathematical conditions for a valid

agar diffusion assay are that the relationship between the logarithm of the enzyme concentration and the zone radius (or its square) must be linear and that the ratio of response for high and low dilutions of the standard must be the same as for the unknown. Carter and Sykes<sup>12</sup> assayed several enzymes by an agar diffusion method, but they failed to find a suitable substrate for the assay of pancreatic lipase. Preliminary experiments in this laboratory indicated that emulsified triglycerides, particularly tributyrin, were suitable substrates and the following thin layer method was developed.

Ten ml. of a 1 per cent emulsion of tributyrin, or equivalent molecular concentrations of other synthetic triglycerides in water (prepared by passing the mixture four times through an Ormerod QP homogenizer at a pressure of 1,000 lb./sq. in.), are added to 90 ml. of a hot solution of Davis agar (1.2 per cent) in M/20 phosphate buffer (usually pH 8). One ml. of this emulsion is spread over an area, 2 in. by 1 in. of a microscope slide, a hole 2.3 mm in diameter is bored with a thin steel tube and 0.004 ml. of the lipase solution added by means of an Agla micro-syringe. The slide is placed in a Petri dish containing moist absorbent cotton wool and incubated at 30° for periods up to 48 h. The diameters of the zones of clearing are measured with Vernier callipers.

Since many workers<sup>13-15</sup> hold the view that the hydrolysis of tributyrin is not an indication that more complex natural triglycerides such as butterfat will be attacked, we have also developed a thin layer agar diffusion method for the detection of lipolytic activity against butterfat. The lipase is added to a phosphate buffered agar gel, on which is then placed a lens tissue (Green, 105), which has been painted with melted butterfat saturated with Victoria blue. The hydrolysis of the thin uniform layer of butterfat is shown as a blue zone against the red background of unchanged dye.

The quantitative validity of these agar diffusion assays was determined with a micrococcus (*M. freudenreichii*, NCDO 1223). The organism was grown in 1 per cent Bacto peptone pH 7.0 and the extracellular lipase concentrated by a factor of 100 by precipitation with ammonium sulphate. The rate of hydrolysis, as measured by the clearing of tributyrin and trioctanoin emulsions, was proportional to the period of incubation (Fig. 1) and remained linear for at least 48 h. Maintaining the lipase preparation at 80° for

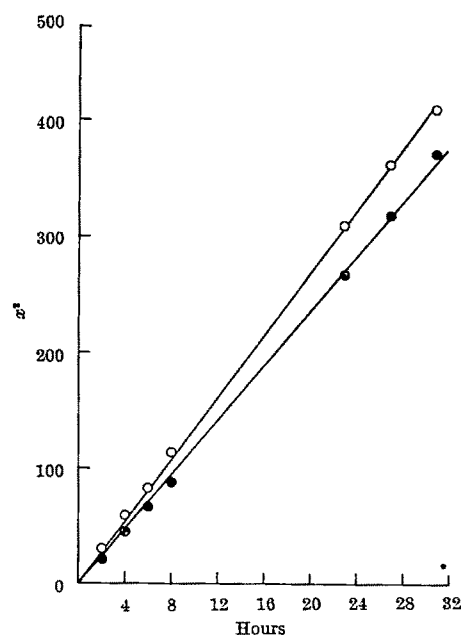


Fig. 1. Hydrolysis of 0.1 per cent (v/v) tributyrin (O) and trioctanoin (●) by a partially purified lipase of *Micrococcus freudenreichii* over an extended period of incubation.  $x$  is the diameter of the zone of clearing of the emulsion minus the diameter of the well.

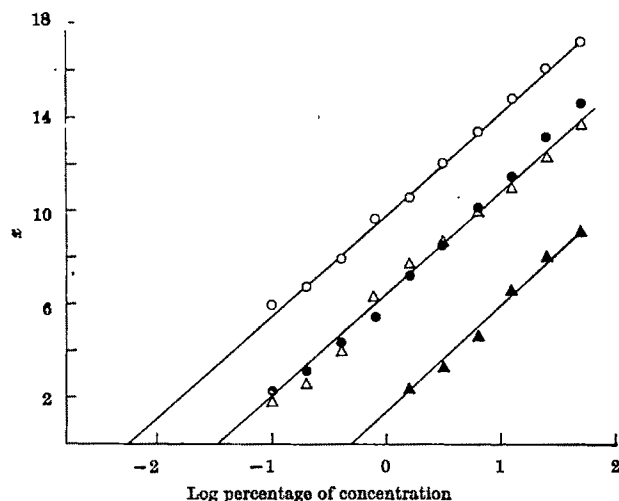


Fig. 2. The rate of hydrolysis of equimolecular concentrations of various triglycerides by dilutions of a partially purified lipase from *Micrococcus freudenreichii* after 19 h at 30°. O—O Tributyrin (0.1 per cent, v/v), ●—● triolein (0.17 per cent, v/v), Δ—Δ tridecanoin (0.2 per cent, v/v), ▲—▲ butterfat-Victoria blue. x is the diameter of the zone of clearing of the emulsion minus the diameter of the well.

2 min completely destroyed its activity, showing that non-enzyme hydrolysis was not responsible. A linear relationship was also obtained when the logarithms of dilutions of the concentrated lipase were plotted against the diameter of the zone of hydrolysis obtained with various emulsions (Fig. 2) after a certain period of incubation at 30° C. The slopes of these straight lines were parallel, indicating that a single enzyme system was responsible for the hydrolysis of all four substrates. The points where these lines cut the axis are the minimal lipase concentrations which give discernible zones with a particular substrate. These could be calculated and confirmed by experiment. A dilution of 1 in 17,500 of the purified lipase was the smallest concentration that gave a zone with tributyrin emulsion, 1 in 2,500 with triolein or tridecanoin emulsion and 1 in 200 for the butterfat-Victoria blue medium. The tributyrin emulsion assay was therefore almost 90 times more sensitive than the butterfat assay. Similar results were obtained with a pseudomonas (*P. fragi*, NCDO 752).

The supernatants from growing cultures of both the organisms used in this investigation readily cleared tributyrin agar emulsions but showed no activity against butterfat. When the lipase in the supernatant was concentrated by ammonium sulphate precipitation, however, the butterfat was readily hydrolysed. The sensitivity of the tributyrin emulsion assay lies not only in the use of a thin layer of agar containing a very low concentration of tributyrin<sup>16</sup> which produces easily distinguished transparent zones. If the concentration of tributyrin is increased the zone of clearing obtained in a given time with a lipase preparation is decreased and a 1 per cent tributyrin emulsion gives zones that are approximately equivalent to those obtained with the butterfat-Victoria blue medium. We consider that the sensitivity of the tributyrin emulsion assay makes it particularly suitable for the screening of micro-organisms for lipolytic activity and that the use of butterfat agar means that many weakly lipolytic organisms are overlooked. Over extended periods this low activity against natural fats, as in the storage of butter or in cheese ripening, could be of great significance.

The thin layer diffusion assay was developed specifically for microbial lipases to overcome the disadvantages of methods which are affected by buffers and the degradation products normally found in microbial cultures. In addition it has been used successfully to determine the activities of lipases from skim milk, pig pancreas and rat adipose tissue, since the ease with which the assay can be

carried out and the small size of the sample needed make it particularly useful for following the purification of lipases by column procedures. The relative lipolytic activities in the fractions off 'Sephadex' columns were identical whether determined as the liberation of free fatty acids in a pH stat (in mμequiv./min) or by the rates of clearing of a tributyrin emulsion.

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## RADIOBIOLOGY

### Peroxidase Isozymes in Barley Leaves from Irradiated Seeds

It is known that ionizing radiations cause an increase in peroxidase activity<sup>1-3</sup>, but it has not yet been explained how they do this. Although multiple forms of peroxidase have been shown in plants<sup>4-6</sup>, no effect of radiations on these forms has yet been described. This communication reports the appearance, in the zymograms of barley leaves which develop from irradiated seeds, of new peroxidase bands with respect to the control.

Plants of barley cv. Esau from irradiated (60 kR. of X-rays) and unirradiated seeds were grown in sand in the greenhouse at 22° C with a 15 h photoperiod. First leaves of 8 day old seedlings were cut and homogenized by grinding in a mortar with 0.066 molar phosphate buffer at 4° C. The homogenate was centrifuged at 20,000g for 20 min and the precipitate was discarded. The supernatant was saturated with ammonium sulphate and the precipitate was removed, 3 h later, by centrifugation at 20,000g for 20 min. The precipitate was dissolved in 2.4 ml. of buffer composed of nine parts of 0.016 molar tris-0.033 molar citric acid (pH 8.1) and one part of 0.02 molar lithium hydroxide-0.076 molar boric acid (pH 8.1)<sup>7</sup> and then dialysed for 20 h against three successive volumes of 200 ml. of the same buffer in a cold room. The final residue in the dialysis bag was again clarified by centrifugation. Starch-gel electrophoresis was carried out as described by Klapper<sup>7</sup> for 3 h at 4° C with a constant current of 25 m.amp (450-400 V) passing through the gel bed (14 × 10 × 0.6 cm). After completion of the run, the gels were flooded for 1 min in benzidine-saturated water containing 0.3 per cent hydrogen peroxide and then in methanol, acetic acid and water (50:10:40). The regions of gel containing peroxidase turned blue immediately and afterwards turned brown. The zymogram of peroxidase from irradiated barley shows five peroxidase isozymes at the cathode and four at the anode, while the zymogram from unirradiated barley shows only three isozymes at

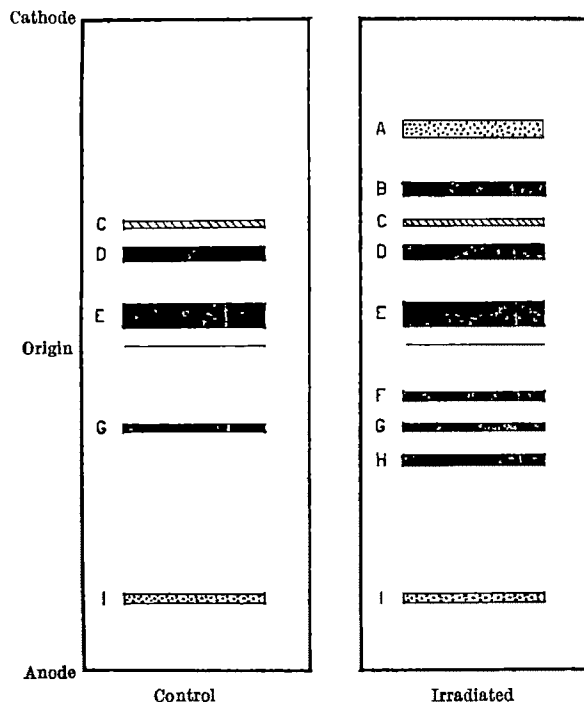


Fig. 1. Electrophoretic pattern of peroxidase isozymes in leaves from irradiated and unirradiated barley seeds.

the cathode and two at the anode (Fig. 1). In the zymogram of the leaves from irradiated seeds, therefore, there is the appearance of two new bands at the cathode (46 mm and 33 mm from the origin) and of two bands at the anode (11 mm and 25 mm from the origin). The staining intensity of all the bands in irradiated material is higher than in the controls, showing a greater enzyme activity. It may be concluded that the irradiation of seeds causes deep alteration of the system which regulate the biosynthetic pattern. This alteration causes a differentiation of forms of the peroxidase isozymes, the biological significance of which remains to be established.

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### Protection by Substrate Analogues from Ultra-violet Damage of Bovine Pancreatic Ribonuclease

THERE is a great deal of evidence that the sensitivity to ionizing radiation of an enzyme is influenced by the presence of a substrate or a substrate analogue in the medium. For example, bovine pancreatic ribonuclease (RNase) is protected by a substrate analogue from damage by  $\gamma$ -irradiation and a modified active enzyme is formed<sup>1</sup>. Little work has been done, however, on the effect of a substrate or a substrate analogue on damage to an enzyme by ultra-violet light. We found that several substrate analogues protected RNase from damage by ultra-violet light.

RNase A solution was obtained by 'Amberlite IRC-50' chromatography<sup>2</sup> from a commercial preparation and dialysis against an acetate buffer (pH 5.6 and ionic strength 0.01) containing  $10^{-4}$  molar ethylenediamine tetraacetic acid (EDTA). The mixture of the enzyme ( $1 \times 10^{-5}$  molar) and a substrate analogue ( $2 \times 10^{-5}$  molar) in the buffer was placed in a quartz cell of optical path 2 mm and irradiated with a low pressure mercury germicidal lamp from a distance of 8 cm at  $2^{\circ}$ – $4^{\circ}$  C. (The pH of 5.6, low ionic strength and low temperature are favourable for making a complex of enzyme and a substrate analogue<sup>3</sup>.) Intensity of the irradiation energy (2537 Å) was measured with an ultra-violet dosimeter calibrated with a chemical actinometer<sup>4</sup>. Immediately after the irradiation, the remaining enzyme activity was measured without any pretreatment to eliminate the substrate analogue from the irradiated mixture. The enzyme activity was measured by an increase in an acid-soluble fraction by the enzyme ( $10^{-7}$  molar) over the substrate (at pH 7.3) which was yeast RNA (0.3 per cent).

The inactivation curves of RNase were log-linear, as shown in Fig. 1, both in the absence and presence of a substrate analogue, and some substrate analogues showed a protective effect. The degree of protection was expressed as follows. A slight correction for shielding of ultra-violet light by a base component of substrate analogue was made. It was estimated as 4 per cent for uridine and cytidine, 6 per cent for guanosine, and 7 per cent for adenosine. The degree of protection was defined as  $(1 - a/b) \times 100$  per cent, where  $a$  and  $b$  are the corrected gradients of the slope in the absence and presence of the substrate analogue, respectively.

As summarized in Table 1, substances which cannot make a complex with the enzyme (which implies all nucleosides) are ineffective for protection, and nucleotides "transparent" with respect to absorption of ultra-violet, namely ribonucleotides of dihydrouracil and uracil hydrate, also have no effect. In general, a 3'-ribonucleotide is more effective than the other isomers, and all nucleotides of cytosine tested have a remarkable protective effect.

The protection by cytidine-2'(+3')-phosphate was not influenced by oxygen in the medium.

Mechanism of the protection by a substrate analogue is not yet clear. Complex formation of the enzyme with the substrate analogue and absorption of ultra-violet energy by the latter are supposed to be involved in the process of the protection.

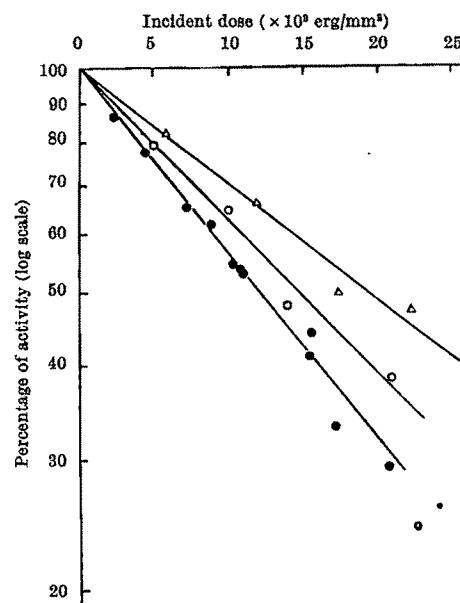


Fig. 1. Inactivation curves of RNase by ultra-violet light with and without substrate analogues. Dose rate is  $4,940 \text{ erg/mm}^2 \text{ min}$ .

Table 1. DEGREE OF PROTECTION BY SUBSTRATE ANALOGUES FROM DAMAGE BY ULTRA-VIOLET OF RIBONUCLEASE

Substance	Degree of protection (per cent)	Complex formation with RNase	Substance	Degree of protection (per cent)	Complex formation with RNase
Nucleoside			$\psi$ -U 2'p	15	Yes
C	7	No	$\psi$ -U 3'p	22	Yes
U	6	No	rT 2' (+3')p	23	Yes
$\psi$ -U	-1	No	A 2'p	-5	Yes
A	8	No	A 3'p	27	Yes*
G	6	No	A 5'p	-5	Yes*
dC	-3	No	G 2'p	-7	Yes*
dT	8	No	G 3'p	28	Yes*
dA	8	No	G 5'p	17	Yes*
dG	9	No	dC 5'p	21	Yes*
Nucleotide			dT 5'p	10	No
C 2'p	15	Yes	dA 5'p	-10	No
C 3'p	39	Yes	dG 5'p	8	No
C 5'p	20	Yes*	MeU 2' (+3')p	2	No
U 2'p	10	Yes	H <sub>2</sub> U 2' (+3')p	0	Yes*
U 3'p	30	Yes	H <sub>2</sub> O-U 3'p	3	Yes*
U 5'p	-5	Yes*			

C, Cytidine; U, uridine;  $\psi$ -U,  $\psi$ -uridine; A, adenosine; G, guanosine; T, thymidine; rT, thymine riboside; MeU, N<sup>3</sup>-methyluridine; H<sub>2</sub>U, 5,6-dihydrouridine; H<sub>2</sub>O-U, uridine hydrate; d, deoxy; 2'p, 2'-phosphate, *et cetera*, for example, dG5'p, deoxyguanosine-5'-phosphate).

Complex formation of a substance with RNase was estimated by the hypochromism\*. In the asterisked cases, the substance shows no hypochromism (hypochromic for G2'p) when it is mixed with RNase, but it inhibits the hypochromism produced by RNase-U3'p complex.

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### Protective Effect of Cystamine and AET given at Various Intervals before Irradiation

Using the spleen colony technique described by Till and McCulloch<sup>1</sup>, we investigated the dose reduction factor for cystamine on haematopoietic stem cells by examining the endogenous colonies in the spleen of control and protected mice. We found that the size of the dose reduction factor varies noticeably with the time which has elapsed between the intraperitoneal injection of cystamine and the irradiation, particularly during the first 15 min after the administration of this radioprotective substance<sup>2</sup>. In the experiments reported here mice were irradiated at a greater dose rate (436 r./min). We were thus able to

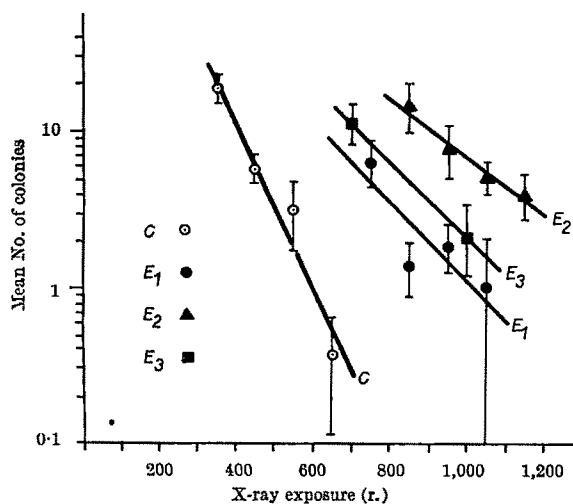


Fig. 1. The relation between the exposure and mean number of colonies/spleen of mice in the first experiment. C, Control group; E<sub>1</sub>, E<sub>2</sub> and E<sub>3</sub>, groups of experimental mice. Vertical bars illustrate the standard deviation of the mean.

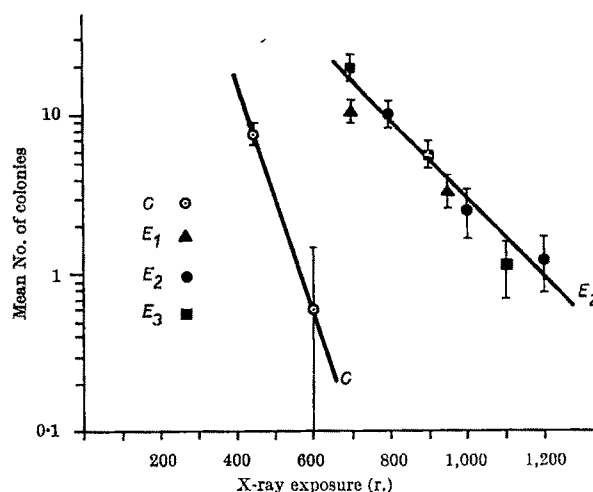


Fig. 2. The relation between the exposure and mean number of colonies/spleen of mice in the second experiment. C, Control group; E<sub>1</sub>, E<sub>2</sub> and E<sub>3</sub>, groups of experimental mice. Vertical bars illustrate the standard deviation of the mean.

shorten the irradiation time and demonstrate that cystamine showed its maximum radioprotective effect from the fourth to the sixth minute after it had been intraperitoneally injected. Similar results were obtained with 2- $\beta$ -amino ethyl isothiuronium (AET). The results will be quoted briefly in this article; they will be published in detail elsewhere.

Mice C57BL were given whole-body X-ray (200 kV, 20 m.amp, half value layer 1.02 mm copper, focus-distance 20 cm, exposure rate 436 r./min) exposures. The animals were divided into four groups; the control mice were only irradiated; the mice in experimental groups were injected intraperitoneally with cystamine (di-2-aminoethylsulphide . 2 HCl; 160 mg/kg) or AET (2-aminoethylisothiuronium . Br . HBr; 200 mg/kg). There were some sub-groups in each group of mice, distinguished from each other by radiation exposure only; ten to sixteen mice were put in each sub-group. All mice were killed nine days after the beginning of the experiment; their spleens were removed and fixed. Macroscopically visible colonies were counted and the standard deviations of the mean numbers of colonies for each spleen were calculated. From these data, the regression lines were constructed for each group.

In the first experiment, the mice protected with cystamine were divided into three groups according to the interval between the injection and the middle of the irradiation time. The duration of these intervals was: in the first experimental group (E<sub>1</sub>) 3 min, in the second (E<sub>2</sub>) 5 min and in the third (E<sub>3</sub>) 8 min. The results are shown in Fig. 1. The dose reduction factors for each experimental group were calculated from the ratio of the effective exposures for four colonies on the regression lines compared with the control group. We found that the radioprotective effectiveness of cystamine was greatest in the E<sub>2</sub> group (dose reduction factor Q2.32), that is, when the injection of cystamine was given 5 min before the middle of the irradiation time. If this interval was shorter (3 min by the E<sub>1</sub> group) or longer (8 min by the E<sub>3</sub> group), the level of the protection was smaller (dose reduction frequency by the E<sub>1</sub> group, 1.61; and by the E<sub>3</sub> group, 1.82).

The arrangement of the second experiment was similar to the first one, but experimental mice were given AET instead of cystamine. The group of control mice included two sub-groups only and the intervals from the injection of AET till the middle of the irradiation time were 4 min in the E<sub>1</sub> group, 6 min in the E<sub>2</sub> group and 9 min in the E<sub>3</sub> group. The results are demonstrated in Fig. 2. In order to make survey easy, only the regression lines for control



and  $E_2$  groups were drawn. Dose reduction factors were calculated in the same manner as for the first experiment. They were: 1.86 for the  $E_1$ , 1.95 for the  $E_2$  and 1.90 for the  $E_3$  group. Thus, the radioprotective effectiveness of AET did not differ in any striking way at all intervals used in the second experiment.

Our results give evidence which favours an assumption that the radioprotective efficacy of cystamine and AET on haematopoietic stem cells in the spleen of irradiated mice is brought about soon after these substances have been intraperitoneally injected. Dose reduction factors for both substances are greatest when administered between 4 and 7 min before irradiation. The dose reduction factor for cystamine is higher than that for AET. After longer intervals the radioprotective effect of cystamine decreases quickly, even as early as at 8 min after the injection the dose reduction factor being no higher than that of AET. We realize that the change of the radioprotective effectiveness of cystamine on haematopoietic spleen stem cells takes place so quickly that we are not able to assess it accurately. Nevertheless, we conclude that the radioprotective effect of cystamine on the haematopoietic stem cells in the spleen of irradiated mice shows a remarkably short peak in relation with AET. This maximum of efficacy may be explained by the difference in the metabolism of these two substances and by the pharmacological properties of cystamine.

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### Transient Changes in the Radiosensitivity of Mammalian Cells on Transfer from *in vivo* to *in vitro* Culture Conditions

CONDITIONS of culture are well known to play an important part in determining the radiation response of micro-organisms. Mammalian cells grown *in vitro* only remain viable within a rather limited range of culture conditions and, perhaps for this reason, only a few reports are available of the radiobiological consequences of changing their culture conditions<sup>1-3</sup>.

The effects of one type of change have been investigated using cells of the Ehrlich diploid mouse ascites tumour which have been grown *in vitro* for 3 yr. It was found that the radiobiological response was profoundly affected if cells which had previously been grown *in vitro* were allowed to grow *in vivo* for some generations and were then transferred to *in vitro* conditions before irradiation. The effect was transient and disappeared within the period of the first *in vitro* cell division.

Cells have been grown routinely *in vitro* as a monolayer on the surface of glass bottles; for survival curve experiments, cells were prepared from these cultures in one or other of two ways. The monolayer was first dispersed with trypsin, then the cells were either plated directly on to Petri dishes or they were inoculated into the peritoneal cavity of a mouse. In this case an ascites tumour, which was a single cell suspension needing no further trypsin treatment, developed in about three weeks. These cells retained their ability to develop into clones when they were plated on to Petri dishes. Cells which were plated immediately after trypsinization of a monolayer culture

will be referred to as "bottle cultured" or *B.C.* cells and cells which were grown in a mouse will be referred to as "mouse cultured" or *M.C.* cells.

Both *B.C.* and *M.C.* cells became attached to the surface of 'Nunc' plastic Petri dishes within 1 h of being plated. The usual plating efficiencies were about 65 per cent and 50 per cent for *B.C.* and *M.C.* cells, respectively. Cells were irradiated, after removal of the growth medium, with 250 kVp X-rays at a dose rate of 253 rads/min.

The radiation sensitivity of the two types of cell was markedly different (Fig. 1). Twice the dose was required to reduce *B.C.* cells to the same level of survival as *M.C.* cells in the presence of oxygen; this result was not affected when *M.C.* cells were treated with trypsin before they were plated. The difference was even more pronounced when the cells were anoxic during irradiation; the ratio of doses producing the same level of survival was 2.5. The additional enhancement in the sensitivity of *M.C.* cells irradiated anoxically was reflected in the oxygen enhancement ratios for the two types of cell, which were 2.7 for *B.C.* cells and 2.0 for *M.C.* cells.

The difference in sensitivity between *B.C.* and *M.C.* cells almost disappeared after the *M.C.* cells had been in *in vitro* conditions for a period before irradiation. In these experiments *M.C.* cells were removed from a mouse and put into culture bottles containing growth medium. These were incubated at 37° C for the selected length of time, after which the cells, by then attached to the glass, were removed with trypsin, plated on to Petri dishes in the usual way and irradiated. The sensitivity of the *M.C.* cells decreased with time spent *in vitro* before irradiation and approached that of the *B.C.* cells (Fig. 2). A dose of 364 rads given in air to cells 1 h after plating killed fifteen times as many *M.C.* as *B.C.* cells. This factor was reduced to 1.5 when *M.C.* cells had been incubated *in vitro* for one day before the irradiation was given. Observations were made on the change in sensitivity over the first 18 h after transferring *M.C.* cells to *in vitro* culture conditions. The sensitivity did not begin to decrease for about 8 h; there may have been a slight increase during this time (Fig. 3).

From these experiments it can be seen that the transition from *in vivo* to *in vitro* culture conditions caused an immediate increase in sensitivity of the cells from which

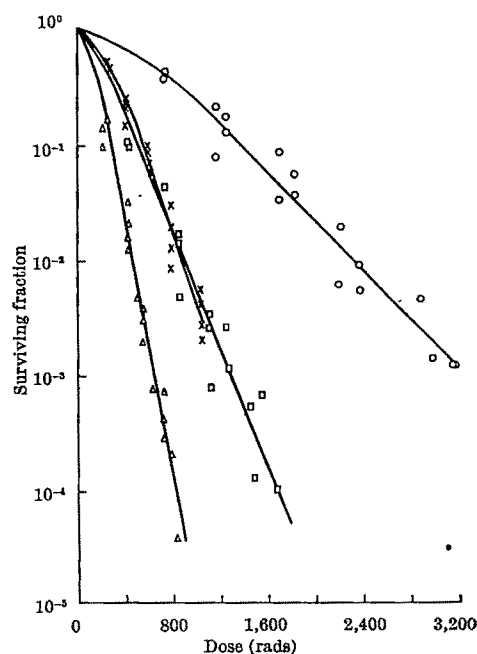


Fig. 1. Survival curves of *B.C.* and *M.C.* cells.  $\Delta$ , *M.C.* cells, oxic;  $\square$ , *M.C.* cells, anoxic;  $\times$ , *B.C.* cells, oxic;  $\circ$ , *B.C.* cells, anoxic.

they slowly recovered. To examine the effect of keeping the cells *in vivo* after irradiation but before plating, a mouse bearing a tumour of *M.C.* cells was irradiated. Immediately after the irradiation and at times up to 4 h later, cells were withdrawn from the mouse, counted, diluted and plated. Because there is evidence to show that a large proportion of cells in a well developed ascites tumour is anoxic<sup>4,5</sup>, the mouse was given pure nitrogen to breathe before and during the irradiation to ensure that the whole population was anoxic. As mice can breathe nitrogen for only about 45 sec before they die, a high

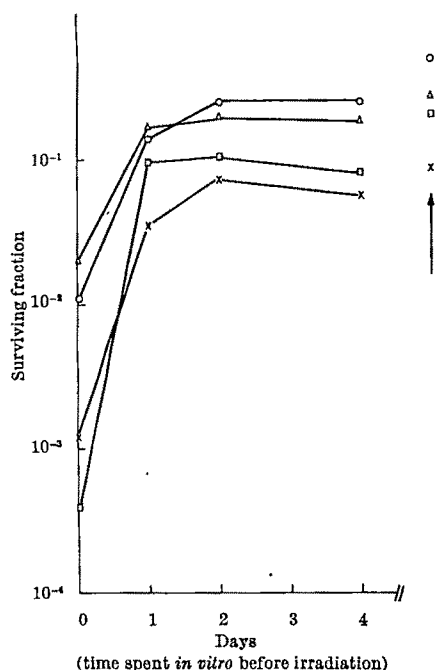


Fig. 2. Change in sensitivity of *M.C.* cells after a period of *in vitro* culture.  $\circ$ , 728 rads, anoxic;  $\Delta$ , 364 rads, oxalic;  $\square$ , 1,246 rads, anoxic;  $\times$ , 622 rads, oxalic. Arrow points to survival of *B.C.* cells to the same dose.

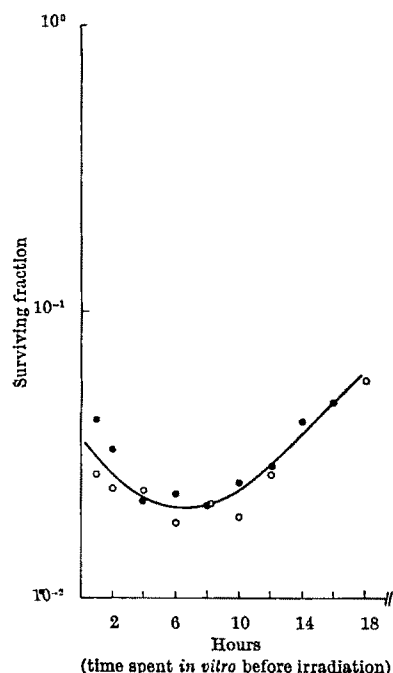


Fig. 3. Change in sensitivity of *M.C.* cells to a dose of 364 rads given in air, after a period of *in vitro* culture. Symbols represent two separate experiments. Arrow points to survival of *B.C.* cells to the same dose.

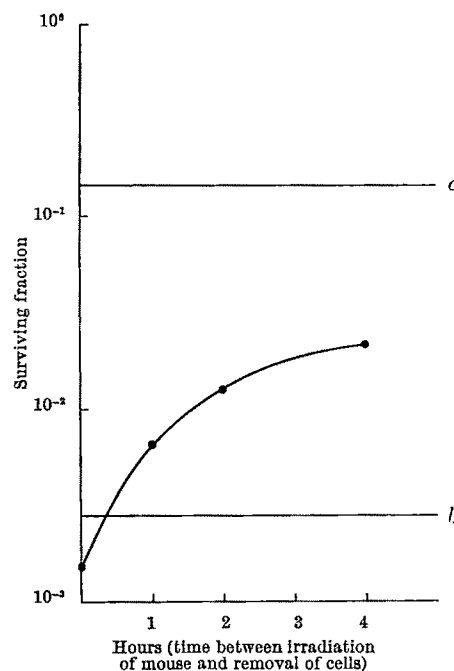


Fig. 4. Change in colony-forming ability of *M.C.* cells with the time they were left in the mouse after irradiation. All samples received 1,200 rads of electrons anoxically. *a*, Survival level of *B.C.* cells and, *b*, survival level of *M.C.* cells, irradiated on Petri dishes 1.75 h after plating.

dose rate was required for the irradiation. This was achieved by using the beam of 8 MeV electrons from the Medical Research Council linear accelerator. The longer the cells were left in the mouse after a given dose the greater was the number of cells able to give rise to colonies when they had been plated (Fig. 4). The increase in surviving fraction when the cells were left in the mouse for 4 h after irradiation was too large to be accounted for by division of surviving cells within the mouse. It may be that the operative mechanism was similar to that which resulted in the increase of survivors observed when *M.C.* cells were kept *in vitro* before irradiation.

Supporting evidence for the transience of the change in sensitivity observed with change in growth conditions of these cells comes from experiments reported by Cullen and Hornsey<sup>6</sup>. Survival curves constructed from results obtained using cells cultured *in vitro* throughout were very similar to those which had been obtained by Silini and Hornsey<sup>7</sup> from experiments in which cells had been grown *in vivo* and subsequently assayed *in vivo*.

The phenomenon in which culture conditions exert a markedly differential action on the sensitivities of cells irradiated in oxia and anoxic conditions has frequently been demonstrated with micro-organisms<sup>8-10</sup>. The only previous demonstration of the effect on mammalian cells seems to have been that by Berry<sup>11</sup>, who found that HeLa cells grown in the presence of "methotrexate" were more radiosensitive than cells grown in normal culture medium and that they also had a reduced oxygen enhancement ratio. Berry<sup>11</sup> suggested that his results might have been caused, in part, by a synchronization of the cell population, but no evidence of synchronization of *M.C.* cells has been found in these experiments.

If cells were normally capable of repairing some of the damage caused by irradiation, these results could be accounted for by the mechanism of repair becoming much less effective for a period after transfer from *in vivo* to *in vitro* culture conditions and gradually becoming more effective again within about 24 h.

Whatever the nature of this repair process, it is more effective when the cells have been irradiated in anoxic conditions so that it must be acting on a component of damage which plays a greater part in these conditions.

This suggests that such damage is associated with an oxygen enhancement ratio which is intrinsically lower than the overall ratio<sup>12</sup>.

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<sup>1</sup> Bases, R. E., *Cancer, Res.*, **19**, 1223 (1959).

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## PSYCHOLOGY

### Ultra-kinaesthetic Judgment of Size

AN investigation has been carried out on some numerical links between visual space and a kinaesthetic sensitivity to spatial relationships. One type of experiment (purely visual) is based on Luneburg's concept of non-Euclidean visual space<sup>1</sup> and on its modification by Blank<sup>2</sup>. I have designed another type of experiment (visuo-kinaesthetic)<sup>3</sup> to investigate some effects of proprioception on vision. In these experiments an observer imagines himself moving towards two distant light points in the darkness and passing his forearm between them. He adjusts the gap between the two light points by remote control so that it equals the length of his forearm. The variables are  $C$ , the size of the gap between two light sources in a frontal plane, and  $R$ , the size of the forearm of the observer. (Other dimensions of the observer's body are considered in other trials.) These variables are compared with  $K$ , Luneburg's curvature of visual space, and  $r$ , the subjective distance of the stimuli from the eyes, as given by Blank in units of  $(-K)^{-\frac{1}{2}}$ . The experiments show that  $C$  (which is approximately equal to  $R$  close to the observer) contracts sharply in the case of negative curvature with an increasing distance from the eyes (to a value several times smaller at a distance of several yards) but remains approximately constant with  $K \sim 0$  and expands with  $K > 0$ .

For a selected experimental condition,  $C$  and  $R$  predict  $K$  and  $r$  (and vice versa) with considerable numerical accuracy. For a wider range of experimental distances and for extreme values of  $K$  and  $r$  some of the numerical accuracy is lost. (This is related to the known difficulties in determining  $K$  and  $r$  for wide ranges.) Statistical differences between the observers, however, remain highly significant. Using a more general approximation to the curves of  $C$  against  $r$ ,

$$C \sim R(\cosh r)^{-1}$$

the meaning of  $C$  as a proportion of  $R$  can be outlined. A small  $C$  (with large  $\cosh r$  and high negative curvature) indicates that an observer uses a small unit in assessing size (allowing for visual angle which diminishes with distance) so that he assesses the size as relatively large. But when for a relatively great physical distance  $C$  approximates  $R$  (with  $\cosh r \sim 1$  and  $K \sim 0$ ) an observer with poor distance perception using a large unit in his measurements assesses the size as relatively small. It is suggested that  $C$  by its proportion to  $R$  establishes a unit of visual measurements which is recalibrated for every phenomenological distance in a system characteristic for an individual observer.

An important feature of this system is that it is expressed in terms of the body size of the observer. This permits a departure from the relativity of the subjective comparisons and allows the assignment of a physical magnitude to a subjective assessment. If, for a distant object of size  $T$ , we were to write,

$$T = nR = nC f(r) \sim nC \cosh r$$

then, in the subjective counterparts of these terms, the proportion ( $n$ ) would not necessarily be affected by the observer's system of measurements, that is, this proportion is most likely to be independent of the observer's personal constants,  $K$ , and the values characteristic for him of  $r$  and  $C$  for great distances. The critical condition required to estimate  $T$  correctly, however, is that the observer must place himself ( $R$ ) and the object ( $T$ ) at the same phenomenological distance ( $r$ ) in his system, and that he must allow for the proportion ( $n$ ). This, it is suggested, is a function of effective kinaesthetic activation in the whole body as well as in the eye orbits.

In an actual experiment which was carried out from an attic window with a wide view, twenty observers were asked to imagine that they were swimming through the air towards distant buildings, or climbing their walls. (Slight muscular exercises designed to promote the kinaesthetic activation within the limbs and the eye orbits were alternated with the tasks.) During certain trials each observer was asked to imagine himself clinging to the face of a chimney (9.5 ft. wide and 130 yd. distant), and to say whether his hands would grip the edges of the chimney or whether they would miss them and by how much, that is, by a hand, by a forearm or by an arm. Each observer was also asked to imagine himself climbing across the width of the Post Office Tower (52 ft. wide and 660 yd. distant) and to say how many times he would have to shift his body with extended arms across the face of the tower while doing so. Afterwards the observers were asked to estimate visually the size of the chimney and of the tower in terms of feet or yards. They also estimated the size of their body members. Finally, the anthropometric measurements of the observer's body were taken.

The results showed that the estimates based on the imaginary tasks and on the observers' size given anthropometrically were better than the visual estimates by a highly significant amount ( $P=0.0006$  in the case of the chimney, and  $P=0.002$  in the case of the tower). The visuo-kinaesthetic estimates were remarkably accurate in the case of several observers and, as expected, they depended neither on their perception of distance nor on  $K$ . They were also independent of an observer's knowledge of the dimensions of his body. Such proprioceptive sensitivity (probably connected with a motor type of imagery and with former visuo-motor experience) would be an advantage in survival tests, as, for instance, in space research. Its neuro-physiological mechanism (which may be linked with the activity of the muscle spindles) and its ties with the visual parameters are a matter for further investigation.

The apparatus for this investigation has been designed by Mr. J. W. Chambers and made in this department by Mr. R. J. Sloan and Mr. C. McManus. I am grateful to the Central Research Fund of the University of London for some parts of equipment, and to my colleague, Dr. Sheila Jones, for helpful suggestions.

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<sup>1</sup> Luneburg, B. K., *Mathematical Analysis of Binocular Vision* (Princeton, Princeton University Press, 1947).

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Wednesday, March 29

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 2.15 p.m.—Meeting on "Performance of Airborne Inertial Navigation Systems".

## Wednesday, March 29—Saturday, April 1

MATHEMATICAL ASSOCIATION (at the Welsh College of Advanced Technology, Cardiff)—Annual General Meeting.

## Thursday, March 30

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Mr. John Karefa-Smart: "The Problems of Nutrition in Developing Countries".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. T. Norris: "Loading of Power Transformers".

INSTITUTION OF STRUCTURAL ENGINEERS (at 11 Upper Belgrave Street, London, S.W.1), at 6 p.m.—Mr. W. I. J. Price: "Expansion Joints for Bridges and Over-Head Highways".

SOCIETY OF INSTRUMENT TECHNOLOGY (at the Stanley Palace, Watergate Street, Chester), at 7.30 p.m.—Mr. A. L. Rankin: "Lasers".

## Thursday, March 30—Friday, March 31

LUTON COLLEGE OF TECHNOLOGY, DEPARTMENT OF SCIENCE (at Park Square, Luton)—Symposium on "The Biochemistry and Genetics of Macromolecules".

## Friday, March 31

BRITISH COMPUTER SOCIETY (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7)—Symposium on "The Computer Service Bureau in Business and Commerce".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 9.15 a.m.—Conference on "New Methods of Increasing Productivity in the Rubber Industry".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. R. W. Stevens: "Acoustic Behaviour of Materials".

## Sunday, April 2—Thursday, April 6

ROYAL COLLEGE OF SURGEONS OF ENGLAND (in association with The Royal Society; The Royal College of Surgeons of Edinburgh; The University of Edinburgh; The University of Glasgow; The University of London; University College Hospital; King's College Hospital; The Lister Institute of Preventive Medicine; The Wellcome Trust; and The Ciba Foundation, at the Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2)—Lister Centenary Conference.

## Monday, April 3

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. Norman D. C. Hammond: "Exploring Ancient Seistan".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER or LECTURER in the DEPARTMENT OF APPLIED MATHEMATICS and MATHEMATICAL PHYSICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (March 28).

LECTURERS and ASSISTANT LECTURERS (with a good honours degree in physiology, biochemistry or related subjects, or with medical qualifications registrable in the United Kingdom) in PHYSIOLOGY—The Registrar, The University, Manchester, quoting Ref. No. 32/67/Na (March 31).

LECTURER (with experience in electronics, electronic physics or plasma physics) in the DEPARTMENT OF PHYSICS—The Registrar, University College of Wales, Aberystwyth (April 3).

ASSISTANT LECTURER (with an interest in extractive or chemical metallurgy) in METALLURGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (April 7).

LECTURER or ASSISTANT LECTURER in BIOCHEMISTRY; and a LECTURER or ASSISTANT LECTURER in FOOD SCIENCE in the DEPARTMENT OF BIOCHEMISTRY, NUTRITION and FOOD SCIENCE, University of Ghana—The Assistant Registrar, Universities of Ghana Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (April 7).

LECTURERS or ASSISTANT LECTURERS in the DEPARTMENT OF BOTANY (one appointment will be made for teaching and research in biochemical genetics and/or plant metabolism)—The Registrar, University Senate House, Bristol 2 (April 7).

ASSISTANT (with an honours degree in botany, some subsequent research experience, and preferably an interest in plant morphology or morphogenesis) in BOTANY—The Secretary of the University Court, The University, Glasgow (April 8).

STATISTICIAN (with a Ph.D. degree in arts or science with mathematics and mathematical statistics as major subjects, or with postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability) in the DIVISION OF MATHEMATICAL STATISTICS, COMMONWEALTH SCIENTIFIC and INDUSTRIAL RESEARCH ORGANIZATION, AUSTRALIA, to take part in the respective Divisional research programmes of the Division of Protein Chemistry and the Division of Animal Health (Animal Health Research Laboratory) which are closely located in Melbourne—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 440/195 (April 14).

LECTURER (with an honours degree in physiology or in a closely related subject, or medically qualified) in PHYSIOLOGY—The Secretary, Queen's College, Dundee (April 15).

LECTURERS/ASSISTANT LECTURERS in ZOOLOGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3 (April 15).

SECOND CHAIR OF PURE MATHEMATICS—The Registrar, The University, Sheffield (April 15).

CHAIR OF CHEMICAL PATHOLOGY at University College Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 17).

SENIOR ASSISTANT LIBRARIAN (with a degree and experience of dealing with scientific material, and preferably professional qualifications)—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey (April 18).

PROFESSOR OF PHARMACOLOGY, and a PROFESSOR OF PHARMACEUTICS at the University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (April 20).

LECTURER and an ASSISTANT LECTURER in PHYSIOLOGY—Secretary of the University Court, The University, Glasgow (April 21).

CHAIR OF PHARMACEUTICS at Chelsea College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 24).

DEMONSTRATOR in PATHOLOGY—The Registrar, The University, Bristol (April 24).

DEMONSTRATOR in PSYCHOLOGY—The Registrar, The University, Liverpool, quoting Ref. RV/420 (April 29).

LECTURERS/ASSISTANT LECTURERS (preferably interested in microbiology, mycology, algology, immunochimistry, genetics, parasitology, vertebrate physiology, fish ecology, higher plant botany, ornithology) in the DEPARTMENT OF BIOLOGY—The Secretary, Royal College of Advanced Technology, Salford 5, quoting Ref. No. B/6 (May 1).

EXPERIMENTAL OFFICER (qualified mechanical engineer) in the DEPARTMENT OF MECHANICAL ENGINEERING to assist in a programme of research connected with the study of turbine and compressor blade vibration—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

LECTURER or ASSISTANT LECTURER in PHARMACOLOGY in the Postgraduate and Undergraduate School of Studies in Pharmacy—The Registrar, University of Bradford, Bradford 7, Yorkshire.

MASTER to teach PHYSICS at all levels—The Headmaster, School House, Rugby.

PHYSICAL CHEMIST for an S.R.C. supported research project on biological fuel cells—Prof. P. N. Rowe, Department of Chemical Engineering, University College London, Gower Street, London, W.C.1.

REGISTRAR (graduate with administrative experience of a high order)—The Secretary, Imperial College of Science and Technology, London, S.W.7.

RESEARCH FELLOW at the Institute of Sound and Vibration Research for a fundamental study of community response to noise from machinery—The Deputy Secretary, The University, Southampton.

RESEARCH FELLOW with research experience in astronomy, optics, spectroscopy or plasma physics) in a Science Research Council supported research group undertaking high resolution solar spectroscopy from rocket and satellite vehicles—Prof. D. J. Bradley, Department of Pure and Applied Physics, Queen's University, Belfast, Northern Ireland.

SENIOR TECHNICIAN (with experience of work in a research or teaching laboratory, able to make up and maintain electrical equipment, and an interest in instruments) in the DEPARTMENT OF ZOOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

## Great Britain and Ireland

Ministry of Technology: Torrey Research Station. The National Collection of Industrial Bacteria—Catalogue of Strains, Second Edition, 1st Supplement. Pp. iii+19. (Edinburgh and London: H.M. Stationery Office, 1966.) 2s. net. [21]

Natural Environment Research Council. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country Around Nantwich and Whitechurch (Explanation of One-Inch Geological Sheet 122). By E. G. Poole and Dr. A. J. Whiteman, with contributions by Dr. D. Magraw, R. V. Melville, Dr. H. C. Ivey-Cook, Dr. D. T. Donovan and B. J. Taylor. Pp. viii+154+8 plates. (London: H.M. Stationery Office, 1966.) 30s. net. [21]

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 772, Vol. 251 (29 December, 1966): A Discussion on Ritualization of Behaviour in Animals and Man. Organized by Sir Julian Huxley, F.R.S. Pp. 247-526+plates 7-32. (London: The Royal Society, 1966.) 140s.; \$21. [51]

Concentration or Competition: a European Dilemma? By D. Swann and D. L. McLachlan. (An Essay on Anti-trust and the Quest for a European Size of Company in the Common Market.) (European Series, No. 1.) Pp. 59. (London: Political and Economic Planning, 1967.) 7s. 6d. [31]

London and Home Counties Regional Advisory Council for Technological Education. Bulletin of Special Courses in Higher Technology, Management Studies and Commerce—1967. Part 2: Spring and Summer Terms. Pp. 146. (London: London and Home Counties Regional Advisory Council for Technological Education, 1967.) 7s. [79]

Central Electricity Generating Board. Sizewell Nuclear Power Station—Descriptive Brochure. Pp. 16. (London: Central Electricity Generating Board.) [51]

Television Research Committee. Working Paper No. 2: Attitude Formation and Change. By J. D. Halloran. Pp. 167. (Leicester: Leicester University Press, 1967.) 21s. [91]

Ministry of Transport: Road Research Laboratory. Road Research Technical Paper No. 56: Traffic Signals. By Dr. F. V. Webster and B. M. Cobbe. Pp. vii+111+8 plates. (London: H.M. Stationery Office, 1966.) 22s. 6d. [91]

Chelsea College of Science and Technology. Annual Report 1965-1966. Pp. 46. (London: Chelsea College of Science and Technology, 1967.) [91]

Building Research Station. Digest 77 (Second Series): Damp-proof Courses. Pp. 4. (London: H.M. Stationery Office, 1966.) 4d. [91]

Bulletin of the British Museum (Natural History). Geology. Vol. 13, No. 6: The British Silurian Cystoids. By Christopher Ronald Charles Paul. Pp. 297-355+10 plates. 44s. Zoology. Vol. 15, No. 1: The Evolution, Host Relationships and Classification of the Nematode Superfamily Heterakidae. By William G. Inglis. Pp. 1-28. 10s. (London: British Museum (Natural History), 1967.) [111]

- University of Cambridge School of Agriculture. Memoir No. 38: A summary of the research in progress and papers published by the members of the Staff of the School of Agriculture and its Associated Research Organizations during the period October 1st, 1965–September 30th, 1966. Review Series No. 21: Research in Applied Plant Physiology—Past, Present and Future. Pp. 26. (Cambridge: The University, 1966.) 3s. [121]
- General Register Office. The Registrar General's Quarterly Return for England and Wales, Quarter ended 30th September 1966. (No. 471, 3rd Quarter, 1966.) Pp. 31. 2s. 6d. net. The Registrar General's Annual Estimates of the Population of England and Wales and of Local Authority Areas, 1966. Pp. 16. 2s. 6d. net. (London: H.M. Stationery Office, 1966 and 1967.) [121]
- The Association of Commonwealth Universities. Third Annual Report of the Council, together with the Accounts of the Association for the year 1st August, 1965, to 31st July, 1966. Pp. 63. (London: The Association of Commonwealth Universities, 1967.) [121]
- University of London. Reorganization 1964–1966. Pp. 158. (London: University of London, 1966.) [121]
- Council for National Academic Awards. Report for the period 1 October, 1965, to 30 September, 1966. Pp. 59. (London: Council for National Academic Awards, 1967.) [121]
- The School Mathematics Project. Director's Report 1965/66. Pp. 47. (London: S.M.P. Office, Westfield College, 1966.) [121]
- Board of Trade (Civil Aviation Department). Aviation Economics and Aircraft Branch (A.E.A.). Operating and Traffic Statistics of United Kingdom Airlines, Calendar Year, January–December 1965. Pp. 29. (London: Board of Trade (Civil Aviation Department), 1967.) [121]
- Commonwealth Mycological Institute. Mycological Papers. No. 104: The Genus *Cylindrocarpus*. By C. Booth. Pp. 56+2 plates. 17s. 6d. No. 105: Microfungi. II: *Brooksia* and *Grallomyces*; *Acrogenotheca ornata* S.P. NOV.; The Genus *Xenosporium*. By F. C. Deighton and K. A. Pirozynski. Pp. 85+1 plate. 10s. 6d. No. 106: Denatiaceae Hyphomycetes. VII: *Curvularia*, *Brachysporium*, etc. By M. B. Ellis. Pp. 57. 17s. 6d. (Kew: Commonwealth Mycological Institute, 1966.) [131]

### Other Countries

- Annals of the New York Academy of Sciences. Vol. 139, Article 2: The Physiology of Diuretic Agents. By Donald W. Seldin and 56 other authors. Pp. 273–539. (New York: New York Academy of Sciences, 1966.) \$7. [21]
- Memoirs of the American Philosophical Society. Vol. 66: Guide to the Archives and Manuscript Collections of the American Philosophical Society. Compiled by Whitfield J. Bell, Jr., and Murphy D. Smith. Pp. vii+182. (Philadelphia: The American Philosophical Society, 1966.) 83s. [21]
- Bulletin of the American Museum of Natural History. Vol. 134, Article 3: Studies on Amphibiae (Amphibia, Reptilia). 3: The Small Species from Southern South America Commonly Identified as *Amphisbaena darwini*. By Carl Gans. Pp. 185–260+plates 37–45. (New York: American Museum of Natural History, 1966.) \$4. [21]
- United States Department of the Interior: Geological Survey. Professional Paper 495-B: Paleozoic Formations in the Wind River Basin, Wyoming. By W. R. Keever and J. A. Van Lieu. Pp. iv+60+plates 1–8. Professional Paper 503-A: Paleozoic Gastropoda from the Moose River Synclinalorium, Northern Maine. Pp. iii+20+plates 1–3. \$0.30. (Washington, D.C.: Government Printing Office, 1966.) [21]
- Indian Forest Bulletin No. 245 (New Series, Entomology): Anti-Termite Characteristics of Jute Board Pieces Treated with "Shirlian". By P. N. Chatterjee and R. S. Thapa. Pp. 4. Rs. 1.00; 2s. 4d. Indian Forest Bulletin No. 255 (New Series, Entomology): Humidity Behaviour of Termites. 1: Effect of Relative Humidity on the Longevity of Workers of *Microcerotermes besseyi* Snyder (Insecta: Isoptera: Termitidae) Under Starvation Condition. By P. K. Sen-Sarma and P. N. Chatterjee. Pp. 6. Rs. 0.65; 1s. 7d. (Delhi: Manager of Publications, 1966.) [31]
- Smithsonian Miscellaneous Collections. Vol. 151, No. 8: The Behaviour of *Ateles geoffroyi* and Related Species. By John F. Eisenberg and Robert E. Kuehn. Pp. iv+63+6 plates. (Washington, D.C.: Smithsonian Institution, 1966.) [41]
- Metropolitan Life Insurance Company. Statistical Bulletin. Vol. 47 (October 1966): Baby Boom Ending. Mortality from Heart Disease—an Insurance Experience. Increased Mortality from Chronic Respiratory Disease. (New York: Metropolitan Life Insurance Company, 1966.) [41]
- Smithsonian Contributions to Astrophysics. Vol. 9 (Whole Volume): Variable Stars in the Small Magellanic Cloud. By Cecilia Payne-Gaposchkin and Sergei Gaposchkin. Pp. iii+205. (Washington, D.C.: Smithsonian Institution, 1966. Available from U.S. Government Printing Office.) \$1.50. [41]
- Bulletin of the Auckland Institute and Museum. No. 5: The Molluscan Families Spigidiidae and Turridae—An Evaluation of the Valid Taxa, both Recent and Fossil, with Lists of Characteristic Species. By A. W. B. Powell. Pp. 154+23 plates. (Auckland, New Zealand: Auckland Institute and Museum, 1966.) [41]
- India: Ministry of Defence. Defence Science Laboratory. Tables of Bivariate Random Normal Deviates. By P. V. Krishna and P. S. Sinha. Pp. iii+120. (Delhi: Defence Science Laboratory, 1966.) [41]
- United States Department of Agriculture. Leaflet No. 540: Periodical Cicadas. Pp. 8. (Washington, D.C.: Government Printing Office, 1966.) [61]
- Barico Fermi Reactor: Use for Irradiation Testing. (Hearing before the Joint Committee on Atomic Energy, Congress of the United States—Eighty-ninth Congress, Second Session, April 6, 1966.) Pp. vi+271. (Washington, D.C.: Government Printing Office, 1966.) \$0.70. [61]
- Mammals Around the Pacific. By George Gaylord Simpson. (The Thomas Burke Memorial Lecture, 1965.) Pp. 24. (London: American University Publishers Group, 1967.) 7s. 6d. [61]
- Carnegie Institution of Washington. Report of the President 1965–1966. Pp. 81+4 plates. (Washington, D.C.: Carnegie Institution of Washington, 1966.) [61]
- Transactions of the American Philosophical Society, New Series, Vol. 59, Part 8: General Tasker Howard Bliss and the "Sessions of the World", 1919. By David F. Trask. Pp. 80. (Philadelphia: American Philosophical Society, 1966.) 82s. [61]
- Mitteilungen des Deutschen Wetterdienstes. Nr. 39 (Band 5): Zur Meteorologischen Begutachtung der Standorte von Kernkraftwerken mit einem Einblick in Grundlagen und Ergebnisse der Ausbreitungsrechnung. Von Anneliese Gutsche, Helga Pfeiffer und Gerhard Seifert. Pp. 63. Berichte des Deutschen Wetterdienstes, Nr. 102 (Band 14): Ergebnisse der Aerologischen Sondermessungen am Meteorologischen Observatorium Hohenpeissenberg im Zweiten Jahr (1965) der Internationalen Jahre der Ruhigen Sonne (IQSY). Von Walter Atmannspacher. Pp. 108. (Offenbach a. M.: Selbstverlag des Deutschen Wetterdienstes, 1966.) [61]
- World Health Organization. Technical Report Series. No. 340: Joint FAO/WHO Technical Meeting on Methods of Planning and Evaluation in

- Applied Nutrition Programs, Rome, Italy, 11–16 January, 1965—Report. Pp. vi+77. 2.40 Sw. francs; 4s.; \$0.80. No. 347: WHO Expert Committee on Nursing—Fifth Report. Pp. 32. 2 Sw. francs; 3s. 6d.; \$0.60. (Geneva: World Health Organization; London: H.M. Stationery Office, 1966.) [91]
- United States Department of Agriculture: Agricultural Research Service. General Catalogue of the Homoptera. Fascicle VI: Cicadelloidea. Part 16: Idioceridae. By Dr. Z. P. Metcalf. Pp. 237. (Washington, D.C.: Government Printing Office, 1966.) \$0.65. [91]
- Canada: National Research Council. Annual Report on Support of University Research 1965–66. (N.R.C. No. 9159.) Pp. viii+224. (Ottawa: National Research Council of Canada, 1966.) \$0.50. [91]
- Institut Royal Météorologique de Belgique. Bulletin Mensuel. Observations Ionosphériques, Novembre 1966. Pp. 26. (Uccle-Fruxelles: Institut Royal Météorologique de Belgique, 1966.) [91]
- International Atomic Energy Agency. Safety Series, No. 19: The Management of Radioisotope Wastes Produced by Radioisotope Users—Technical Addendum. Pp. 81. 42 schillings; 12s.; \$2. Radioisotopes in the Detection of Pesticide Residues: Proceedings of a Panel held in Vienna, 12–15 April, 1965. Panel Proceedings Series. Pp. 118. 65 schillings; 18s. 8d.; \$2.50. (Vienna: International Atomic Energy Agency; London: H.M. Stationery Office, 1966.) [101]
- Australia: Commonwealth Scientific and Industrial Research Organization. Eighteenth Annual Report of the Division of Coal Research, 1965–1966. Pp. 34. (Chatswood, N.S.W.: Commonwealth Scientific and Industrial Research Organization, 1966.) [101]
- Proceedings of the California Academy of Sciences, Fourth Series. Vol. 34, No. 1 (July 26, 1966): Population Biology of the Pacific Sardine (*Sardinops caerulea*). By Garth I. Murphy. Pp. 1–84. (San Francisco: California Academy of Sciences, 1966.) [101]
- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Wheat Research Unit, 1965–1966. Pp. 13. (Ryde, N.S.W.: Commonwealth Scientific and Industrial Research Organization, 1966.) [101]
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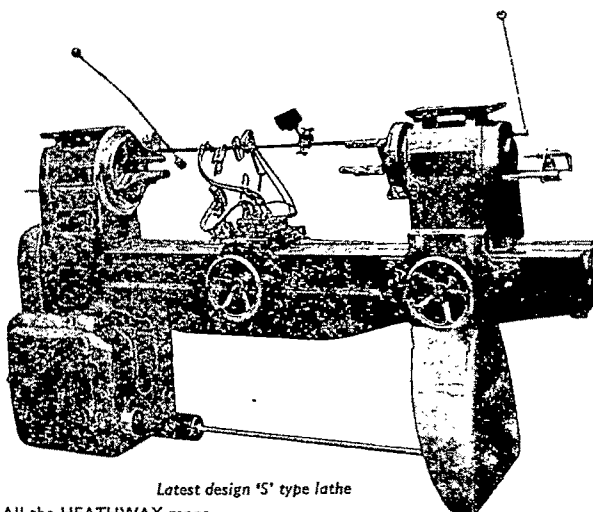
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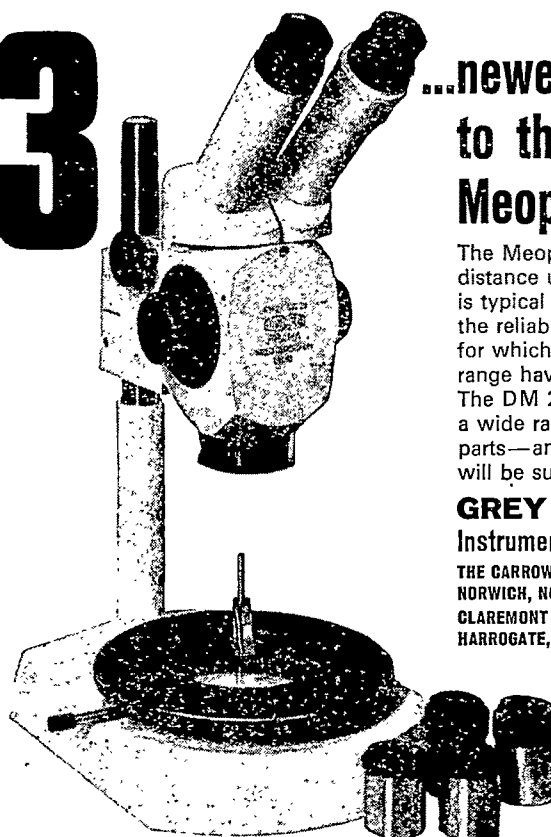
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Further information and application forms may be obtained from the Administrative Assistant, Department of Chemistry, The University of Aston in Birmingham, Gosta Green, Birmingham 4. Please quote: 541/6. (1274)

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